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# Insights from Norway: Using Natural Adaptation to Breed *Varroa*-Resistant Honey Bees

Melissa A. Y. Oddie  and Bjørn Dahle

## Introduction

*Varroa destructor* shook the beekeeping world when it hit. The parasite, once it made the jump onto *Apis mellifera*, was quick to breed itself out of control (Rosenkranz et al., 2010; Traynor et al., 2020). As if the new parasite was not enough, it also developed the potential to vector common viruses. Once those viruses had an easy way to infect bees quickly and thoroughly, the problem became a lethal one (Dainat et al., 2012; Dietemann et al., 2012). The incredible thing about the natural world though, is its ability to shift back to a state of equilibrium after a large disturbance, with species adapting to one another to find some form of novel balance between them. This is exactly what Western honey bees have done.

We now know of several populations of our beloved honey bee living successfully with *V. destructor* and little to no human-mediated control. Some of these populations were derived from domestic stock (Locke et al., 2012), but the most interesting thing is that there are populations in possession of surviving traits that are currently used for honey production (Oddie et al., 2017), so not only can they survive, but they survive well enough to support a professional honey operation too.

In the next few pages, we hope to bring you knowledge and experience from the bee breeding community, centered around the story of a beekeeper in Norway, how he and his bees overcame *Varroa*, and what we have learned about *Varroa* resistance through studying them.

Terje Reinertsen has been breeding within his own stock of about 400 hives since before *Varroa* was recorded in the area where he works. When the mite was detected in 1993, he began to treat his colonies as per the recommendations given

by the Norwegian Beekeepers Association, but he decided to stop treatment in 1997. Initially the losses were high, but in just a few years there were rapid improvements: the bees recovered, mites were still present, but they were few in number, and colony mortality fell back into acceptable rates, between 5% and 15%. Now Terje's stock does not require any mite treatments, and continues to produce competitive quantities of honey every year. Terje's bees are *Varroa*-resistant, meaning they have the ability to reduce the population growth of *Varroa* within a hive so that their levels are balanced (Figure 1), rather than the bees simply existing with high mite loads almost continuously; this is a trait we will call mite tolerance. We will be focusing on mite resistance in this article, but mite tolerance is also a survival mechanism used by some.

Terje watched his bees very carefully. His attentiveness was likely what allowed him to see the changes in his bees and then help them along, rather than try to fight the mite by himself using only conventional treatments. So, what is the recipe? How can this become a simple plan that anyone can follow? There is still a lot of work left to do; we do not yet know exactly how the bees are overcoming *Varroa*, but we have a few clues, and by the end of this article we hope to present some steps that will point you in the right direction.

## What Makes a Surviving Bee?

Scientific research so far has pointed to multiple traits working together to provide resistance to *Varroa* (Mondet et al., 2020), and these traits likely vary among populations, depending on the environment, locality and the demands made by human need (such as honey production) (Le Conte et al., 2020). The consensus of many literature reviews is

that measurable, physical traits and their assessments are the key to developing mite resistance in honey bee stocks (Guichard et al., 2020; Mondet et al., 2020). On this subject there are a few practical things we know: (1) Nearly all bees with a natural *Varroa* resistance have a trait that reduces the reproductive success of the parasite. This trait we will call "Suppressed Mite Reproduction" or SMR (Locke, 2016; Mondet et al., 2020) and for the purpose of this article, this term will include mite non-reproduction. This trait exists in wild, feral and domestic honey bee populations across continents: bees in Africa display this trait (Nganso et al., 2018), as well as African hybrids (Corrêa-Marques et al., 2003) and pure European (Western) honey bee domestic stocks (Locke et al., 2012). (2) Of the resistant populations of Western honey bee, most tested populations have displayed high levels of another trait called "cell recapping" (Oddie et al., 2018), which has been identified as a very useful trait when examining a colony's ability to resist *Varroa* (Mondet et al., 2020, p. 202), both because of its consistent presence and the fact that it can be easily seen without a large time commitment or unique tools: The holes in cell caps can sometimes be identified from the top of the cell, but forceps or a fine needle are all that is needed to invert the cap and examine the mended hole left there by hygienic bees (Figure 3). If you can see it, you can measure it, so this trait can be very easily tracked in colonies and may prove very useful when selecting queens for resistance breeding. The direct link between SMR and cell-recapping has not yet been defined, but their paired presence in surviving populations is quite clear.

## SMR

Let us go back to the concept of equilibrium between a parasite and its host: The plain truth is that *Varroa* is likely here to stay. With the number of domestic hives increasing globally (FAOSTAT, 2008;

Moritz & Erler, 2016), and hive densities in many areas inflated well beyond the natural levels (Graystock et al., 2016), parasite spread may be impossible to control, however parasite density and population growth can be balanced to permit the growth of healthy colonies. It is simply a matter of adaptation, and examples of this occur all the time in the natural world. One of the most famous tales of invasion comes out of Australia: The cane toad (*Bufo marinus*) is a species that can eat almost any animal smaller

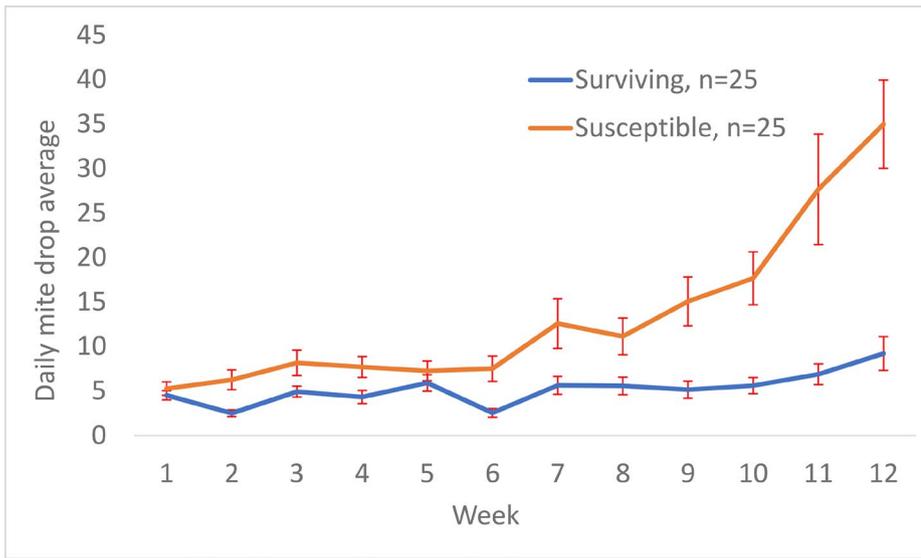
than itself and it is very resistant to predation due to the toxins it produces from glands in its skin. Several species in Australia, that usually prefer preying on toads, have developed an aversion to this species, increasing their probability of surviving an encounter with the toads (Greenlees et al., 2010). In the context of the bees, this type of equilibrium has manifested as bee colonies being able to reduce the number of offspring a *Varroa* foundress can produce in her reproductive cycles.

This second graph (Figure 2) displays the proportion of failed reproductive cycles by *Varroa* foundress mites in one summer across four geographically separate populations. The Norwegian population is the commercial stock used by Terje. The bees do not remove *Varroa* from the hives completely, but SMR plays a very distinct role in creating the population patterns seen by that blue line in our first graph (Oddie et al., 2017). The result is a honey bee population that still has *Varroa*, but at a level that can permit both parasite and host to exist and even thrive in balance. So, we now have a good idea of what is happening in these populations, but *how* is it happening?

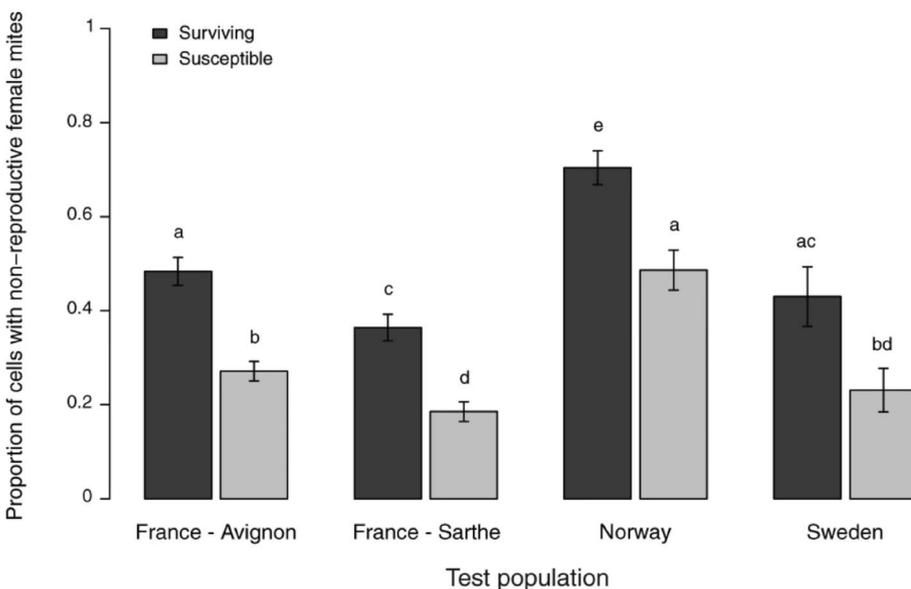
**Cell Recapping**

The shortest answer to the “how” is, we are still not sure. There are many ways SMR can be achieved: *Varroa* sensitive hygiene (VSH), for example, where the infested brood cells are removed completely, and the cycle of mite reproduction is stopped (Harris et al., 2010). VSH would produce SMR, if bees choose not to target cells where the *Varroa* foundress has laid fewer eggs, leaving only those “rejected” cells to be measured by scientists. There might be changes in the brood pheromonal signals (Broeckx et al., 2019), which the mites rely on almost exclusively to begin their egg-laying cycles (Garrido & Rosenkranz, 2003). A more rapid development of the young bees, i.e. a reduced post-capping period could also contribute to SMR (Oddie et al., 2018). *Varroa* foundresses need good nutrition to be able to produce offspring, and increased grooming (de Guzman et al., 2008) may decrease their chances of feeding on adult bees, resulting in malnourished foundresses producing fewer eggs. More or less evidence can be provided for each of these hypotheses, but there is one trait that has been observed consistently in surviving populations:

Cell uncapping is a behavioral trait that is very natural in all honey bee colonies to assess the health of pupating brood; it is the first step to removing diseased or dead brood (Figure 3) (Danka et al., 2013; Harris et al., 2012; Oddie et al., 2018). The sensory cues used to perform brood removal are likely many, and linked. If not all cues are present, the behavior may only be partially done, meaning brood cells may be left open for bees to recap them sometime afterwards. Generally, bees do not leave their healthy, pupating brood cells open. This could create a pathway for a new trait that has the potential to control *Varroa*. *Varroa*



**Figure 1.** A graph presenting the average mite fall counts for two experimental apiaries of two different honey bee stocks each week of the summer (n = 25 colonies per stock; standard error shown). Terje’s *Varroa*-resistant bees (blue) which were not treated at any point, and a Carnica control stock (orange) that was treated regularly every year, up until the fall of 2018. Terje’s resistant population had much lower mite counts in the final week of measurement than the control population ( $t_{\text{final week}} = 4.99, df = 48, p < 0.001$ ). Data was collected in the summer of 2020 (May 19–August 18), a t-test was performed on final count averages.



**Figure 2.** The rates of failed reproduction of *Varroa* foundress mites in four geographically distinct populations of *Varroa*-surviving Western honey bee. Rates of failed reproduction are consistently higher in all surviving populations than their local control groups of susceptible bees. This figure was first published in Oddie et al. (2018).

offspring rely on consistent temperature and humidity inside the cells where they are developing (Kraus & Velthuis, 1997; Le Conte et al., 1990). Disturbances in these constants may be sufficient to reduce the number of offspring that hatch and survive to adulthood. It may even be as simple a case as the opening of a brood cell frightens the foundress to the point where she is prevented from laying eggs, or disturbs the offspring mating, which occurs exclusively in bee brood cells. Cell uncapping and recapping can occur multiple times on the same cell, and hypothetically this could result in consistently lower reproductive rates in *Varroa* foundresses colony wide. Theories aside, there is striking evidence that cell recapping is both increased in surviving colonies and targeted on *Varroa*-infested brood cells:

The data in this third graph (Figure 4) were taken from the same four populations where *Varroa* reproductive success was measured: These bars depict the levels of recapped cells found on the same pieces of comb, which was sampled from a number of colonies in each population. The most interesting thing about these numbers is that they are from very separate populations, with no consistent contact, being in four very different regions in Europe, and yet levels of recapping and the *Varroa*-targeting are consistently high in all four. It is direct evidence to support the convergent evolution of this trait. Beyond these four, recapping has more recently been documented in other surviving populations (Martin et al., 2020): *A. m. scutellata*, *African hybrids* and *A. m. capensis* all displayed higher rates of recapping than control populations, and

this trait did not seem to correlate with freeze-killed brood tests. Cell recapping was simply targeted to *Varroa*-infested cells. It was also correlated with SMR in a study done on several more Western honey bee populations in Germany, Croatia and Austria and it was highlighted that this would be a trait to aid in queen selection (Büchler et al., 2020). This correlation would not happen if recapping did not play at least a partial role in achieving the equilibrium between the bees and *V. destructor*.

So now we know a bit about what is going on, and we have an idea of how it is occurring, but the question still remains: what can beekeepers do to take advantage of these natural adaptations, and bring them into domestic operations? Is there some recipe that can be built from what we have learned?

### What is the Recipe?

In nature, there are two things that push changes in a species or population: trait variation and a pressure from the environment that favors some traits over others.

Genetic diversity provides variation in traits, and honey bee biology is designed to maximize genetic diversity: queens naturally mate with multiple drones (Winston, 1991), and their genetic recombination rates (how the genes from both parents are



Figure 3. With a little practice, cell recapping is not hard to observe at a glance: (a) Top of a recapped cell, with a clear depression where the bees have opened the cell and covered it back over with wax. If you carefully remove the cell cap and turn it over, the hole in the pupal cocoon becomes even more visible, like in (c). (b) Complete cell cap, for comparison.

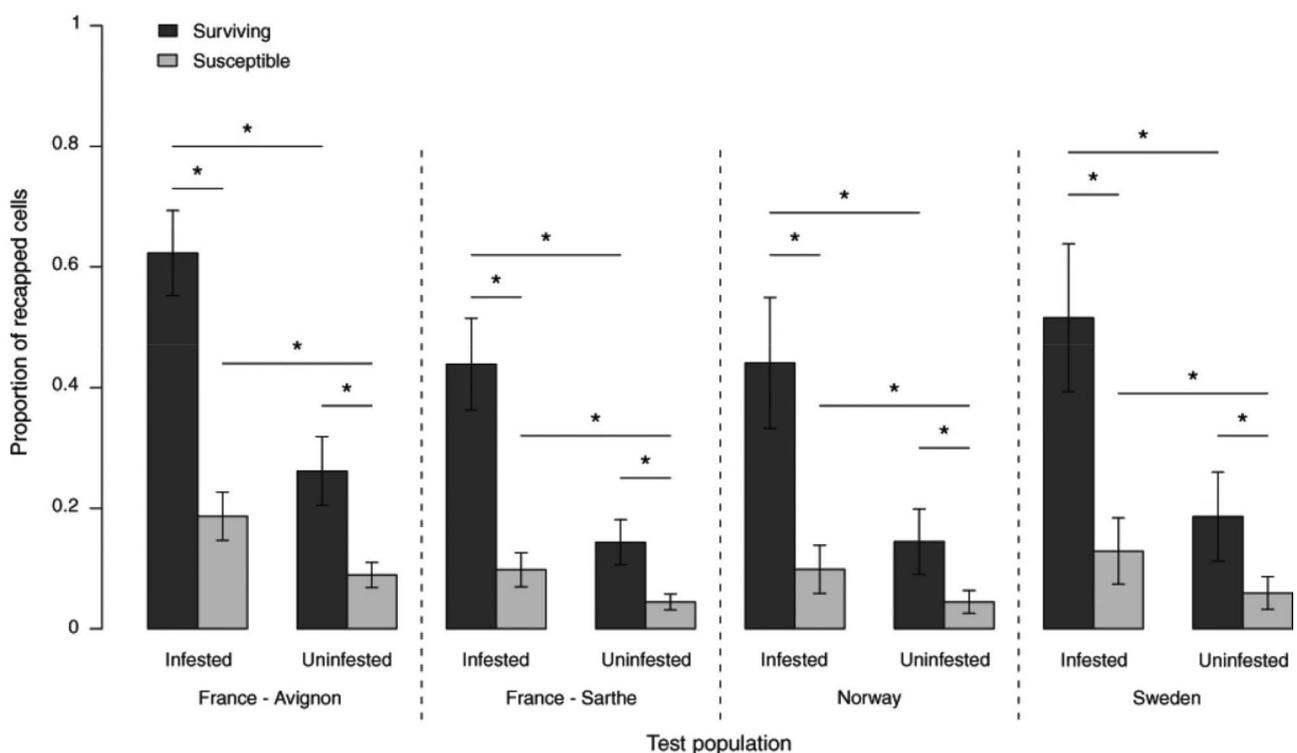


Figure 4. Recapping rates in four geographically distinct populations of *Varroa*-surviving Western honey bee. Rates of recapping are displayed for both cells containing mites (infested) and cells free of mites (uninfested). The graph shows that for surviving colonies (dark grey) recapping was much higher, and the highest rates in cells that contained *Varroa* mites. This figure was first published in Oddie et al. (2018).

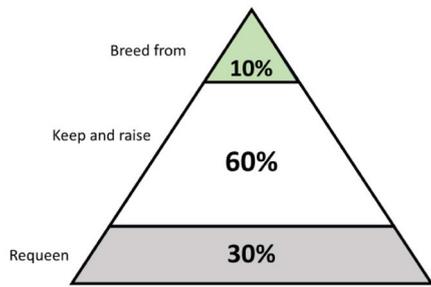


Figure 5. Terje's population management structure where the top 10% in green are the colonies that do the best overall in a model where mite treatments are not used. It is recommended to control mite levels in the colonies that are deemed unfit for breeding, to prevent excessive spillover across your hives and neighboring beekeepers.

mixed to produce offspring) are the highest of any animal on earth (Kent et al., 2012). Despite these strengths, honey bees can still undergo inbreeding depression: this is when the genetic diversity in a hive or population becomes too low due to breeding related individuals and detrimental mutations are repeated more frequently. A lower diversity both in colony and population also reduces the presence of beneficial traits and makes a population more susceptible to new threats like *Varroa*.

Keeping the genetic diversity high by having a large number of breeding individuals in your population aligns with the natural strategies of the bees, and though it may make beneficial traits more variable, it will ultimately make healthier bees (Neumann & Blacquière, 2017).

The second ingredient is selection pressure. In order to spread beneficial traits within a population, the individuals without those traits must be removed from the breeding pool. In this context, *Varroa* could be used as an indicator of which colonies to breed from and which to avoid. In nature, individuals that do not possess the traits that give them an edge generally do less well than individuals that do possess these traits, and probability of death slowly decreases their numbers. The good thing about keeping bees is you do not have to let a colony die to prevent it from breeding. In the case of Terje, he simply breeds queens from the colonies that did the best in the previous year. Terje finds the best 10% of his population each year and breeds queens from that best 10% to replace the worst 30% (Figure 5). His drone colonies are cycled each year, so there are always new colonies (with queens of varying ages) contributing drones to the free mating congregations the queens fly to. In terms of "best," we will not try to define that here, as what is "best" will be completely dependent on case and preference, so take this as is simply "your best" for the operation you have.

"*Varroa* is not a focus, it is a variable," Terje says (Figure 6), and this approach can be applied to a lot of issues that may appear in a population. Being aware of how nature works, especially when keeping bees, is very useful, and we can help evolution along with selective breeding while also refining the traits we need.

That being said, any selection needs to be done responsibly. In most cases concerning *Varroa*, we have to deal with a system that has not yet reached its natural equilibrium (Blacquière & Panziera, 2018; Rosenkranz et al., 2010), and the problem has the potential to get out of hand very quickly. Responsible breeding would involve controlling mites in colonies that are losing the battle to prevent excessive mite loads which could hamper your selection efforts and make life difficult for neighboring beekeepers. The selection pressure remains in the system because those colonies that do not require any (or as much) treatment will continue to do better than the colonies that do and slowly you should be able to shift an entire population towards *Varroa* resistance, just like our ancestors made these same bees docile enough to keep in the first place.

Now that we know something of how the system works, we know a bit about natural adaptation and the recipe for selection, how does one actually start breeding *Varroa*-resistant bees? There are a few practical things that can be recommended and we will close out this article with them.

## What Can Beekeepers Do?

One of the most important things to remember is that bees are unique in the world of livestock: They cannot be fenced in like other animals and they require interaction with the natural environment. By extension, they cannot be prevented from interacting with other bees, so keeping all your colonies healthy will ultimately serve not only your own operation, but your beekeeping community too, and that is crucial as it is the community that will ultimately be the biggest driving force to the improvement of beekeeping as a whole. Cleanliness practices like changing wax in the brood room to reduce disease spread and not sharing frames between colonies are basic beneficial steps towards healthy beekeeping. For *Varroa*-specific problems, we have three central suggestions, which have been based on the review of scientific literature that we have touched on above, as well as on the experience of working with and being a part of the beekeeping community for many years. These suggestions are not exclusive, and by no means should they be considered rules, but they may help in endeavors to obtain a naturally surviving stock of domestic bee.



Figure 6. Terje Reinertsen, the Norwegian beekeeper who has developed his own stock of *Varroa*-resistant bees. Photo credit: Anita Reinertsen.

### Count Mites

The first thing to do? Count mites. At the beginning of any breeding attempt, it is very important that you are aware of parasite levels in your hives so you can make decisions about when or what to treat. Sugar shakes or soap washes are more reliable (De Jong et al., 1982; Devlin, 2001), but bottom board counts are less invasive and both methods can give you a good general picture of the mite situation in each hive you test. It is good to take more than one test in a season. A spring, summer, and autumn count can give you an idea of how much the mite population has grown. Remember also that the mite level in a hive is tied to the amount of brood (Wilkinson & Smith, 2002), so it is good to take tests once a week for at least two weeks in a row. The mite levels can change quite a lot, especially in summer and autumn. Consider the size of your colony as well, how many frames are your bees covering? A lot of mites in a small hive are worse than a lot of mites in a big one. The damage threshold varies by area and also throughout the season (Büchler et al., 2014) so local knowledge about damage thresholds should be used when deciding which colonies to treat with acaricides and which colonies to use for breeding.

### Breed Some of Your Own Queens

Even if you do not have a queen breeding operation, it may pay to try and breed from your own stock. Local adaptation does play a role in the health of colonies (Büchler et al., 2014), and you have a much better idea of what traits your bees have over those of bees imported from a breeder far away. Working with queen breeders in your area is also an option. They may agree to graft some of your bees' larvae for you, and of course, genetic diversity is key, so try to keep your breeding pool wide within a local context (Neumann & Blacquièrre, 2017). The magic number for Terje is 10% of his stock. Changing queens will be a regular process when selecting for *Varroa* resistance. You do not need to kill a colony that is struggling, simply choose a better queen.

### Share Experience

Finally, the step that we believe is the most important: Share your experience. The beekeeping community is one of the most cohesive, collaborative, and innovative communities we have ever had the pleasure of working with and that is a massive strength when taking on the daunting task of adapting an entire population to *Varroa*. As said before, bees cannot exist in a closed system; to achieve

population-wide changes, we will need the collective efforts of many. Especially, if your operation is limited in size, the cooperation with your neighboring beekeepers will be of utmost importance. Start bee clubs, speak to other beekeepers, and record what you have learned so it can be passed on. Do not be afraid to reach out to your community for help if you are just starting out, or if you want to change things up in an older operation. If bees have taught us one thing, it is that there is *always* something more to learn.

## Conclusion

As research continues to reveal more and more about the “how” of honey bee resistance to *V. destructor* our methods and results will improve. We know that adaptation to this parasite is very complex, but that does not mean it cannot be understood and used. Easily quantified traits like recapping could be the key to choosing domestic bee colonies that already have an advantage and strengthening stock at a local level. There will be no one “super bee” that will solve the world's parasite problems, but individual beekeepers and communities both apicultural and scientific working together just might.

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Julien Perrin, Abdelhak Boukadiri, Pascal Boyard, Jean-Baptiste Soubelet & Jean Xavier Mazoit:

**Hygienic behavior in honey bees and prediction of *Varroa* non-reproduction in single-drone inseminated (SDI) colonies**

<https://www.tandfonline.com/doi/full/10.1080/00218839.2019.1673550>

The two standardized assays for testing hygienic behavior in *Apis mellifera* in the field are the freeze-killed brood (FKB) and the pin-killed brood (PKB) assays. Correlation between the two tests is still in debate. It has been argued that the PKB assay was predictive of *Varroa* nonreproduction. We measured the agreement between the two methods in two apiaries with 36 and 59 colonies, respectively. The agreement between the two assays was very poor with a 95% limit of agreement greater than 100%. These assays and the SMR (Suppression of Mite Reproduction) trait were also measured in 21 single drone inseminated colonies infested with *Varroa* and with varying SMR efficacy. A PKB assay result of >46% at 24 h predicted an SMR trait >40% with a sensitivity and a specificity of 0.727 and 0.90, respectively, whereas the FKB assay did not exhibit any predictive value. In conclusion, the PKB and FKB assays are not correlated, but the PKB assay predicted SMR with a good accuracy.