BREEDING BEES RESISTANT TO VARROA

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WHY BREED FOR VARROA RESISTANCE?

For a number of years we have been breeding bees for general disease resistance on the European, African, North and South American continents. At the beginning when we had our first contacts with the varroa mite we utilized all the existing chemical treatments. It quickly became clear that other more efficient non-chemical techniques would have to be found. We realized that chemical treatments were short term measures having serious disadvantages such as high costs and residue problems. To further complicate the situation, when mites become resistant to a treatment new chemical treatments must be developed and so you must start again from zero. At this point you are on a "chemical treadmill" and you cannot advance. Some chemicals have negative effects on the health of queen bees (Haarmann et al. 2002) Also your own health can be affected. So there are a lot of good reasons why chemical treatments should be phased out.

At the beginning of the varroa infestations we did not know if resistance to varroa mites existed in the various races that we were breeding from. The reason was that we had been chemically treating our hives every year because we were too afraid of loosing all our hives. This kept the varroa populations down but at the same time masked the occurance of any varroa resistant hives. In 1993 several of us participated in an experiment testing Apis intermissa in Southern France to see if they would show the same resistance to varroa in France as they had in Tunisia under different climatic conditions in an area where varroa mites kill hives. These bees and their interacial hybrids did show a resistance (Kefuss et al 2004). This indicated to us that varroa mites could be genetically controlled. It was at that point we seriously started thinking about breeding for varroa resistance.

A major goal of bee breeders at the present time is to develop a honey bee " resistant to the varroa mite". Differing definitions for resistance exist (Kefuss et al. 2003,2004). All definations have their weak points and none are completely satisfactory. **So before going on , let us clarify what we mean by the terms varroa tolerance and varroa resistance**.

Commen Sense Definition

We define tolerance as the passive acceptation of varroa reproduction in a colony up to a critical threshold. This critical threshold is colony death. Resistance occurs when a colony actively maintains it's varroa population below the critical threshold (Fig.1).

Veternary Definition

The term "resistance" usually means that an organism cannot be infested at all. Resistance is the capacity not to permit the normal development of a parasite. It is the lower level of receptivity. Receptivity is the possibility of an animal to permit the presence of a parasite and the normal development of its life cycle. An animal resistant to a parasite "refuses" the parasite (zero receptivity). A honey bee resistant to varroa does not permit varroa to parasitize it.

Tolerance is the capacity not to develop clinical symptoms when a parasite develops on or in an animal. It is the lower level of sensibility. Sensibility is the ability of an animal to exhibit the symptoms of a parasitic infestation. A tolerant animal is parasitized but not hindered by the parasite (zero sensibility). Thus a honey bee that is varroa tolerant has parasites but is not bothered by them. Susceptible animals have both receptivity and high sensibility to parasites. Honey bee colonies that are susceptible to varroa must be treated for them to survive.

Resistance is multifactoral and can take the form of **better hygienic behavior or lack of varroa reproduction** in the brood for example (Harbo and Harris 1999). It can also be a **combination of diverse factors** which may or may not be expressed depending upon environmental conditions. Bees for example are more hygienic when food is coming in (Palmquist-Momot and Rothenbuhler 1971). This means that bees resistant under specific local conditions, may show varying resistance at that location and perhaps no resistance somewhere else. **Hygienic behavior is much more complicated** than most people think. In 1996 we put forward the hypothesis that hygienic behavior was controlled by at least 20 to 30 differe nt genetic characters (Kefuss,Taber, Vanpoucke and Rey 1996) but we did not have any hard data to back it up. In 2002 Lapidge, Oldroyd and Spivak demonstrated that hygienic behavior is indeed more complex and pointed out that "many genes are likely to contribute to this behavior.They found seven quantitative trait loci (QTL) associated with hygienic behavior. Jurgen Gadau a geneticist from Wurzburg Germany interprets their results as meaning that at least 70 different genes are involved in hygienic behavior. So that gives us a lot of possibilities for selection (Gadau 2004).

HOW DO YOU SELECT FOR VARROA RESISTANCE?

Before you start selecting for varroa resistance you need to determine what type of bee you need. What is your main source of income? Is it honey, pollination, queens, package bees, pollen, royal jelly, propolis or other bee products. It makes no sense to have a varroa resistant bee if it is not economically viable for you. So the first thing to do is select bees that fit your economic needs. You need to develop simple tests that give clear results with low labor input. Selection is essentially a process of elimination.

Pollen production is fairly straight forward to select for because it has high heritability, can be easily measured and does not require any expensive testing equipment (Table 1). The same is true for royal jelly production as evidenced by the work done in China (Shibi et al. 1993). Honey production is very difficult to select for because there are so many non-genetic variables involved which you cannot control. However you can select for a trait which is associated with honey production such as egg laying (Cale and Gowen 1956) or hoarding behavior (Kulincevic and Rothenbuhler 1973). **Once you have made the first basic selection for economic chacteristics, then it is worthwhile to start selecting first for general disease resistance and then for varroa resistance .**

When we select for general disease resistance, the first thing we do is make a hygienic test on the best production hives (Table 2). We use the frozen brood method and not the pin test because the results are more conservative (Kefuss et al.). The pin test has been demonstrated to artifically increase hygienic behavior (Gramacho et al. 1999). The hygienic test allows us to quickly eliminate hives succeptible to american foulbrood and chalkbrood. Hygienic colonies have lower levels of varroa on the adult bees (Spivak and Reuter 1998). In Chile we have found that hygienic colonies have less varroa in the capped brood ($P<.001$) (Fig.2). So the hygienic test is actually our first step to select for varroa resistance. After we have screened our hives with the hygienic tests we bring the best hives which are 100% hygienic after 48 hours to the same location and stop all anti-varroa treatments. We called this survival test the **Bond Test**, i.e.," **Live and let die**". The results are very easy to interpret. Your colonies either remain alive or are dead. At the present time we have **Bond Test** colonies in France that have survived since 1993. This test automatically selects against colonies that produce non-resistant drones which in turn speeds up your breeding program and lowers costs. **Dead hives** don't produce drones! At the same time, while dying out, the non-resistant hives produce varroa mites which helps to maintain selection pressure on the other hives. Varroa mites are very expensive when you have to buy them. Under natural conditions certain resistance mechanisms may require several years to reach equilibrium. The **Bond Test** allows this to happen. The main disadvantage of this test is that it is slow.

To go faster in a selection program that is already in progress it is important to make higher infestations than normal from time to time to provoke strong sanitary reactions in the colonies which will eliminate colonies with lower resistance. One way to do this is to place frames of highly infested brood (40 or more varroa /100 cells) in colonies that have no varroa infestations. This eliminates about 90% of the test colonies in six months. We call this second survival test "**Bonds Accelerated Test" (BAT)** i.e., **survive or die now.** This very destructive method shortens the **Bond Test**. If not enough breeding material exists in reserve, test queens which you wish to keep can be transferred to a healthy colony just before its colony dies out. **"BAT"** reduces testing time and for this reason we think that it is cheaper than other methods of selection. It is a simple technique that can be used to speed up any varroa resistance program. The defect of **BAT** is that you cannot see weak resistance reactions.

HOW DO YOU KEEP YOUR BEES RESISTANT?

Your best breeder queens are not going to survive very long. In fact much shorter than you think. If you do not produce daughters from them immediately, you will waste all your breeding efforts and a lot of genetic material. This is a point we cannot over emphasize! Once their daughters are in your bee yards the mother queen is not so important. So the first thing to do, is to change out queens in as many of your bee yards as possible, with daughters from each of your resistant breeder queens. The more different groups of daughters at the same yard, the better. Here their drones and future virgin queens will have a maximum impact on the local gene pool and allow new genetic mixing to take place! This mixing can help your bees evolve in their resistance to future changes in the varroa population. The more genetic diversity you have the better. **At Pacific Queens in Chile we try to work with about 14 different breeder queens to keep inbreeding at a minimum.**

Once resistant material is spread throughout all your hives the next problem is to decide **what is the best way to phase out all chemical treatments?** You probably do not know why your bees are resistant. So here you have a real psychological dilemma complicated by your "fear of the unknown". One way is to stop treatments at a few of your bee yards and see what happens. That way you reduce your overall risks and if your hives continue to produce correctly and have normal hive mortality you can gradually phase out treatments in your other yards. The problem with this method is that it can take a lot of time to put into practice and it is more difficult to eliminate non-resistant hives.

In France we decided after several years of reflection to take the "sink or swim" approach and **ovcr 6 years ago** we stopped treatments in all our hives, including the genetic lines purchased from other queen breeders. **During the first two years we lost over two thirds of our hives.** Normally we thought we would loose a lot more. So we were encouraged by the results and began to make new colonies. Our situation is still not ideal because our winter losses are about 15%. However not all these losses were due to mites. Varroa populations are very low in our hives and anti-varroa treatments are not justified. So now we are saving time and money in France because we do not have to make anti-varroa treatments. In Chile we stopped treatments only on the breeder hives because we need 4500 hives for our pollination and pollen collection business. For this reason it is taking longer to obtain resistance (Fig.3).

SOME PRACTICAL ADVICE

The main problem in selecting resistant bees and keeping them resistant, is the beekeeper . If he treats he maintains susceptibility in his bees because normal genetic evolution cannot occur. **We suggest that any hives used for breeding should never be treated against varroa.** Since non-resistant colonies usually occur in larger numbers the presence of their drones will be counterproductive to any genetic advances. This means that the beekeeper will have to continue testing and changing queens all the time to maintain genetic pressure on the open breeding population.

How do you know if your bees are really varroa resistant? If you are a queen breeder the answer is really quite simple. Your customers stop treating against varroa and their hives survive.

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Figure 1. Varroa infestations per 100 bees on varroa tolerant and varroa resistant bees in France. (Using commen sense definition)

Figure 2. Absence or presence of varroa in capped brood at different levels of hygienic behavior.

Figure 3. Results of varroa resistance breeding programs in Chile and France .

Table 1. Selecting breeder queens for pollen collection according to the group averages of their naturally mated daughters.

Table 2. Selecting the best pollen production queens for hygienic behavior