BEE DOC - M6.1 Popular version for publication

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Evaluation of *Varroa* mite tolerance

Summary

Breeding honey bees for specific defined characteristics to obtain varroa mite tolerant bees appears to be difficult. Instead it is suggested that the daily rate of mite population growth during optimal mite breeding conditions is used to determine the breeding value for mite tolerance in evaluated colonies. The precision needed to determine mite population growth will determine if samples of adult bees at different occasions will suffice, or if more detailed measurements of the mite population is required. Threshold levels for mite population densities before mite control is required need to be determined for different geographical regions and foraging conditions.

Background

As part of an effort to promote the development of *Varroa* mite tolerant stocks of bees, the BEE DOC project has surveyed breeding programs and available literature on the subject to formulate recommendations suitable for practical breeding purposes. The initiative has been a collaborative effort with Swedish honey bee breeders (Svensk Biavel AB).

Varroa mites (*Varroa destructor*), with the viral diseases where the mite acts as a vector, means that the vast majority of colonies that are infested in a Nordic climate collapse 3-4 years after the parasite first becomes established, with up to 10 000 mites in some colonies, if the mite population growth is not limited (Korpela et al., 1993). In more southern climates in Europe the collapse is likely even faster. When the parasite has been established in a population of bees the extent of viral infections are likely to increase and colony collapse may occur even at lower infestation levels. For beekeeping not to be eliminated, varroa mites must be controlled. A recent review of the parasite's biology and how it can be combated by different methods can be found in Rosenkranz et al. (2010).

Varroa mite on our European bees, *Apis mellifera*, comes from the Asian honey bee, *Apis cerana*, where there are more species of varroa mites, which have not made the host change we have seen by *V. destructor* (Anderson & Trueman, 2000). The Asian bee, is not considerably damaged by the attacks because they have developed certain characteristics that make them tolerant. The most important feature is that reproduction of the parasite in practice only occurs in drone brood, because infested worker brood is quickly cleaned out (Rath & Drescher, 1990). Infested drone brood takes a long time to clear out because of the strong cocoon, and as the drone pupae are sensitive and often die from multiple varroa females in the cell, the mites will die with the host (Boecking, 1993). An effective grooming behaviour in which bees are helping each other to attack the mites have also been documented in *A. cerana* (Peng et al., 1987), but it has not been shown that the fallen mites are more damaged than in European bees (Fries et al., 1996). Simulations show that with reproduction in drone brood only the varroa population is unlikely to increase to harmful levels (Fries et al, 1994), especially if the mites are buried in the cells when multiple attacks occur that kill the pupae. The problem with the varroa mite of European bees is that reproduction work well in both worker and drone brood, although the latter is preferred (Fuchs, 1990) and produce more offspring per mite-cell attack (Ifantidis, 1984, Martin, 1995). It seems unlikely that European bees will develop the character (solid cocoons of drones) that allows the parasite to become buried in heavily infested drone cells. However, it seems more likely that the ability to detect and remove infested larvae could be improved, because the property already exists in varying degrees in European bees (Arathi & Spivak, 2001). Bees selected for the removal of dead brood, known as removal or hygienic behaviour, results in lower average infestation of varroa mites in field colonies (Spivak and Reuter, 2001) but the ability to specifically detect and eliminate cells with the reproduction of varroa (Harbo & Harris, 2009) has a greater impact on the mite population development (Ibrahim et al., 2007).

In recent years it has been shown that *A. mellifera* can survive the attack by varroa mites. Early on it appeared that the Africanised bees in South America did not succumbed to the attacks, at least partly because a large proportion of mite females were infertile in worker brood (Camazine, 1986, 1988). Later, the reproductive potential on worker brood improved, but still without damaging effects by the mite infestations, as mite population growth appears to slow down when the density increases (Vandam et al., 1995, Medina et al., 2002). An important reason for mite tolerance in Africanised bees, despite fertility of the mites being similar to infestations of European bees, appears to be a higher mortality of the mite offspring, also in the males, which suppresses the mite population growth (Mondragon et al., 2006). When the varroa mite came to the African continent in the late 1990s (Allsopp et al., 1997), it turned out very soon that the parasite did not have to be controlled for the bees to survive, although the reproductive potential of the parasite initially suggested damages to be expected (Allsopp, 2006). A more developed removal behaviour of African bees (Fries & Raina, 2003) may have been part of the greater mite tolerance (Frazier et al., 2010), but the absence of pesticides against mites may also contribute to the evolution of mite tolerance (Frazier et al., 2010). Populations of European honey bees also appear to have developed different levels of mite tolerance in Europe (Fries et al., 2006; Le Conte et al., 2007), as well as in the US (Seeley, 2007) through natural selection.

Selection for specific characters may improve the tolerance to varroa mites in honey bees. However, because of the difficulties in recording the specifics needed this is probably not the best way forward. Ultimately mite tolerance is likely to be a combination of qualities, a combination that may vary in different geographic areas and among different bees. In most places the infestation of varroa mites will probably not eradicate the species *A. mellifera*. Nevertheless, the vast majority of bees are likely to die if no mite control is practiced, at least in Europe, but after a decade or so populations may recover to build up new viable populations despite attacks by varroa mites. Unfortunately, the bees that survive through natural selection may have lost desirable properties for profitable beekeeping. Exposing the European bee population for natural selection in this context is not acceptable, with the implications for pollination and beekeeping in general that would result. On the other hand, efficient mite control masks any differences in tolerance leading to a continuous need for mite control. The conclusion must be to find a strategy that makes it possible to distinguish colonies of greater and less resistance to varroa mites. Taking into account what has been said above regarding the selection of individual parameters, probably the only realistic alternative is to study and compare the growth rate of mites in colonies of different genetic background, while allowing the mite population in all colonies to develop. In short, the success of beekeeping with varroa mites in most of Europe is about producing healthy winter bees that have not been heavily parasitized by mites. It is fully compatible with both good honey harvest and good wintering to have a relatively high mite population in bee colonies in spring and early summer. Therefore, it must be during the time of optimal growth of the mite population that the growth rate is monitored but that mite control is employed in time to produce healthy winter bees, if certain thresholds (in debris counts of mites or infestation rates of the bees) are exceeded for a predetermined part of the summer. What such thresholds should be, when they should be located in time and for what geographic location need further investigation.

Recommendations

Selection criteria

In light of what has been reported, it may be realistic to limit the selection for increased mite tolerance to two characters that are relatively easy to measure.

i) the hygienic behaviour and
ii) the mite population growth rate.

The hygienic behaviour issue has been covered above. As previously indicated, selection for hygienic behaviour has only limited effect on varroa mites, but since there are also positive, albeit limited effects on resistance to varroa, this character should be included also in this context.

Measuring the mite population growth rate in different colonies should give the best measure for varroa tolerance. The methods used need to be simple for practical beekeeping, but have as high information value as possible. In addition, the method must allow for measurements in colonies with different mite levels, because it is unrealistic to standardize infestation levels. We can assume that the mite population growth rate is exponential (Fries et al., 1994) with a growth rate of approximately 2.5% per day if there is free access to brood and that the mite infestations are not large enough to affect colony development (Calatayud & Verdú, 1993, 1995). By estimating the mite population size between two dates with free reproduction of the mites a growth rate can be obtained that is comparable between different colonies, regardless of infestation level and at least in part independent of the number of days the measurements include. With this information the growth rate can be calculated from

I. $\chi = e^{r * d}$

where

χ = the number of multiples by which the population has grown

e = the natural logarithm

r = growth rate per day

d = number of days during which the measurement occurred

Example: The measurement took place during 65 days (d = 65). Mite population is estimated to have increased from 100 to 580 (= 5.8). Formula I can now be written as

II. $r = \ln\chi / d$

hence $r = \ln(5.8) / 65 = 0.027$

Thus a growth rate of 2.7% per day in this case. This measurement should provide a basis for assessing the varroa tolerance. Measurements of mite population growth should be undertaken only in full strong colonies (a lower limit defined) and with fully functional queens. It is proposed that measuring begins a few days after the bees could fly on flowering willow for the first time. This is because only with proper access to fresh pollen brood rearing takes off. A sample of approximately 300 live bees are taken in the brood room and the number of mites washed off, giving a measure of the number of mites per bee. Investigations in the field show that such sampling gives a surprisingly good prediction of the overall infestation rate in a hive (Lee et al., 2010), with a precision that may be sufficient in this context. To increase the precision if needed, samples from both brood and bees, and measurements of brood and bee numbers can be used (Lee et al., 2010). A second sample of bees (or both bees and brood with estimates of bee and brood numbers) is taken sometime in early July or mid-July in the same way and the number of times the mite numbers have doubled is calculated (number of mites per bee in test two (or in a colony) / number of mites per bee in sample one (or in a colony). Thereafter, the growth rate, as described in formula II., is used to compare colonies of different genetic backgrounds for their relative resistance to mites. Varroa populations must be allowed to grow to levels that make measurements meaningful. Only if certain pre-determined thresholds, as previously discussed, are reached, should mite control be practiced. In Germany, the threshold for mite control is set to 10 % infestation of adult bees in July (Büchler et al., 2010).

Concluding remarks

The aim of this milestone has been to determine how to evaluate the colonies relative tolerance to varroa mites to be able to use this information for breeding purposes. There are many indications that the main characters of bees that resist *Varroa* is specific mite directed hygienic behaviour (VSH) and / or decrease in fertility and maternal fecundity of mother mites. These characters are very laborious to measure, so the most practical solution seems to be to monitor mite population growth, regardless of the underlying characters. What is proposed is mainly based on a German approach for evaluating mite tolerance (see Büchler et al., 2010 for details), but here based solely on samples of bees, or on samples of brood and bees combined with population estimates, if this precision is needed. This latter consideration needs to be evaluated in the field..

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