A small shift in VSH-gene frequency instead of rapid parallel evolution in bees. A comment on Oddie et al. 2018

We refute a recent claim that parallel evolution in four European populations of honeybees has resulted in a not previously reported behavioural defence mechanism of the bees against the parasitic mite *Varroa destructor*, i.e. the ability of uncapping/recapping to reduce mite reproductive success. There are no data to support this claim, while there is a more plausible alternative interpretation of the reduced mite reproduction, i.e. reduction of mites through *Varroa* Sensitive Hygiene. We provide evidence why the former mechanism cannot explain resistance against Varroa in honeybees and the latter is instrumental in reducing *Varroa* populations.

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A small shift in VSH-gene frequency instead of rapid parallel evolution in bees. A comment on Oddie et al. 2018

By

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Introduction

Recently Oddie et al.[1] claimed that parallel evolution in four European populations of honeybees has resulted in a not previously reported behavioural defense mechanism of the bees against the parasitic mite *Varroa destructor*, the ability of uncapping/recapping to reduce mite reproductive success. Their study does not provide the data to support this claim, it does not consider a more plausible alternative interpretation of the results (the reduced mite reproduction through Varroa Sensitive Hygiene) and lacks experimental evidence to distinguish between both hypotheses.

Background

Varroa destructor is an external parasitic mite of honeybees that shifted from its original host *Apis cerana*, the Asian hive bee to *Apis mellifera*, the European honeybee. The parasite spread rapidly and colonized western Europe and North America in the early eighties, where it has been the major mortality factor of honeybees ever since. Varroa mites are vectors of several bee viruses and at high mite densities these viruses cause colony collapse.

On its original host, the mite is an innocuous parasite. One reason why *Varroa* is so virulent on *A. mellifera* is that it can breed in worker brood and so obtain a much longer reproductive season, while in *A.cerana,* mite-infected pupae are always removed from worker cells [2] and breeding is restricted to the short season when drones are produced [3].

In western Europe and North America, hives are frequently treated with acaricides, natural acids or essential oils to control *Varroa*, or *Varroa* reproduction is disrupted by other apicultural measures [4]. Moreover, a large proportion of the hives are regularly requeened with non-resistant pure-bred queens. These practices are thought to prevent natural selection from selecting for resistance against *Varroa*.

The traits that provide resistance against *Varroa* in *A. cerana*, are also present in European *A.mellifera* populations, albeit in low frequency: auto- and allo-grooming can results in the removal of phoretic adult mites and inflict mortality among them[5]. In addition, the uncapping of *Varroa*-infected cells and the subsequent removal of parasitized pupae, together described as “hygienic behaviour” [6 7 2] and more precisely as “*Varroa* sensitive hygiene” (VSH) [8 9 10 11 12], results in the removal of mite offspring before they have been able to reproduce successfully. Bees with the alleles for VSH recognize cells containing reproducing *Varroa* [13 14]. They uncap these cells from which the pupae are subsequently removed, thus interrupting the reproductive cycle of the *Varroa*. The proportion of workers in a colony expressing the VSH behaviour is positively correlated with the proportion of non-reproducing mites in the brood [15]. This is because VSH bees preferentially attack cells with reproducing mites [10]. The subsequent removal of reproducing mites results in an increase of the proportion non-reproducing mites [9 15].

Natural selection for these characters would be possible in groups of colonies that are neither treated against *Varroa* nor requeened. A number of natural [16 17 17] and designed experiments [18 20] have reported survival of such colonies and suggested that the surviving bees have developed tolerance or resistance against *Varroa*. Some of these studies started more then 17 years ago and reported bee colonies surviving *Varroa* already more than ten years ago [21] [22]. Given the magnitude of the *Varroa* problem and the associated economic losses, this begs the question why *Varroa* surviving bees have not become widely available to apiculture.

Now, Oddie et al.[1] suggest that parallel evolution by natural selection in some of these populations (two French, Avignon and Sarthe [22], a Norwegian[19] and a Swedish population, Gotland[21]), has resulted in a common and not previously reported behavioural mechanism in the European honeybee to reduce reproductive success of the mites. They claim that the bees in the four populations (1) recognize cells with reproducing Varroa-mites, (2) that they open these cells and then (3) close them again. (4) That the brief period during which the cells are open interrupts the Varroa breeding cycle and kills the offspring. (5) That this behaviour is the mechanism that causes the lower reproductive rate of *Varroa* in comparison with that of control colonies from the same geographical areas not exposed to natural selection. (6) They also claim that this behaviour is superior to the alternative of removing infested pupae (*i.e* VSH). The first two behavioural steps of the mechanism that they suggest are identical to those of VSH behaviour.

Oddie et al.[1] did not measure VSH and did not exclude it as a cause for their results. As we will show, the data they supply can better be explained as caused by VSH behaviour, hence, Oddie et al.[1] provide no evidence for the existence of an opening-recapping strategy. Moreover, we will argue that an opening recapping-strategy would not be not superior to VSH behaviour in terms of survival of colonies as suggested by Oddie et al.[1] We also will show that the *Varroa* resistance of these four populations is incomplete and that beekeepers' practices help the populations to survive.

Recapping or VSH?

Oddie et al. [1] found a higher frequency of recapping in surviving colonies when compared to *Varroa*-susceptible ones. This easily can be explained by the fact that surviving colonies have a larger proportion of bees that express VSH. Therefore, more infested cells are opened and hence there is more opportunity for workers that do not express VSH behaviour to recap cells [10]. Thus, the observation cannot serve as evidence for an "opening-recapping" strategy.

Oddie et al.[1] found no significant difference between recapped and undisturbed cells in the proportion of non-reproductive mites, in contrast to what they expected. They argue that bees preferentially open cells with reproducing *Varroa* (a preference that is typical for VSH [13,14 15]) thus interrupting reproduction of the mites, making that the difference between undisturbed and opened cells would disappear. To test this hypothesis they compared experimentally uncapped cells with undisturbed cells. The reproductive rate of *Varroa* was lower in the uncapped cells than in the undisturbed cells. However, this does not prove that uncapping-recapping was instrumental in lowering the reproductive success of *Varroa*, as the frames with uncapped and undisturbed cells were placed back into hives in which bees with VSH genes were present at an unknown frequency. The VSH behaviour of bees in these colonies will have resulted in the removal of pupae with reproducing *Varroa* mites from the uncapped cells. This would leave opened cells with non-reproductive mites more often untouched and available for recapping, thus accounting for the lower reproductive rate in recapped cells as compared to undisturbed cells.

Hence, there is neither proof that uncapping-recapping results in mite mortality, nor in disruption of mite reproduction and hence no evidence that it reduces reproductive success in *Varroa*.

Recapping explained

The genes for VSH occur in all European honeybee populations at low frequencies. Moreover, there is evidence for the Norwegian population that it expresses VSH[19] and for all populations for suppressed mite reproduction, a trait typically associated with VSH [20] [23] [24] .

Oddie et al.[1] do not take into account that the worker bees differ in genetic make-up, because the queens of the four populations mated naturally and worker bees in each colony originate from 10 to 20 different drones.

Therefore, only a part of the workers express VSH genes. They uncap cells and remove larvae infested with reproducing *Varroa* mites. Another part of the workers lack copies of the VSH alleles. When they encounter an uncapped cell, they will recap it, thus counteracting the actions of the VSH bees.

Support for this interpretation comes from a study[10] that used an experimental design that allowed to discriminate between the effects of VSH behaviour and recapping. It compared bee colonies that expressed VSH for about 70%, and control colonies that expressed VSH for about 25%, A much higher incidence of recapping occurred in the high VSH colonies. The frequency of infertile mites in recapped cells was not significantly different between the two types of bees, suggesting that uncapping and recapping of brood cells is not a major cause of infertility of mites, while the VSH-behaviour resulted in a reduction of 70% of Varroa-infested cells

A Cost-Benefit analysis of VSH and Opening and Recapping

Oddie et al[1]. claim that the costs of VSH exceed those of opening and recapping cells. They do not provide any data to support this claim but simply state that colonies would not be able to sustain high rates of killing of their own offspring. They even suggest that VSH could be instrumental in the destruction of a colony, when workers are lost at a faster rate than they are being replaced, but do not explain how the dynamics of the interaction between bees and *Varroa* could ever produce such an effect.

The proper way to compare the two strategies and predict which of the two would be favoured by natural selection, would be to measure both costs (in number of workers killed) and benefits (in number of mite offspring killed) of the two strategies and to determine how these affect the population dynamics of the mite-bee interaction. We give three reasons why VSH is likely to be the better strategy.

(1) The costs of VSH (and that of recapping) increase with *Varroa* infestation rate, but since colonies that show a high rate of VSH have very low *Varro*a infestation rates, the costs in numbers of workers lost are negligible, once a colony is resistant.

(2) The probability of interrupting the reproductive cycle of Varroa is 1.0, in VSH, while mites often reproduce successfully after opening-recapping[10]. Hence, the probability of interrupting the reproduction is much smaller than 1.0. for opening-recapping.

(3) Even when uncapping results in disruption of the reproduction of the *Varroa* mites, host bees surviving after recapping would already be infected with viruses such as DWV and be instrumental in dispersing the virus in the colony. As these viruses ultimately cause the collapse of the colony, killing the infested pupae should be better. This is why VHS may have long-term benefits that more than compensate for the costs.

The presence of VSH in these populations creates the logical problem why the two strategies would co-exist. It is difficult to see how a mixed strategy would be stable, as all worker genotypes would benefit from the strategy that most efficiently reduces reproduction of *Varroa*. Selection would thus remove the less efficient strategy.

Resistance to Varroa

It is clear that *Varroa* mites have lower reproductive rates in the four surviving populations than in the control populations they were compared with. The most plausible cause for this reduced *Varroa* reproduction is VSH behaviour. A bee colony is fully resistant against *Varroa*, when the population growth rate of *Varroa* in the colony is zero. Values of W_r (= the number of fertilized daughters per breeding cycle) exceeding 0.7 result in growing *Varroa* populations. W_r= 0,9 already results in exponential growth[25]. The W_r value for Norway is 0,87[19], those for Avignon and Gotland have not been published but data on the Varroa reproduction in these populations indicate that they are higher than that of the Norwegian population[20] . Hence, the suppression of mite reproduction in these populations alone is not strong enough to prevent the populations of *Varroa* from growing and eventually causing colony collapse. Other heritable traits, like resistance to viruses could play an additional role [23]; [24]. In addition non-heritable traits like small colony size and swarming frequency [17] [26] [16] could explain the survival of these colonies, while bee-keeping practices in the four populations also play an important role [27] [24]. As example a quotation on the Avignon and Sarthe bees[24] "*What has happened to these bees since we published those results in 2007? Once every two years, we graft queen larvae from the three best colonies in each apiary (west and south of France) to get 20 colonies. The queens are naturally mated by local drones. About 30–35% of the colonies die within 18 months, but the rest of the colonies are good candidates for surviving to the mite, so the stock still survives efficiently*". This quote shows that mortality rate of the surviving colonies is not different from that of *Varroa*-sensitive ones. It also shows that beekeeping

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practices interfere with natural selection, by creating a large number of new queens and by artificial selection. Nevertheless, the bees do not perform any better than *Varroa*-sensitive ones, as was shown in a large comparative study[28]. The Avignon colonies did not survive any longer than *Varroa*-sensitive strains.

Conclusions

The results of Oddie et al. (2018) *i.e.* (1) a reduction in *Varroa* mite reproductive success and (2) a higher frequency of recapping behaviour in surviving colonies and (3) a higher proportion of non-reproductive mites in recapped cells can easily be explained by incomplete VSH behaviour in these colonies.

The observed reduction in *Varroa* mite reproduction rate W_r is not enough to allow the colonies to survive. Survival of the populations is partly due to apicultural practices. In fact, these colonies do not survive better than *Varroa*-sensitive colonies. A modest reduction in *Varroa* mite reproduction in a period of almost 20 years is not exceptional and should not be called "rapid parallel evolution" .

For more than 35 years *Varroa* has been the major threat for apiculture. The scale of the damage and the costs of its control make it a very urgent problem. The study of "surviving" colonies has, so far, not resulted in a lasting solution for the beekeeping community. It seems time for the research field to shift its attention to more efficient ways of obtaining *Varroa*-resistant bees.

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J.v.A and BJ.F. wrote the main manuscript text. Both authors reviewed the manuscript."

Competing interests

The author(s) declare no competing interests.