Varroa Destructor and the Sustainability of *Apis Mellifera* - an Overview

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Bulletin UASVM Animal Science and Biotechnologies 77(1)/ 2020

Print ISSN 1843-5262; Electronic ISSN 1843-536X

DOI:10.15835/buasvmcn-asb: 0023.19

Abstract

Varroa destructor is a parasitic mite that represents a major global threat for the Western honeybee *Apis mellifera*. This parasite managed to switch from its natural host the Eastern honeybee, *A. cerana*, and within a few decades, it spread among *A. mellifera* populations around the world. Today beekeepers are using a variety of different acaricides to keep the parasite population under control. However, for many of these substances, the parasite evolved resistance asking for the development of novel compounds. Hence the treatment is less suited as a sustainable tool in honeybee health; consequently, other alternative options are needed, and breeding of *Varroa* resistant honeybees have been suggested as a more sustainable solution. Here we reviewed the successful efforts and the apicultural procedures needed to be implemented to achieve resistant honeybees. We also describe the underlying resistance mechanisms and discuss the benefits of breeding within regional populations, considering the biodiversity aspects of *A. mellifera*.

Keywords: *A. mellifera*, breeding, resistant honeybees, *Varroa destructor*

1. Varroosis as a major global killer of honeybee colonies

Global losses of honey bee colonies have been a major concern over the past decades. Due to the importance of the bees in agriculture, as they have a significant role in pollination (vanEngelsdorp and Meixner, 2010; Hung *et al.*, 2018) and the large variety of products with nutritional and medical implications for humans (Waykar and Alqadhi, 2016), as well as new applications in biomonitoring (Bromenshenk *et al.*, 2015) they managed to attract the attention of many research fields.

Like other species of animals, bees confront themselves with many diseases that can be

bacterial, viral, fungal or parasite origin and various pests. However, the most harmful so far is represented by *Varroa* infestation and is the main driver for the colony losses at the moment (Potts *et al.*, 2010; vanEngelsdorp and Meixner, 2010).

2. Biology and life history of *Varroa*

The biology of *Varroa destructor* has been intensively well studied (Anderson and Trueman, 2000; Rosenkranz *et al.* 2010). The life cycle can be divided into two main phases. The first one is the phoretic phase, where the parasite is typically located between abdominal tergites and feeds on the hemolymph of the adult bees (Rosenkranz *et al.*, 2010). A more recent study that involved bio-

staining procedures suggests that the primary food source for *Varroa* is represented by honeybee body fat rather than hemolymph (Ramsey *et al.*, 2019). The phoretic stage is important for female mites as they can transfer to other bees or larva inside the colony or other honey bee colonies using an adult bee as a carrier (Rosenkranz *et al.*, 2010; Piou *et al.* 2016).

Moreover, the mite can feed on the adult bee fat body by making a hole through the intersegmental abdominal membrane (Ramsey *et al.*, 2019). After some time that may vary between one and ten days depending on the availability of brood, the female mite switches to the reproductive phase leaving the adult bee and entering a cell with brood larva (Beetsma *et al.*, 1999). The length of the phoretic phase is particularly essential for the maturation of young female mites and less for adult females (Piou *et al.*, 2016).

The mite prefers the drone brood larva in detriment of worker larva. Drone brood has a more extended period of incubation and gives the female mite more time to reproduce (Martin and Cook, 1996; Kuenen and Calderone, 2000; Rosenkranz *et al.*, 2010).

Once the female mite finds a suitable cell to infest, she switches to the reproductive cycle. The female mite chooses and infests the brood cell a few hours before the nurse bees cap the cell (Evans and Cook, 2018). The reproductive cycle of the parasite is perfectly adapted to its host; the female mite chooses and infests a brood cell, a few hours before the nurse bees close the cell. A few hours after the cell is sealed the *Varroa* female feeds from the larva. This step is necessary to activate its ovary system (Garrido *et al.*, 2000). Host factors appear to initiate ovary activation of the female mite. Larval volatiles, followed by a cascade of other factors have been suggested to trigger the further growth of the activated oocytes (Garrido *et al.*, 2000; Garrido and Rosenkranz, 2003). Artificial infestation experiments on bee larva in different stages it seems that female mite has approximately 12 hours after the cell is capped for ovary activation and is suggested that specific substances produced by larva trigger this activation (Garrido and Rosenkranz, 2004; Frey

et al., 2013). Since it is entirely dependent on its host, the female mite regulates its reproductive cycle after the host. Female mites with activated ovaries transferred in a new brood cell on the exact developmental stage continued their cycle, while most female mites transferred from the white eyes pupa on newly capped brood cells produced a new male (Garrido and Rosenkranz, 2003). In less than three days after the cell is sealed the first egg is laid this is always an unfertilized egg from which will hatch a haploid male. After that, she lays up to five eggs at regular intervals of approximately 30h each the female mite lays a female egg (Rosenkranz *et al.*, 2010). Inside an infested cell we have specific areas of interest for the mites, the feeding point made and maintained by the adult mite on the larva, in this area the mite and all her offspring feed. There was also identified a specific region inside the cell where the female mite lays her eggs and another area where the mite defecates; in this area the male mite mates with his sisters (Donzé and Guerin, 1994; Frey *et al.*, 2013). The mother must maintain open the feeding area for her offspring as they are unable to break the bee pupa cuticle (Donzé and Guerin, 1994).

At the end of a reproductive cycle, the mother mite and her mated female offspring leave the cell along with the hatching drone (or worker bee) while the male mite dies (Boecking and Genersch, 2008).

3. Global parasite of the Western honeybee *A. mellifera*

The first recorded description of the *Varroa* mite was made in 1904 when it was described as an external parasite found on *Apis cerana* (Oudemans, 1904). The estimated time of the host switch is pointed at the beginning of the 19th century when the productive *A. mellifera* colonies were brought to Asia with the trans-Siberian train (Oldroyd, 1999). Presented with a new host, the mite grabbed the opportunity and made the host switch. Given the virulence of the parasite and present on a host with no resistance, the mite was able to spread at the global level (Oldroyd, 1999; Moritz *et al.*, 2005). Also, due to the lack of a host-parasite relation, the naive *A. mellifera* was

unable to respond correctly and take appropriate measures against an already adapted mite (Peng *et al.*, 1987).

Due to the effect of cryptic speciation and morphological stasis phenomenon frequently encountered in Acari species, *V. jacobsoni* was considered responsible for the host switch (de Guzman and Rinderer, 1999; Skoracka *et al.*, 2015). Only after a profound morphologic and molecular analysis, it was revealed that the host switch was made by the *Varroa destructor* more specifically two haplotypes the Korean and Japan in this process other variants of the *Varroa* were also revealed (Anderson and Fuchs, 1998; Anderson and Trueman, 2000)

After it was proved that *Varroa* is more than one species, later studies of mtDNA of *Varroa* has revealed differences of haplotypes, and by 2004 there was reported other haplotypes of *V. jacobsoni* and *Varroa destructor*. Moreover, it was suggested that there exists natural genetic isolation between *Varroa* genotypes that infest different populations of bees in China (Anderson and Trueman, 2000; Fuchs *et al.*, 2000; Zhou *et al.*, 2004). In later studies confirmed the presence of two new variants of the Korean haplotype and two of the Japan haplotype. That might be a potential threat for *A. mellifera* colonies in Europe. Also, it was suggested that an invasion of new haplotypes of *Varroa destructor* on *A. mellifera* is dependent on the time of exposure (Navajas *et al.*, 2010). Two studies in Serbia suggest the presence of two new haplotypes Serbia 1 and Peshter 1. The authors mention that the source for this variability is still not clear and will require further study (Gajic *et al.*, 2013; Gajić *et al.*, 2016).

In recent years there was an increasing trend of the beekeepers from Asia for *A. mellifera* colonies, and this leads to situations where both species can be found in proximity. The presence of different *Varroa* populations still present on the original native host *A. cerana* can lead to this scenario as well as the possibility that specific virulent types of *Varroa* to switch back from *A. mellifera* on the original host (Beaurepaire *et al.*, 2015).

4. Parasitic role in the natural host *A. cerana*

In the original host, *A. cerana*, the parasite seems to have his reproductive success limited on the drone brood inside the colony. The lack of reproduction in worker brood of *A. cerana* has a significant impact on *Varroa* mite population as the drone brood grows only in a specific interval of time (Boot *et al.*, 1999), however more recent studies suggest that the Korean haplotype of *V. destructor* is able to initiate the reproductive phase on *A. cerana* workers but with a low rate of success (Lin *et al.*, 2018). Recently there was identified that *Varroa jacobsoni* mites started shifting the host from *A. cerana* to *A. mellifera*. There is a difference in gene expression for the mites that reproduce on *A. cerana* and *A. mellifera* (Andino *et al.*, 2016). This proves that the mites tests *A. mellifera* colonies as a potential new host and brings the danger of a new mite for *A. mellifera* colonies.

For *Apis cerana* and *Varroa*, there was a long co-evolution relation because of thus *A. cerana* seems to have a few resistance mechanisms. First is mentioned an auto-grooming mechanism that seems to manifest almost instantly and in some cases, they also perform allo-grooming. Still grooming behaviour alone does not explain lower infestation rate on *A. cerana* (Boecking *et al.*, 1993; Fries *et al.*, 1996; Boecking and Spivak, 1999). Another interesting mechanism in *A. cerana* worker is the ability to uncap and remove worker brood cells, but in case of drone brood, they make this operation only if there is a single female adult one the mite inside. If there are two or more mites inside or if they notice bacteria disease, they seem to close the pore isolating the parasite and diseased brood inside. This one seems to be a non-removal mechanism and carries the name of "entombing" (Boecking and Spivak, 1999). In this situation, the drone brood seems to remain without sufficient energy and will be unable to hatch and will die inside the cell; as a result, the parasites also remain blocked along with the dead brood. Moreover, *A. cerana* workers seem to delay the dead brood removal (Boecking and Spivak, 1999; Rath, 1999). As a result, *A. cerana* uses different strategies to cripple mite population uncapping and removal

alongside with entombing. Since this entombing strategy is used for other pests and pathogens, it might not be a specific mechanism for *Varroa*. However, it seems to be very efficient and affects the population dynamics of the mite. Having multiple mother mites trapped inside a brood cell results in a decrease in mite population and at the same time is a way of reducing the genetic variation of the mite population (Rath, 1999).

5. Negative effects on *Apis mellifera*

Since *Varroa* is a parasite without a free-living stage, it is not aiming to kill its host; however, on the new naive host, we can see numerous negative effects.

At the individual level, it was recorded a significant weight for parasitized individuals (Duay *et al.*, 2003). For infested drones have a decrease in flight performance (Boecking and Genersch, 2008). Infested worker bees have a shorter lifespan, and the infested individuals seem to have affected the immune system (Amdam *et al.*, 2004; Rosenkranz *et al.*, 2010; Kurze *et al.*, 2016). A more significant impact is seen on worker brood that develops in winter bees as they fail to develop specific characteristics for winter bees, resulting in a depopulation of the colony due to the mite load, and a greater risk for the colony to die over winter (Amdam *et al.*, 2004; Daiant *et al.*, 2012). The feeding activity of the mite affects the surface hydrocarbons of bee cuticle. As a result, this influences the honey bee capacity to regulate water exchange (Annoscia *et al.*, 2012). Moreover, the damage to the adult bee is directly influenced by the mite phoretic stage duration (Piou *et al.*, 2016).

Field tests show modifications in forager's flight behaviour of infested bees, and a tendency to spend more time outside of the colony followed by and a lower rate of return compared with uninfested colonies (Kralj and Fuchs, 2006).

The parasite acts as a vector and can carry and spread 18 different types of viruses of the bees. Under normal conditions (without *Varroa* infestation) a healthy bee colony can manage by itself a virus outbreak, and very often they happen to pass unnoticed by the beekeeper (Boecking

and Genersch, 2008; Rosenkranz *et al.*, 2010). For parasitized bees, it was also recorded a decrease in cognitive abilities. The precise cause is not fully understood, but there is a down-regulation of genes involved in behaviour and cognition and an up-regulation of genes involved in neuron excitability (Navajas *et al.*, 2008).

Since the external digestion phenomenon of the mite affects the fat body of the bee, some of the negative effects are severe; the fat body has a direct implication on growth, metamorphosis, pesticide detoxification, immune function, metabolic activity of bees (Ramsey *et al.*, 2019). This could also explain the altered gene expression for bees exposed to infestation as they presented different expression in genes involved in metabolism, immunity stress response and nervous system function (Zanni *et al.*, 2017).

At colony-level, it has been observed that the infested colonies produce a smaller number of swarms a reduction in the population growth rate; this happens when the mite population inside the colony is still low (Rosenkranz *et al.*, 2010). A high number of mites/ bee population over the winter period result in low survival rates (Fries *et al.*, 2006; Rosenkranz *et al.*, 2010). More recent studies have proven that worker bees infested with *Varroa* mites during the developmental stage seem to be less involved in hive activities (Annoscia *et al.*, 2014).

6. Use of acaricides

Once the mite started to make economic damage (both in the production of the colony and the colony numbers), the beekeepers called the chemical option, using various substances with acaricide effect to fight this parasite. In time various treatment methods were developed, including the use of substances against *Varroa* such as flumethrin, coumaphos, amitraz, formic acid, lactic acid, oxalic acid, thymol, etc. (Wallner, 1999; Boecking and Genersch, 2008; Tsuruda *et al.*, 2012). However, these substances have a limited effect over the mite population, as some of them manage to survive a new problem appeared once mites became resistant to the treatments leaving the beekeepers with a limited number of options

(Pettis, 2004; Dietemann et al., 2012; Kamler et al., 2016). Many acaricides can also harm the bees, and residues have been found in the bee products (Wallner, 1999; Tsuruda et al., 2012). Moreover, the purchase of substances to treat the bees incurs undesired expenses and can be time-consuming. Therefore obtaining bees resistant to *Varroa destructor* is suggested as a more long-term viable solution Dieteman et al., 2012; Tsuruda et al., 2012).

Since the reproductive cycle of the mite favours the mite population to fixate alleles for resistance to acaricides inside a colony (Beaurepaire et al., 2017), this suggests that the use of acaricides alone cannot work as a long term viable solution.

Due to widespread on the new host and to its growing resistance to acaricides, new control strategies are necessary. Moreover, since genetic approaches for mite control seem to be the most promising long-term solution, a genetic study was made in order to make available *V. destructor* genetic sequences and assembly. This information could be used as a foothold in further studies of transcriptome analysis towards a better understanding of host-parasite mechanisms (Cornman et al., 2010).

7. Resistant populations of *A. mellifera*

Even though in theory *V. destructor* has a great virulence and can decimate *A. mellifera* populations if the beekeepers do not help them, various honey bee populations able to withstand the infestation without any treatment against *V. destructor* have been identified.

Gotland population was created around the year 1999 from 150 *A. mellifera* colonies obtained from different apiaries placed on Gotland Island and left untreated for ten years (Fries et al. 2006). These colonies seem to have a reduced mite population growth that seems to be given by host adaptation to mite infestation (Fries and Bommarco, 2007). The population from Gotland was tested for resistance. The drone brood was probed in the stage of brown eyes and yellow body, and the mites within each cell were observed (number of offspring's/ female mite, number of dead offspring and the absence of offspring).

This information was used to determinate the reproductive success of the mite population. A female mite was considered able to reproduce if she was able to produce at least one female offspring that has succeeded mating (that means at least one male mite and one female mite that we're able to hatch, grow and mate) (Locke and Fries, 2011; Locke et al., 2012). The resistant colonies were compared to susceptible ones and as a result bee population of Gotland, has registered a higher rate of infertility of the female mites. As a result, these bee colonies seem to have a host-parasite relation, and they can limit mite population growth (Locke and Fries, 2011). A later study on this population identified 61 candidate genes that could play an essential role in this population resistance to *V. destructor* as some of these genes have implications in prepupal development and metamorphosis of the larva (Conlon et al., 2018).

The Avignon population was made in 1994 from colonies of untreated bees that had survived without acaricide treatment for at least three years and later were added untreated colonies from other beekeepers. The resistant colonies were monitored from 1999 to 2005, and the results were compared with controls. As a result, the Avignon population seemed to maintain the mite population under control by having a stable host-parasite relation with the mite. The exact mechanism at that moment was unknown, and the possibility of another environmental factor that favoured these bees was not excluded (Le Conte et al., 2007). In order to analyse the resistance mechanism, they compared the gene expression in 2 colonies of *Varroa* tolerant bees (Avignon) and two susceptible colonies. They have examined drone pupa that was infested with *Varroa* and pupa that was not infested. The results of this study suggest that *Varroa* mite infestation causes changes in expression of genes that are involved in embryonic development, cell metabolism, immunity. Also, drones from resistant colonies seem to have a different expression in genes that regulate neural development, olfaction and neuronal sensitivity (Navajas et al., 2008).

In a later study the population from Gotland (Sweden), and population from Avignon (France)

were both compared with control colonies for specific characters of the mite (presence or absence of the eggs of the mite, number of offspring and the delay of the mother in laying eggs). As a result, both resistant populations of bees had managed to reduce the average reproductive success of the female mite (Locke *et al.*, 2012).

In a study made on Gotland population resistant to *Varroa* infestation has been compared with susceptible colonies from Uppsala. The study aimed to prove the inheritance of the reduced mite reproductive success of the Gotland population. They obtained a significant reduction of mite reproductive success for all populations that had in their composition Gotland genetic material. These results prove that the genetic structure of the host is responsible for the reduced mite reproductive success, and this trait could be used in breeding programs. However, the exact mechanism that causes this trait remains unknown (Locke, 2016b). Moreover, the number of colonies used in this experiment might be too small, and the resistance of the hybrid colonies might be due to the heterosis effect.

Toulouse bee population was formed using queens from *Apis mellifera intermissa* considered to be resistant to *Varroa*, brought from Tunisia, and placed in queenless hives in Toulouse. Their offspring were left to naturally mate with local *Apis mellifera carnica* as a result they obtained hybridized colonies that managed to resist to *Varroa* infestation without treatments (Kefuss *et al.*, 2004).

Samples from Gotland population were harvested, in order to find candidate genes that suppress mite reproduction. The study aimed to identify specific genomic regions in the honey bee genome that are responsible for reducing the reproductive success of the mite. In order to do that they mated a resistant queen (from Gotland population) with drones from an apiary that does not manifest any sign of resistance to *Varroa* mite. They used BSA (Bulk Segregant Analysis) method and managed to identify three regions on chromosomes 4, 7 and 9 that seem to influence the ability of the drone brood to suppress mite reproduction (Behrens *et al.*, 2011). Based on

this study for the region on chromosome 7, a microsatellite scan was made in order to find a selective sweep of the genes involved with the reduction of the mite reproductive success. Gotland population structure from 2000 was compared with that one obtained in 2007. The result inside the selective sweep was the obtainment of eleven candidate genes that might be involved in the reduction of the mite reproductive success; one of this candidate gene is GMCOX18 (glucose-methanol-choline oxidoreductase). However, further studies are necessary in order to identify the exact mechanism and candidate genes (Lattorff *et al.*, 2015).

Based on these studies, a similar experiment was done in **Østlandet region, Norway**, on a population of honey bees (having mixed origin), that has been kept without treatment for approximately 19 years. Their results are confirming previous studies that local populations of honey bees can develop resistance to *V. destructor* by reducing the female mite reproductive success (Oddie *et al.*, 2017). In this case, due to the mixed origin of the colonies (Buckfast), the heterosis effect cannot be excluded.

Primorsky Russian honey bee is represented by a population of *A. mellifera* honey bees that originates from the Primorsky region Russia. It was considered that this population has entered in contact with both *A. cerana* and *Varroa*, and because there was no high mortality present, it was considered resistant. Based on this information, this honey bee population was taken to the USA to evaluate its resistance (Rinderer *et al.*, 2001). This population is noted to be also resistant to *Acarapis woodi*, the resistance mechanism attributed for this resistance being autogrooming and assumed to be genetic and it was considered that this mechanism was also involved in *varroa* resistance (de Guzman *et al.*, 2005). Extensive studies on this population were made and seem that has lower infestation rates, a strong expression of the hygienic behaviour and a higher rate of brood removal. Over the years, the proven resistance of this population has attracted the interest of an association of beekeepers and this population is now commercially available (Rinderer *et al.*, 2010).

VSH honey bees population was formed using selective breeding and artificial insemination techniques with colonies selected from USA stock and were suspected of mite resistance (Harbo and Hoppingarner, 1997). They managed to obtain more colonies from those hives, and they mated each queens coming from those colonies with a single drone (Harbo and Hoppingarner, 1997; Harbo and Harris, 1999). This breeding program has also reached the commercial point and the selected queens were available on Glenn Apiaries until 2012, (Rinderer *et al.*, 2010; <http://www.glenn-apiaries.com>). This mechanism seems highly variable and difficult to use in the population. It is estimated that this is quantitative, and several genes are involved in the expression of this character (Lapidge *et al.*, 2002; Oxley *et al.*, 2010).

Separately another study pointed out four candidate genes involved in olfactory sensitivity related to this mechanism. This experiment focused on the ability of the worker bee to identify the infested brood cells and uncap them, suggesting it as an critical component for varroa resistance and could have great potential in breeding programs and had at its base a assay specifically design SNP assay designed for the analysis of this behaviour (Spötter *et al.*, 2012; Spötter *et al.*, 2016).

African honey bee resistance to *Varroa* populations of *A. mellifera scutellata* and *A. mellifera capensis* seem to have some resistance traits to mite infestation. A study revealed the fact that *Varroa destructor* can reproduce inside brood cells at a similar level with the one registered at European honey bees, and it was a matter of time until the negative effect would appear (Martin and Kryger, 2002). A study from 2013 that had the aim to reveal the level of parasite and pathogens has highlighted *V. destructor* as the most common parasite. Still, with the lack of chemical treatments, the average rate of mites inside a colony was at 4%. Also, the natural resistance of these honey bees to other pathogens has to be noted (Strauss *et al.*, 2013). A detailed description of African honeybees populations along with the Africanized honey bees (killer bees), who also seem to have a genetic resistance to parasites and pathogen,

resistance attributed to the African bee origin (Calderón *et al.*, 2010; Locke, 2016a).

Arnot Forest, Ithaca, NY, USA population.

The particularity of this population is that bee colonies are harboured in hollowed tree cavities (Locke, 2016a). However, in this case for this population, the survival of colonies seems to be attributed to the mite avirulence and the distance between colonies; the study does not reveal a clear mechanism for resistance (Seeley, 2007). A later study is suggesting that this population of bees is self-sustaining and could have a natural resistance to *Varroa destructor*, as they manage to survive without treatment even if they cannot eliminate environmental factors or the mite avirulence as a consequence for their survival (Seeley *et al.*, 2015).

Also, a population of *A. mellifera ligustica* located on **Island of Fernando de Noronha** has been found resistant to *Varroa* mites. Both populations have been reviewed by (Locke, 2016a). One interesting fact about the mite population from Fernando de Noronha has been identified as Japanese haplotype (Strapazzon *et al.*, 2009), which is less virulent. However is noted that over the years the relationship between the host and the parasite remained stable despite the lack of treatment; therefore, the presence of a natural resistance mechanism cannot be excluded and the hygienic behaviour mechanism is suggested since the colonies managed to resist without treatment against mites over 32 years (de Mattos *et al.*, 2016; Se Brettell and Martin, 2017).

8. Breeding schemes and the use of drones for selection

According to Anderson and Fuchs's (1998) observations establishing the mite genotype and virulence are an essential step in order to start a breeding program. Also, similar studies had proven the fact that adaptations might occur in *A. mellifera* families and mite populations, as well. That might lead to a stable host-parasite relation and a way for bees to fight against the parasite. The primary reasons that stop the beekeepers to use this natural selection method are: major population loses inside the colony, the risk to lose

the colony and major production losses (Fries and Bommarco, 2007).

In North America, 2 major breeding programs (VSH and RHB) are already implemented at commercial level; one of these programs has led to the foundation of Russian Honeybee Breeder's association while the VSH germplasm is available through Glenn Apiaries (Rinderer *et al.*, 2010).

In Europe, most of the breeding programs are focused on using pure subspecies of *A. mellifera*. Except for the Buckfast bee program (that uses crossbreeding) (Büchler *et al.* 2010). Significant progress in breeding of *A. mellifera* has been made after the BLUP- animal model has been adapted for honey bee particularities (Bienefeld *et al.*, 2006).

Another strategy used in order to breed resistant colonies is AGT (The Arbeitsgemeinschaft Toleranzzucht founded in 2003); this method consists of two major parts. First is a selection of a large honey bee population. This phase is followed by monitoring and testing the selected population of bees, and applying a natural selection for the drones in the infested population (Büchler *et al.*, 2010; <http://www.toleranzzucht.de>).

Even if there were made extensive programs and complex breeding strategies, they seem to be less effective when compared with more simple selective breeding strategies based on natural selection. These populations were made with untreated local colonies of *A. mellifera*, coming from Avignon and Gotland (Fries *et al.*, 2006; Le Conte *et al.*, 2007). In both cases, these colonies were obtained from local populations of honey bees that have survived untreated a longer period.

Selective pressure under *Varroa* infestation will favour the resistant drones. Given the infestation effect on flight performance, only the fittest will pass their genes to the next generation, and those resistance genes should be passed in the next generation (Jandricic and Otis, 2003; Büchler *et al.*, 2010).

In the end, host-parasite co-evolution has led to colonies in which *Varroa* mites are unable to reproduce on worker brood. This strategy proves to be effective in *A. cerana* colonies since they can subdue the mite population growth inside the colony. A selection strategy based on

lowering the reproductive success of the parasite seems to be a viable solution (Boot *et al.*, 1999). To accomplish this, it is essential to determinate the *Varroa* mites reproductive success for each haplotype, along with a better understanding of the resistance mechanisms of *A. cerana*, that could help fight against this parasite (Lin *et al.*, 2018). This might represent the key to fighting against this pathogen for *Apis mellifera*, too. Moreover, there are available breeding methods for genetic resistance to the parasite that can be implemented for commercial beekeeping programs (Kefuss *et al.*, 2016). Another more recent developed breeding method suggests taking full advantage of natural selection by forming a population of 25-30 colonies of local origin completely unrelated (no sister queens). The colonies will be split in order to mimic the natural swarming phenomenon and the virgin queens will be left to mate with drones from the local population. All new colonies will be selected based on the survival and the proliferous development of the colony, as they have to survive over winter untreated against *Varroa*. For the surviving colonies, the protocol is repeated at least for 4 consecutive years. The developers of this protocol suggest that in this way the genes involved in mite resistance should come to surface, and therefore to obtain resistant populations (Blacquière *et al.*, 2019).

9. Benefits for natural selection

In normal conditions, a host-parasite relation favours the parasite, but in the case of *A. mellifera* and *V. destructor*, we meet there are a few conditions that favour the bees. It seems that honey bees genome seems to have a high recombination rate (ten times higher) than most of the eukaryote organisms studied so far (Beye *et al.*, 2006), a high recombination rate should favour adaptation of an organism to changing environments and influence the genetic diversity inside a population (Stapley *et al.*, 2017). The recombination rate in *A. mellifera* can reach up to 197 cM/Mb in specific hotspots associated with gene conversion content and gene diversity. Still, their results suggest that immune-related genes have a low recombination rate, while genes associated with behaviour have a

high recombination rate (Liu *et al.*, 2015). On top of that, the queen can mate with up to 12 drones; this also gives a high diversity of the offspring and its positively correlated with colony fitness (Fuchs and Moritz, 1999; Delaplane *et al.*, 2015).

Since *Varroa* infestation induces changes in gene expression of the infested colonies of *A. mellifera* as a natural response of the immune system, this strategy can be used to pinpoint differences in gene expression in drone brood to locate the specific genes that are responsible for **reducing the reproductive success of the female mite** (Navajas *et al.*, 2008).

Evaluation of the suppression of mite reproductive success on drone brood can be made after (Locke and Fries, 2011) method. In their method, the development of each drone pupa was recorded based on (Martin, 1994) description. A further advantage of using this selection trait is that the phenotype can be directly controlled by analyzing the honey bee brood. This gives the possibility to identify the responsible genes for this mechanism and use them later in marker-assisted selection programs (Behrens *et al.*, 2011). Some molecular studies made in this direction already suggest potential candidate genes that could be involved in the ability of the bees to reduce the mite reproductive success for Gotland and Toulouse populations (Conlon *et al.*, 2018; Conlon *et al.*, 2019).

Since *Varroa* mites prefer to reproduce inside drone cells, obtaining resistant drones that can stop the mite reproductive cycle may prove a serious advantage. Even if just half of the drone brood has this type of resistance, the result will be a drop in the population growth rate of the mite. Also, if this resistance mechanism will affect the parasite at the beginning of the infestation, the mite population growth will be slower. Moreover, as a result, the virus speeding vectored by mites is slower, too. Furthermore, because of the male haploid reproduction, the drone has only one set of chromosomes, and that can make the natural selection less complicated (Kurze *et al.*, 2016).

Moreover, it seems that *Varroa destructor* genetic structure has an almost clonal origin and a low genetic variation (the study was made only

for the Korean and Japan haplotypes) (Solignac *et al.*, 2005).

A protocol based on natural selection and adaptation for local populations of honey bees could lead to resistant colonies of *Apis mellifera* and could prevent the spread of other invasive species. Moreover, this protocol can be used even if the exact mechanism for resistance is not yet duly understood (Blacquire *et al.*, 2019).

Conclusions

Treatments for *Varroa destructor* become less efficient and new strains of *V. destructor* have been identified (Lin *et al.*, 2018). A promising long-term solution could be the breeding of resistant colonies. A breeding scheme based on drone selection promises to give a simple breeding method that can be applied at the apiary level (Jandricic and Otis, 2003; Blacquire *et al.*, 2019).

In 2006 „The Honeybee Genome Sequencing Consortium” managed to sequence the whole *A. mellifera* genome for the first time. This study gave the researchers new possibilities for study. Among the particularities of this species, it was noted that the genome of *A. mellifera* has fewer genes for innate immunity and more genes for odorant receptors (Weinstock *et al.*, 2006).

The reduced number of immune genes results in a limited ability of the honeybees to fight against the pathogens as an individual, considering that their immune system is focused on a small group of pathogens (Weinstock *et al.*, 2006).

Recent studies have made available genomic and transcriptomic analyses for the Asian honeybee (*A. cerana*) giving new perspectives for analysis. For example, if compared with *A. mellifera*, the genome of *A. cerana* seems to have more immune system genes than *A. mellifera*. Moreover, they presented fewer genes that encode odorant-binding proteins and olfactory receptors compared to *A. mellifera*. Since *A. cerana* has a superior response to *V. destructor* infestation, this might suggest that grooming behaviour is regulated by other mechanisms and is not triggered just by visual and olfactory stimuli. There might be other genetic factors involved (Diao *et al.*, 2018).

Having available the genomes and transcripts for *A. cerana*, *A. mellifera* and *V. destructor* as tools, the researchers might be able to understand the host-parasite interaction better by analysing the transcriptomic and proteomic data. This could help identify candidate genes that could be used in breeding schemes based on MAS (marker-assisted selection), in order to obtain *Varroa* resistant populations of *A. mellifera* (Weinstock *et al.*, 2006; Cornman *et al.*, 2010; Diao *et al.*, 2018).

Recently a new assembly for *V. destructor* genome was released along with a genome assembly for *V. jacobsoni* providing a better understanding of this parasite genome and an update for the gene list (www.ncbi.nlm.nih.gov; Techer *et al.*, 2019). The availability of the genomic resources for this parasite could help researchers to identify the mechanisms used to exploit their host and possible ways to disrupt them (Evans and Cook, 2018).

Until now, the mechanisms underlying the initiation of the reproductive cycle of the *Varroa destructor* are not fully understood. The study of this mechanism at the molecular level could lead to a better understanding of the host-parasite relationship, and transcriptomic analysis may lead to the identification of gene cascades involved host resistance mechanisms to this parasite (Ji *et al.*, 2014).

Acknowledgements. This work was supported by the project PN-III-P4-ID-PCE-2016-0637, contract number 162/2017, financed by UEFISCDI.

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