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Review Article

Varroa destructor: how does it harm *Apis mellifera* honey bees and what can be done about it?

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Since its migration from the Asian honey bee (*Apis cerana*) to the European honey bee (*Apis mellifera*), the ectoparasitic mite *Varroa destructor* has emerged as a major issue for beekeeping worldwide. Due to a short history of coevolution, the host–parasite relationship between *A. mellifera* and *V. destructor* is unbalanced, with honey bees suffering infestation effects at the individual, colony and population levels. Several control solutions have been developed to tackle the colony and production losses due to *Varroa*, but the burden caused by the mite in combination with other biotic and abiotic factors continues to increase, weakening the beekeeping industry. In this synthetic review, we highlight the main advances made between 2015 and 2020 on *V. destructor* biology and its impact on the health of the honey bee, *A. mellifera*. We also describe the main control solutions that are currently available to fight the mite and place a special focus on new methodological developments, which point to integrated pest management strategies for the control of *Varroa* in honey bee colonies.

Introduction

The mite, *Varroa destructor*, has been the subject of thorough investigations, since the 1980s after its introduction in Europe to *Apis mellifera* honey bee populations [1]. *V. destructor* continues its worldwide expansion and has now been reported in most countries. In the last 10 years, new invasions were noted in Hawaii [2], Reunion Island [3], Madagascar [4], Mauritius Island [5] and also African countries such as Uganda [6] and Ethiopia [7]. In early 2020, only Australia, several countries in Africa and a few islands have yet to report the presence of *V. destructor* in their *A. mellifera* populations [8,9]. Despite the extensive literature on the mite (see [10–12] for reviews), its biology and impact on its honey bee host remain partially unknown. The scientific community has also put many efforts into the development and validation of control methods to fight the deadly mite in *A. mellifera* colonies. This review highlights the main findings revealed by the rather large corpus of literature published between 2015 and early 2020 on *V. destructor* biology, the mite's pathogenic effects, and solutions developed to fight the mite.

Biology of *Varroa destructor*

Between 2015 and 2020, the behaviour of *V. destructor*, its genetics and physiology, have become more precisely understood. Its life cycle can be separated into two phases: the phoretic phase and the reproductive phase. The phoretic phase only concerns the female mite which uses the adult bee as a 'transportation vector' and food source. During this phase, bees unintentionally take part in the spreading of *Varroa* within and between honey bee colonies. The reproductive phase begins when the mite enters into an unsealed brood cell containing a 5th stage bee larva, to lay eggs. Recently, Häußermann *et al.* revealed the possibility of a virgin mite beginning the phoretic phase. In that case, the mite invades a brood cell, lays an unfertilised egg in a new comb, and mates with its male offspring [13]. This finding confirms that female *Varroa* do not need to mate to lay eggs, and thus, have an arrhenotokous parthenogenetic reproduction system. While it was first thought that *Varroa* feeds

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on honey bee haemolymph, recent research shows that the parasite also feeds on the honey bee fat body [14]. Analyses of the whole-life transcriptome and proteome of the mite have helped to understand the gene expression and protein variations during its different life stages and have provided data for further investigations of mite physiology [15,16]. The morphology of *Varroa*'s heart has been described, its heartbeats have been recorded [17] and the sensory pit organs of the male have been scanned by electron microscopy [18], complementing the morphological research on female pit organs [19]. The identification of the chemosensory receptors on *Varroa* forelegs confirms the importance of their front legs in understanding their chemical environment [20]. Iovinella and collaborators performed the proteomic analysis of the chemosensory organs of the mites. This study identified novel semiochemical proteins, including odorant-binding proteins which are particularly abundant in mouths and forelegs [21]. Further chemical ecology investigations have indicated that the male *Varroa* detects female pheromones with its sensory pit organs present on their front legs [18]. All these findings are important to better understand *V. destructor* mating behaviour, detection and invasion of brood cells close to be capped. We know that *V. destructor* has the ability to mimic the cuticular hydrocarbons of their *A. mellifera* hosts at different stages [22]. It seems that this mimicry can rapidly adapt as *Varroa* is able to harbour the cuticular hydrocarbon profile of a new, artificially-given host [23,24]. Furthermore, *V. destructor* from *A. cerana* are better in mimicking new hosts than *V. destructor* from *A. mellifera* [23]. To do so, *Varroa* adapts its *n*-alkane:alkene ratio to fit with the honey bee's chemical profile [25]. This mimicry stands as a passive ability, because even when dead, *Varroa* continues to imitate host cuticular hydrocarbons [24]. Nevertheless, the mite needs to have access to the cuticular hydrocarbon of the host in order to adapt its own cuticular hydrocarbons [24].

Varroa destructor genetics

V. destructor infestation of *A. mellifera* was described as two partially-isolated clones in early 2000 [26], but it now seems that the genetic population of *Varroa* is more variable than expected [27]. Different haplotypes of *V. destructor* can be found within a given apiary or colony, ensuring the genetic flow in the mite population [28,29]. *V. jacobsoni* and *V. destructor* show very different evolutionary trajectories since their divergence [30]. Despite these differences, it seems that the two subspecies have a potential to hybridise [31]. If that is the case, hybridisation events could result in a new species of *Varroa*, possibly more detrimental, that would become a greater risk for honey bees.

Varroa-virus duo

The host-parasite relationship between honey bees and *Varroa* should actually be considered as a three-way relationship, as *Varroa* presence is closely associated with several bee viruses in colonies [32]. However, clear vectoring by *Varroa* has only been described for two viral species, deformed wing virus (DWV) and acute bee paralysis virus complex (ABPV) [33–35]. Bee viruses have several routes of vertical and horizontal transmission within the colony, but the vectoring ability of *Varroa* opens up new, very effective horizontal routes of transmission [36,37]. In the last 5 years, new viruses and variants linked to *Varroa* have been discovered in honey bees infested by the mite [38–42], and the association between DWV and the mite has been confirmed, but only for specific DWV variants [2,32]. New variants and viral species have also been described specifically in the mite, as demonstrated by VDV replication in the mite [43], in which, VDV-5, VDV-3 and VDV-2 can replicate in *V. destuctor* and not in *A. mellifera*, suggesting that their presence in honey bees is due to the *Varroa*'s feeding behaviour [38].

It is thought that some of the viruses infesting mites can change *Varroa* behaviour, which presents a new perspective for finding targets to kill the mite [43]. Similarly to its impact on the virus community infecting bees, the mite also alters the honey bee's bacteriome [44,45], but the impact of such an association remains unknown.

What can it do?

Impact of the mite at the individual level

The parasitism of honey bees by *V. destructor* decreases the body weight and water content of young emerging bees [46]. The lowered weight of the future adult bee increases with the number of mite foundresses [47,48]. In honey bees, the number of spermatozoids is correlated with drone body size [49]. By decreasing the size of drones, *Varroa* induce a deficit in sperm production, and thus, in reproductive fitness [50]. *Varroa* also alters flying, homing and orientation abilities in foragers [51], which in turn, limits efficiency in their ability to collect resources needed for colony development. Non-returning to the colony can also be considered as a mechanism of defence from parasited bees. The impact of *Varroa* on bee behaviour may be explained by its ability to alter neural processes [52], disrupting the bee-host's non-associative learning abilities. Indeed, parasited bees have a lower sugar responsiveness and a faster habituation to olfactory stimulation [52]. Moreover, *Varroa* provokes the down-regulation of immune gene expression in emerging infested adults [45], as well as proteomic changes in the honey bee's immune response [53–55]. It disrupts the bee's immune response by interfering in the cascade immune response [56]. For instance, *Varroa* reduces the number of haemocytes circulating in the haemolymph, and lowers the expression of prophenol oxidase involved in the synthesis of melanin [57,58]. Both haemocytes and melanin permit the encapsulation of pathogens during infection or wounding, and thus play a role in insect immune response and healing mechanisms [59]. The weakening of honey bee immunity [60,61] may be linked to the finding that mites feed on fat bodies, given that these organs play a major role in immunity [62].

The impact of Varroa coupled with other stressors of the colony

Varroa can interact with other biotic and abiotic stressors, such as environmental factors, other parasites and pathogens, pesticides or viruses. Climate change induces longer periods of brood rearing in honey bee colonies and foraging because of longer warm seasons. Longer brood period means more *Varroa* reproduction cycles and may lead to an increase in mite populations [63]. *Nosema* infection reduces the effectiveness of bee defences against the mite. In addition, neonicotinoid pesticides and *Varroa* both contribute to the decrease of winter honey bee population of the colony [64,65]. Together with another neonicotinoid, imidacloprid, *Varroa* decreases the bee's flying ability [66]. Monchanin *et al.* [67] demonstrate the negative impact of *Varroa* coupled with the neonicotinoid insecticide thiamethoxam on honey bee homing behaviour. Also, the *Varroa*-virus duo plays a key role in the weakening of the colony [68]. Emerging adult bees that were parasitised during the pupal stage show a higher infection rate of DWV compared with non-parasitised individuals [45]. DWV infection induces pathological effects such as crumpled wings and reduced body size leading to behavioural impairment. The DWV titer in honey bees increases as the immunity of the bee decreases [69]. DWV virus can also immunocompromise bees, which may result in a beneficial effect on *Varroa* reproduction [70].

As colonies are constantly exposed to different stressors at the same time, further research needs to focus on the interaction between two or more stressors at the same time to investigate their combined impact on honey bee fitness. For example, to determine interactions between co-occurring viruses and Varroa on honey bee health.

Honey bee colony dynamics favours Varroa population growth

The population of *V. destructor* in a colony is directly related to the amount of brood and, by extension, the colony's population size. One way for a colony to decrease the mite population is to create a broodless period. To do so, an increase in the frequency of the reproductive behaviour of swarming can be an adaptive defence mechanism of the honey bee colony against the overpopulation of mites. [71] (Figure 1).

Moreover, the number of colonies within an apiary can be advantageous for mite populations. Indeed, colonies of high-density apiaries have a greater infestation rate than that of low-density apiaries [72]. Colonies treated for *Varroa* can be reinfested when foragers rob food stores from a dying colony or drift into another colony [73], and when drones rest in foreign colonies during the mating period [74].

What can be done?

Different approaches are used by beekeepers to control *Varroa* infestation of their colonies. Synthetic and organic acaricides as well as essential oils provide good results to prevent colony losses and the latter, represent interesting tools for organic beekeeping [75]. Depending on the country, methods may differ due to variations in the laws governing the use of chemicals.

Chemical control

Conventional control using synthetic miticides has been used for more than 40 years against *V. destructor* [76]. However, only a limited number of molecules are available (Figure 2). They include the pyrethroids tau-fluvalinate (e.g. Apistan®, Klartan®, Mavrik®) and flumethrin (e.g. Bayvarol®, Polyvar yellow®), the formamidine amitraz (e.g. Apivar®, Apitraz®) or the organophosphorus coumaphos (e.g. Checkmite®, Asuntol®, Perizin®)

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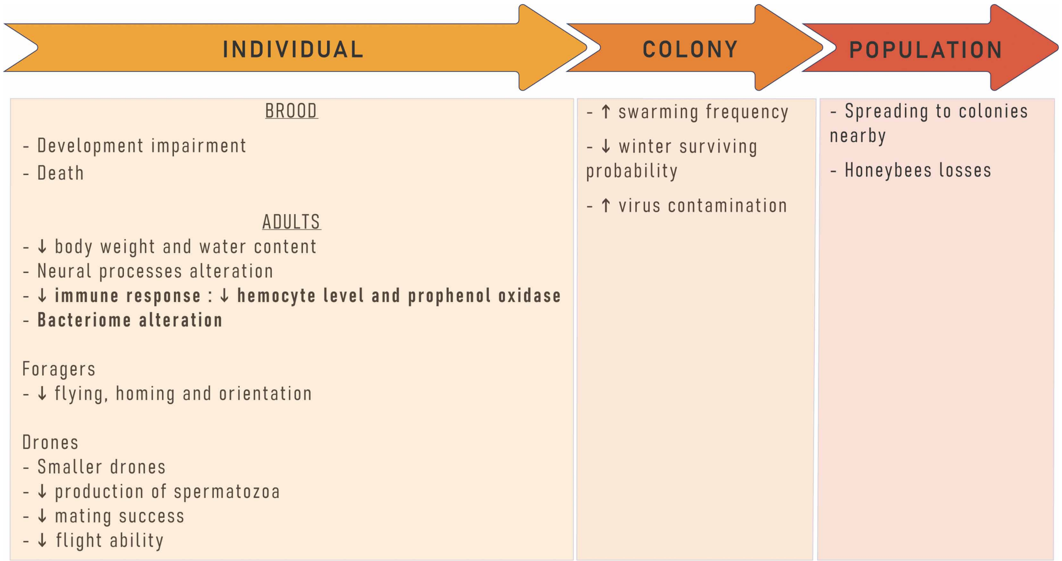
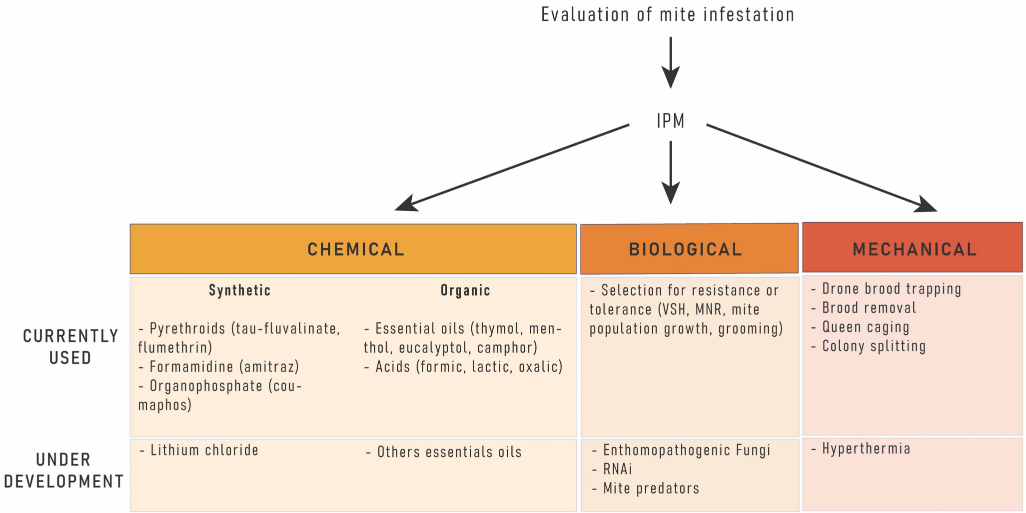


INDIVIDUAL	COLONY	POPULATION
BROOD - Development impairment - Death ADULTS - ↓ body weight and water content - Neural processes alteration - **↓ immune response : ↓ hemocyte level and prophenol oxidase** - **Bacteriome alteration** Foragers - ↓ flying, homing and orientation Drones - Smaller drones - ↓ production of spermatozoa - ↓ mating success - ↓ flight ability	- ↑ swarming frequency - ↓ winter surviving probability - ↑ virus contamination	- Spreading to colonies nearby - Honeybees losses

Figure 1. Impact of *Varroa destructor* parasitism on *Apis mellifera* honey bees.
Varroa's impact can be described at individual, colony and population levels. Bold terms correspond to findings published between 2015 and 2019.

[10,77]. All miticide products kill only *Varroa* on adult bees, as they cannot target the reproducing mites hidden in the capped brood. To overcome this shortcoming, products made of strips releasing the acaricide compound over time have been successfully developed and mites are killed when they emerge from the brood cells. These strips are easy to use and efficient but have important limitations, like the resistance developed by the mite and the drawback of accumulating as residues in bee products [78].

Mites are becoming increasingly resistant to acaricides [79–81]. *Varroa* resistance to fluvalinate is now widespread [82], due to DNA mutations [83,84]. Molecular tools have been developed to detect this resistance in *Varroa* populations [85,86]. A provisional solution to limit and temporally bypass *Varroa* resistance to



	CHEMICAL		BIOLOGICAL	MECHANICAL
	Synthetic	Organic		
CURRENTLY USED	- Pyrethroids (tau-fluvalinate, flumethrin) - Formamidine (amitraz) - Organophosphate (coumaphos)	- Essential oils (thymol, menthol, eucalyptol, camphor) - Acids (formic, lactic, oxalic)	- Selection for resistance or tolerance (VSH, MNR, mite population growth, grooming)	- Drone brood trapping - Brood removal - Queen caging - Colony splitting
UNDER DEVELOPMENT	- Lithium chloride	- Others essentials oils	- Enthomopathogenic Fungi - RNAi - Mite predators	- Hyperthermia

Figure 2. Methods currently used or under development to treat honey bee colonies against *Varroa destructor* parasitism.
Methods can be coupled within an integrated pest management scheme (IPM). VSH: *Varroa*-sensitive hygiene; MNR: Mite non-reproduction.

miticides is to switch between molecules with different molecular targets. Synthetic acaricides could then provide good results for preventing colony losses [75]. Growing data have been published on acaricide residues in honey bee colony matrices, particularly in wax, which have the chemical properties to store the used lipophilic acaricides at concentrations that could even be toxic to the bees [78,87,88].

Because of the adverse impact that conventional synthetic acaricides have on bees and bee products worldwide, beekeepers are increasingly using organic control methods. Organic methods are usually less efficient compared with conventional synthetic acaricide treatment, but still effectively control mite populations [89]. The most common are essential oils such as thymol, and organic acids like oxalic acid and formic acid [77] (Figure 2). Organic acids are naturally found in bee products and have lower risk for triggering resistance in mites [77], but can still have some negative effects on bees, such as decreasing worker populations, increasing capped brood removal or decreasing drone sperm quality [90].

The use of organic control mostly involves flash treatments and thus needs a broodless colony to be efficient in killing phoretic *Varroa*. As such, organic product applications coupled with mechanical methods to exclude the brood can provide a good *Varroa* control solution [91,92]. Queen caging or brood removal can artificially create a broodless colony that keeps the mites on adult bees, making them accessible to acaricides [91,93]. A recent study suggests that while brood removal may result in a decrease in honey production, this loss can be compensated for by avoiding the use of acaricides, enabling a better price of hive products to be achieved [94]. Recently, a new formulation, to be used when brood is present and based on strips releasing oxalic acid, was tested successfully for *Varroa* control, creating a real opportunity in the organic control of *Varroa* [95].

Need for new active compounds

Although several products that efficiently control the mite are available, there is an urgent need for new active compounds because of the risk of *Varroa* resistance. A screening approach combining *in silico* screening (virtual screening of a chemolibrary of homology sequence models) with *in vitro* experiments to search for selectively inhibiting *Varroa* acetylcholinesterase was successfully developed leading to the discovery of new compounds that have the potential to become new treatments against the mite [96]. Lithium chloride has also been demonstrated as a potential compound against *Varroa* [97], as well as other essential oils and their combinations [98]. Recently, Bendifallah *et al.* [99] demonstrated the biological activity of sage essential oil as a *Varroa* control. However, currently there are no registered products available based on these new bioactive sources. The difficult part about the search of new acaricides to control the mite is that the compounds need to be safe for the bees. Development of *in vitro* rearing of *Varroa* under laboratory conditions will provide an efficient platform for rapidly screening activity of new compounds which are potentially useful in *Varroa* control.

Alternative approaches to the use of chemical treatments

Different techniques have been previously described to limit *Varroa* infestation, such as the 'trapping comb technique' or the use of screen bottom boards to trap the mites. A recent study showed that splitting colonies, which mimic swarming events that can control *Varroa* growth, could be an effective method for decreasing the mite populations [71].

Biological control methods using parasitic fungi have been developed successfully in controlled experiments [100,101]. Hamiduzzama *et al.* [100] showed varying virulence of entomopathogenic fungi on *V. destructor*. Unfortunately, most field experiments on honey bee colonies were not yet successful as the fungi can cause detrimental effects on brood development, queens and worker mortality as well as decreasing of weight in newly emerged adult bees [100]. Another experiment using a Beauveria strain showed an effect on *Varroa* mortality in the field but no visible negative effect on honey bee health [101]. To date, there is no available method on the market for beekeeping using this technique.

The use of predators has shown mixed results and was unsuccessful when applied to honey bee colonies, as was recently the case in an evaluation of the predatory mite *Stratiolaelaps scimitus*, which demonstrated interesting results in vials but no effect on honey bee colonies [102].

Hyperthermia has been used since the 1970s, and is based on the better heat resistance of the bees compared with *Varroa* [103]. Artificially heating the hive stops *Varroa* reproduction and kills the parasite, without harming the bees, because *Varroa* reproduction is significantly compromised at 36.5°C and mites die at 38°C [104,105]. Heating systems have been proposed to control the mites in different parts of the world, and there are a few systems available on the market at the moment [103,106].

One other option is to use RNA interference to knock down specific genes of *Varroa*, which has been studied since 2012 with successful results [43,107,108]. To date, there is no product available for beekeepers [109] (Figure 2).

We have the tools for integrated pest management (IPM)!

A variety of tools available for beekeepers make the development and use of the IPM concept in *Varroa* control possible (Figure 2). IPM is partly based on limiting pesticide use to when it is only necessary. This requires regular monitoring of the *Varroa* population levels, in order to detect critical infestations and decide on a treatment. As critical infestation levels differ worldwide, these must be defined for specific regions and biotopes.

Efficient but time-consuming techniques exist to diagnose a *Varroa* infestation [110]. Mites can be counted in the brood, on adults or on debris at the bottom of the hive. Brood examination consists of opening capped cells to verify *Varroa* infestation by removing the pupae and counting mites [77,111]. Mite count on adult bees is better documented and increasingly used. It consists of collecting 200–300 bees, separating the mites from bees with a surfactant substance, such as chloroform, alcohol, icing sugar or acaricide treatment, and counting the removed mites [77]. Debris examination can be operated with a sticky sheet placed on the floor of the hive with a thin wire mesh on top of it to prevent bees from cleaning out the fallen mites. Fallen mites stick to the board and honey bees are not able to remove the parasite from the hive [77]. As it is a time consuming and tedious method, a stratified method has been proposed to make an accurate estimation of the mites [112].

New techniques, using technological developments such as gas sensors or computer vision systems, are being tested to estimate *Varroa* infestation [113,114]. They have yet to be transferred to the field and to the beekeeping community.

Selective breeding of naturally resistant or tolerant honey bee populations

In parallel to the development of control solutions to fight the mite, quantitative geneticists and bee breeders have started to search for a longer-term, sustainable solution: selecting honey bee populations that can survive mite infestation without treatments. Several surviving honey bee populations have been identified or bred throughout the world (for a review of these efforts, see [115–117]). Examples of large-scale use of such populations in beekeeping are scarce [118,119] and currently limited by the lack of tools that allow selection of surviving honey bee colonies in the field.

Such tool development relies on the identification of specific phenotypes that characterise these populations. To do so, a better understanding of the mechanisms that undergird the ability to survive is necessary. Surviving can occur through the expression of resistance or tolerance traits, with resistance involving a reduction in *Varroa* growth, while tolerance reduces parasitic burden despite similar levels of *Varroa* growth [120]. A wide range of traits involved in honey bee survival to *Varroa* have been identified, and mainly relate to resistance mechanisms [121]. Tolerance has so far been suggested only in cases (e.g. in the Gotland population in Sweden) where colonies are able to support *Varroa* burden due to mechanisms of tolerance or resistance to the viruses that are associated with *Varroa* infestations [42,122].

Recent investigations highlight the importance of behavioural defences displayed by *Varroa* resistant honey bee populations (Figure 3). Hygienic behaviour specifically targeting *Varroa*-infested capped brood cells (VSH, *Varroa*-sensitive hygiene) has been confirmed as a major trait contributing to reduced mite population growth in European and African bee populations [123–125]. Two other adult bee traits, grooming [126] and recapping [127,128], have been confirmed as important mechanisms for *Varroa* resistance. At the colony level, swarming can enhance resistance in surviving populations living in the wild [71,129]. Brood traits could also be involved in resistance abilities, if they confer hypersensibility of the brood that leads to the accelerated death or an increased rate of removal, thereby preventing the spread and reproduction of *Varroa* [110,130]. Altogether, these traits participate in limiting mite population growth as characterised by high levels of mite non-reproduction within the brood.

Research has mostly focused on host traits to explain the survival of untreated colonies, but the parasite itself may play a central role too. Indeed host fitness can directly be affected by parasite fitness, as suggested by studies showing that *Varroa* infesting surviving colonies are genetically distinct from *Varroa* infesting neighbouring susceptible colonies [131,132].

Progress in understanding the mechanisms that underlie resistance abilities along with the recent boost in genomic tool development has opened the possibility of devising a diagnostic tool of resistance based on

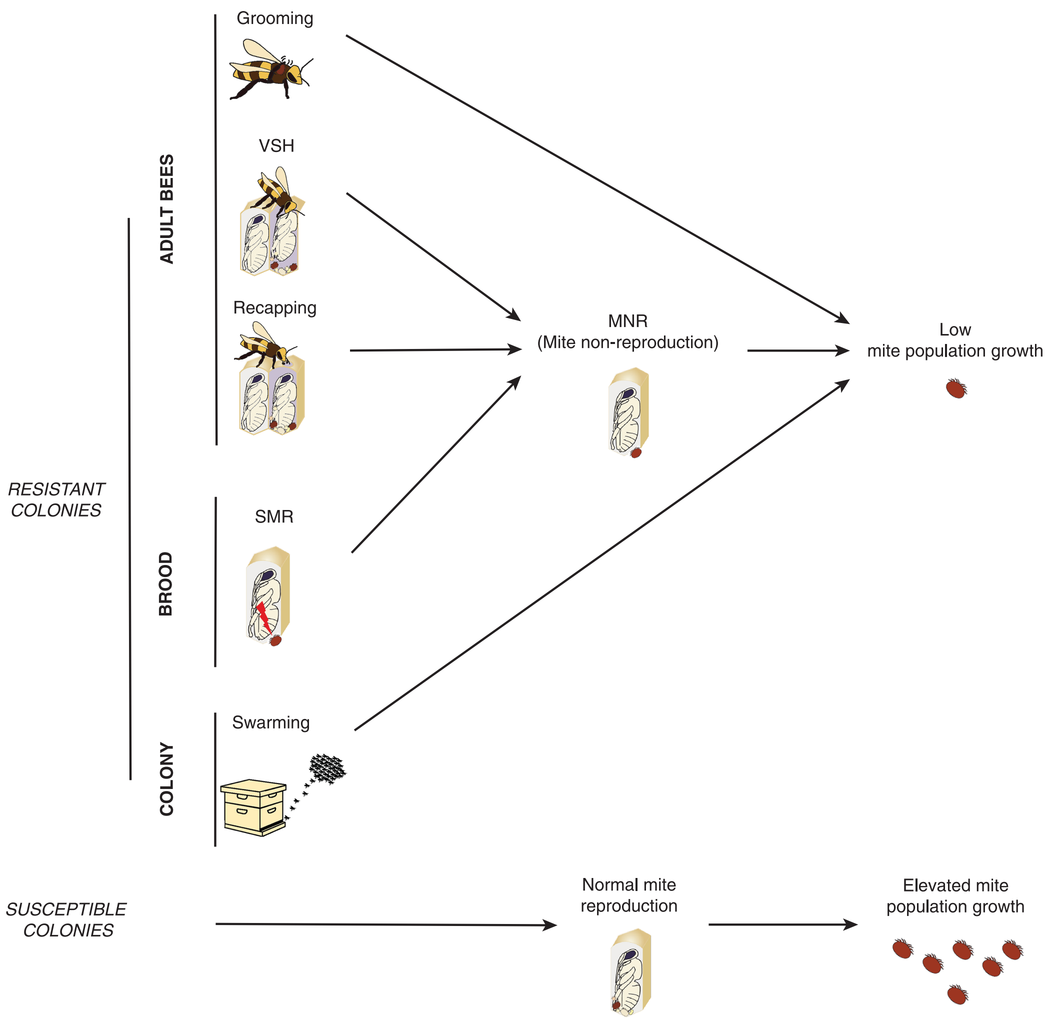


Figure 3. Main behavioural and physiological traits involved in the natural resistance of honey bees to the parasite *Varroa*.

VSH (*Varroa*-sensitive hygiene), recapping and SMR (suppressed mite reproduction) contribute to MNR (mite non-reproduction). Together with grooming and swarming these traits lead to colony resistant through low mite population growth.

phenotype, directly accessible to beekeepers. So far, available methods are very tedious and difficult to apply in the field on large numbers of colonies. This is true for measures of mite population growth, mite non-reproduction or hygienic behaviours. The recent identifications of molecular and protein markers of several traits related to resistance (grooming, hygiene, VSH and mite non-reproduction), although very limited in overlap between different studies [121], opens up the possibility for marker-assisted selection [133–140]. It would allow beekeepers to easily select their colonies for on the basis of interesting resistance traits when the phenotype are difficult to characterise. To date, there are no products available on the market and research is continuing in this area.

Another perspective for the development of selection tools originates specifically from a detailed understanding of the mechanisms of VSH behaviour, a trait in which honey bees are able to specifically detect *Varroa*-infested brood. Strong evidence suggests that the recognition step involves the detection of *Varroa* infestation-associated semiochemicals [16,141–144]. Evaluation of the bee response following application of such candidate compounds in colonies, by acting as a reliable proxy of the VSH activity of the colony, could result in a practical field tool to phenotype resistant colonies.

Conclusion

Despite the amount of research done on the *A. mellifera*–*V. destructor* host-parasite model, *Varroa* remains a major issue for beekeeping throughout the world. Future developments, both in fundamental and applied research, are necessary to generate sustainable control solutions for this deadly parasite.

Summary

- The latest findings on the physiology of *Varroa* and its behaviour provide a better understanding of its negative impact on bee health.
- IPM methods can be used to limit the use of acaricides to control *Varroa*. The selection of resistant or tolerant honey bee populations could bring a sustainable mite control solution for beekeeping and wild honey bee populations.
- Research needs to focus on the development of control methods, especially new active compounds to counter the mite's resistance against acaricides and to efficiently fight *V. destructor*.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Author Contributions

A.N., F.M. and Y.L.-C. designed and wrote the manuscript.

Abbreviations

ABPV, acute bee paralysis virus complex; DWV, deformed wing virus; IPM, integrated pest management; VSH, *Varroa*-sensitive hygiene.

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