Insectes Sociaux

Genetic bases of tolerance to *Varroa destructor* in honey bees (*Apis mellifera* L.)

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Abstract Currently, the *Varroa destructor* mite is the most serious parasite of honey bees (*Apis mellifera*) and has become a nearly cosmopolitan species. The mite not only causes damage by feeding on the haemolymph of honey bees, but it also transmits viruses, which have been implicated in colony collapse disorder. The major research goal has been to breed mite-tolerant honey bee lines in order to reduce the amount of pesticide used, because pesticides can promote the evolution of resistance in mites. In this review, we describe different behavioural traits and genes that may be part of the defence against the *Varroa* mite. Specifically, we review grooming behaviour, *Varroa*-sensitive hygiene and the suppression of mite reproduction. A large number of candidate genes have been identified by Quantitative Trait Loci studies, and through gene expression studies their function and effect have been elucidated. Results from the studies discussed can be used in apiary practice.

Keywords *Varroa destructor* · Tolerance behaviours · Candidate genes · QTL · Gene expression

Introduction

Honey bees are the main pollinators worldwide and are essential for many agricultural crops (rape, sunflower, pulses) and in the conservation of natural plant biodiversity. Delaplane and Mayer (2000) estimated that ∼35 % of human food consumption depends directly or indirectly on insect-mediated pollination. Agriculture dependence on pollinators has increased by 50 and 62 % in the developed and developing world between 1961 and 2006 (Aizen et al., 2009). Globally, insect pollination has been estimated to account for approximately 9.5 % of the total value of agricultural production (Gallai et al., 2009). The global production of honey was estimated at 1.07 million metric tonnes in 2007, representing an important international commodity. The total number of managed honey bee colonies worldwide was estimated at 72.6 million in 2007, representing a 64 % increase since 1961. However, in both Europe (−26.5 %) and North America (−49.5 %) the number of managed colonies has significantly decreased, while large increases have occurred in Asia (426 %), Africa (130 %), South America (86 %), and Oceania (39 %) in the period between 1961 and 2007 (FAO, 2009).

Varroa destructor is the most dangerous parasite of the honey bee (*Apis mellifera*) (Rosenkranz et al., 2010). By feeding on the haemolymph of developing and adult bees, it acts as a vector for several highly pathogenic honey bee viruses (Boecking and Genersch, 2008). Mites have spread around the world and killed hundreds of thousands of honey bee colonies. Mites from the *Varroa* genus have adversely affected the apiculture industry in every single country and have been responsible for economic losses of billions of dollars since the parasite arrived in the USA. *Varroa* is currently represented by four species of obligately ectoparasitic mites: *Varroa jacobsoni*, *Varroa underwoodi*, *Varroa rindereri* and *V. destructor* (Rosenkranz et al., 2010). *Varroa jacobsoni* was the first ectoparasitic mite of *Apis cerana* found in Java (Oudemans, 1904); Delfinado-Baker and Aggarwal (1987) first described *V. underwoodi* from *A. cerana* in Nepal; *V. rindereri* was found from *Apis koschevnikovi* in Borneo (De Guzman and Delfinado-Baker, 1996), and *V. destructor* was formerly erroneously also

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classified as *V. jacobsoni* (Anderson and Trueman, 2000). There are two haplogroups of *V. destructor:* the Korean haplogroup (Europe, the Middle East, Africa, Asia, America and New Zealand), which is found worldwide on *A. mellifera,* and the less common Japanese haplogroup which occurs in Thailand, Japan, and America (Anderson and Trueman, 2000).

Apiculture is impossible in Europe without chemical acaricides to control *Varroa* (Rosenkranz et al., 2010). Unfortunately, it is likely that the mite will eventually become resistant against these chemicals and their efficiency will be reduced (Pettis, 2004). Control treatments often cause contamination of the honey and pollen, for example, acaricide residues (Martel et al., 2007). *Varroa* tolerance may be caused by very different traits because the interaction between the host and the mite is complex.

There are several European honey bee populations that have never been infested by *Varroa* mites (Island of Ouessant in France, northern Sweden and the Finnish island Aland). Such populations are essential for understanding the genetic, evolutionary and epidemiological mechanisms driving tolerance against *Varroa* (Moritz et al., 2010).

In this review, we outline the latest findings from molecular genetic studies of *Varroa* mite tolerance in the honey bee.

Defence reactions

There are a wide range of behaviours that might impair the survival and reproductive success of *V. destructor*; two of them are "*Varroa*-Sensitive Hygiene" (VSH) and the "grooming behaviour" (Evans and Spivak, 2010). Harris (2007) described another characteristic known as "Suppressed Mite Reproduction" (SMR).

Grooming behaviour

Peng (1988) noted that bees possibly remove *Varroa* mites via allogrooming behaviour. Physical damage has been found on the bodies on the *Varroa* in *A. mellifera* colonies caused by the mandibles of honey bees (Ruttner and Hänel, 1992). *A. mellifera* workers groom themselves (autogrooming), as well as other honey bee bodies (allogrooming) (Peng et al., 1987). This behavioural pattern is being described as a grooming dance performed by workers (Milum, 1947), and it could be considered as selection trait in future breeding programmes to reduce the susceptibility of *A. mellifera* colonies to *V. destructor* infestation (Delfinado-Baker et al., 1992). Autogrooming seems to be a highly variable (Büchler, 1994) behaviour, suggesting it may have strong potential for selective breeding. However,

it is unclear how high the heritability of autogrooming is in European honey bees (Harbo and Hoopingarner, 1997; Moretto et al., 1993). Research has shown that Africanised honey bees exhibit higher average levels of both grooming behaviour and VSH compared to European bees (Mondragon et al., 2006).

Varroa-sensitive hygiene

Rothenbuhler (1964) first described hygienic behaviour as the uncapping and removal of dead, diseased or parasitised brood. These two forms of behaviours define the hygienic behaviour collectively and each controlled by an independent locus. The first behaviour (*Uu* and *UU* workers) is not able to uncap dead cells, whereas the homozygous-recessive *uu* workers are able to do this. The second describes when workers (*rr*) are able to remove dead pupae from uncapped cells, whereas workers that are heterozygous *Rr* and *RR* are not able to remove dead brood. Homozygous-recessive *uurr* genotypes need to be present for VSH. These reactions are apparently complex and include repeated uncapping and removing of infested brood cells (Rosenkranz et al., 1993). The removal of mites from the brood can cause a break in the reproductive cycle of the *Varroa* mite, a prolonged phoretic phase or even the death of the mites. Previous studies have shown that in *A. cerana*—the original host of the *Varroa* mite— experimentally inoculated with mites, workers removed 97 % of mites from open brood cells within a few minutes (Peng et al., 1987), while *A. mellifera* removes a lower number of mite-infested pupae under the same time (Boecking and Ritter, 1993).

Suppress mite reproduction

The mite's life cycle is synchronised with the developmental time of pupae (Garrido and Rosenkranz, 2003). Ruttner et al. (1984) detected that honey bee colonies often had a high proportion of non-reproducing mites in their brood cells. This non-reproductive trend was independent of the origin of the bee brood (Fuchs, 1994). The number of mites was 50 % lower in the untreated population of *A. mellifera* on the island of Gotland (Locke and Fries, 2011; Fries and Bommarco, 2007), and mite infertility was one of the parameters found to be influencing the reduced reproductive success of the mite (Locke and Fries, 2011).

Suppressed mite reproduction is a heritable trait that has been shown to control *V. destructor*. The bees removed reproductive *Varroa* mites more often than non-reproductive ones (Harbo and Harris, 2005). The results of the study by Harbo and Harris (2005) suggest that the SMR trait

may be identical to *Varroa*-specific hygiene described by Boecking et al. (2000).

Approaches to study tolerance against *Varroa*

In the future, artificial selection will include the use of conscious molecular genetics. In 2006, the Honey Bee Genome Project was completed and provided the possibility to better understand disease resistance in a highly social organism (Weinstock et al., 2006). Evans et al. (2006) introducing a genome-wide analysis of immunity in honey bee. *A. mellifera* has one-third of the insect immunity genes compared to *Drosophila melanogaster* and *Anopheles gambiae*. There are three signal pathways associated with immunity: the Toll pathway, immunodeficiency pathway (IMD) and janus kinase/signal transducers and activators of transcription (JAK/STAT) pathways that have been identified in the honey bee genome. Oligonucleotide microarrays with all annotated genes of the honey bee ($\sim 13,400$ genes) are available. The transcriptome has been screened to reveal differential genome responses to specific infections (Moritz et al., 2010).

During the 1990s, molecular genetic tools were first used in *Varroa* mite research to look for variation among and within the mite population (Kraus and Hunt, 1995). Cornman et al. (2010) published the results of the *Varroa* Genome Project. It provides direct tools for the control of *Varroa* mites by the identification of *Varroa* genes. The haploid genome size of the *V. destructor* (estimate of 565 ± 3 Mb) is larger than the genome size of most insects and smaller than the genome size of most mites. The rate of single nucleotide polymorphisms (SNPs) in the mite is 6.2×10^{-5} per base pair (Cornman et al., 2010).

Molecular genetics studies have led to the identification of multiple genes or genomic regions that affect traits of interest in livestock. When genes are identified that have an influence on resistance, gene-assisted selection can be used and the rate of genetic improvement can be accelerated. By definition, gene-assisted selection is aimed directly at the favourable allele at a gene (Harris, 2007), and is a powerful means of selection for traits with low heritability, that are expensive to study or impossible to measure early in life (Dekkers, 2004). In order to better understand the mite tolerance behaviour of the bees, it is important to identify regions that contain candidate genes (quantitative trait loci, QTL) and conduct gene expression studies to elucidate gene function.

Quantitative trait loci (QTL) studies

A honey bee genetic map revealed a higher rate (19 cM/Mb) of meiotic recombination (Hunt and Page, 1995), which was an order of magnitude higher than in *Drosophila*. This high rate of recombination facilitates QTL mapping. However, mapping studies require a large number of markers (Weinstock et al., 2006).

The *thelytoky* (*Th*) and *complementary sex determination* (*csd*) genes are currently the only known honey bee-specific genes (Moritz et al., 2010). Thelytoky (produce female offspring parthenogenetically) is controlled by a single gene (*th*) and it has been shown that this gene also influences other traits, including egg production, related to the queen phenotype and queen pheromone synthesis (Lattorff et al., 2007). It is important to identify novel genes that control host–pathogen interactions, as many pathogens are highly specific to the honey bee system (Moritz and Evans, 2007). Most traits are controlled by large numbers of genes. Seven suggestive QTLs have been associated with hygienic behaviour (Lapidge et al., 2002). Lobo et al. (2003) sequenced an 81-kb genomic region and found an association between *sting-2* and aggressive behaviour. Oxley et al. (2010) identified three QTLs and candidate genes that influence hygienic behaviour: one locus influences removal behaviour and two loci influence uncapping behaviour. The four other genes were involved in learning olfaction, social behaviour and one gene has an effect on circadian locomotion.

Behrens et al. (2011) described honey bee populations from the island of Gotland, Sweden, that survive mite infections and performed QTL mapping for this trait. Parasitised and non-parasitised drones were separated, and the genome was screened for potential QTLs using a total of 216 microsatellite markers. Three candidate target regions were found on chromosomes 4, 7, and 9, but the strong epistasis among these three loci complicated application in a breeding programme. Tsurada et al. (2012) investigated the genetic architecture of VSH and identified one major QTL on chromosome 9, containing the gene "*no receptor potential A*" and the *dopamine receptor* gene. The latter gene plays a role in vision and olfaction in *Drosophila*. Dopamine signalling has been previously shown to be required for identifying mites within brood cells. Arechavaleta-Velasco et al. (2012) used a QTL mapping approach to identify a single chromosomal region on chromosome 5, and other candidate genes for honey bee mite-grooming behaviour. This region contained only 27 genes including *Atlastin*, *Ataxin* and *Neurexin1*, which have potential neurodevelopmental and behavioural effects.

Genes and proteins impinge on *Varroa* resistance

Honey bees have been considered a model organism for the study of the social dynamics of disease transmission and immunity (Royet et al., 2005). Humoral defences include

proteins, enzymatic pathways and antimicrobial peptides (Schmid-Hempel, 2005), while cellular defences are those mediated by haemocytes such as phagocytosis and encapsulation (Strand, 2008). *A. mellifera* has about 30 % fewer immune system genes compared to dipteran species (Evans et al., 2006). Immune genes that were upregulated in workers subject to *Varroa* parasitizing belonged to the antimicrobial peptide family or were key activators of immune pathways. Certain immune genes do not necessarily play a major role in immune responses, while certain genes from the immune signalling pathways might have different roles (Alaux et al., 2011).

The phenol oxidase (PO) enzyme has been studied mainly in haemolymph. PO is important for defence reactions against microorganisms and parasites; in addition, it plays a role in cuticle pigmentation (Zufelato et al., 2004) and encodes the precursor of the key enzyme in the melanin synthesis pathway (Andersen et al., 1996; Ashida and Brey, 1998). The PO enzyme is known to have an important role in insect defence, and was described as a zymogen (inactive enzyme precursor) (ProPO) in haemolymph and cuticles that can be activated by the serine protease (SP) cascade (Lai-Fook, 1966). Quantification of the prophenoloxidase mRNA levels by qRT-PCR showed increased amounts of transcripts in haemocytes and integument from young pupae injected with 20-hydroxyecdysone (Zufelato et al., 2004). Lourenco et al. (2005) characterised the first proPO (Am-proPO—*A. mellifera* prophenoloxidase) cDNA in *A. mellifera,* a gene that occurs in a single copy. In that study, a higher amount of AmproPO transcripts were found in the whole body of adults and older pupae than in younger pupae. The parasite inhibited an aspect of the host's immunity, which was consistent with suppression of the host's immune system (Alaux et al., 2011). Expression of *AmproPO* was detected in haemocytes and integument, and it has been suggested that AmproPo plays a role in melanisation and differentiation of the exoskeleton in adults (Lourenco et al., 2005).

Varroa destructor adaptively suppresses the immune responses of the bees. Gregory et al. (2005) studied the expression level of antibacterial peptides, such as abacein and defensin. Their results indicated that the expression levels for these two peptides changed non-linearly with respect to the number of mites parasitizing the host. Defensin acts by penetrating pathogenic cells through the cytoplasmatic membrane (Cociancich et al., 1993).

Individual, colony-level or social immunity occurs in social insects (Cremer et al., 2007). Individuals have an innate immune system that has specific memory (Kurtz, 2005). The antimicrobial *defensin1 and 2* genes (Klaudiny et al., 2005) are inducible components of the insect immune system that may also play a role in limiting the development of parasites (Lowenberger et al., 1999). Sequences of the two *defensin* genes revealed their different structure. The defensin protein encoded by *defensin1* gene, which contributes to social immunity (Klaudiny et al., 2005), can be detected in the haemolymph of workers infected by bacteria. *Defensin1* mRNA was detected in hypopharyngeal, mandibular and salivary glands (Ilyasov et al., 2012). The *defensin2* gene, which encodes a novel honey bee defensin (Klaudiny et al., 2005), was found to be responsible for individual immunity (Ilyasov et al., 2012). Richard et al. (2008) showed that *defensin2* was upregulated after treatment with Bacterial Coat Lipopolysaccharide, consistent with a boost in the individual immune system. The expression of the *defensin2* gene increased in the fat bodies in 7-day-old workers after LPS injection. Ilyasov et al. (2012) confirmed that the d*efensin2* gene was expressed in dorsal vessel, fat body, ventral diaphragm, midgut and dorsal diaphragm. Defensins are controlled by the interaction of Toll and IMD signalling pathways as well as antimicrobial action (Ilyasov et al., 2012).

The Serine protease (SP) cascade also plays a major role in insect defence mechanisms, and is activated by ProPO (Ashida and Brey, 1998). Serine protease in the S1 family is involved in various physiological processes, including the defence response (Rawlings and Barrett, 1993). In the genome of *A. mellifera,* 44 SP and 13 serine protease homolog (SPH) genes have been identified. The *A. mellifera* genome includes potential SP inhibitor genes (*serpin-1*, *serpin-2*, *serpin-3*, *serpin-4* and *serpin-5*). Three genes of SP (*proPO*, *spätzle-1*, and *spätzle-2*) are supposedly substrates (Zou et al., 2006). Serine proteases and thioredoxin peroxidase, which probably play a role in the innate immune system of insects, are upregulated in mite-tolerant workers when the colony is infested with mites (Cardoen et al., 2011). A gene expression study has identified 116 genes that regulate in tolerant workers during parasitism by mites. Differences were found in the expression of genes that regulate neuronal development, neuronal sensitivity and olfaction. In addition, parasitism by mites caused changes in the expression of genes related to immunity, cell metabolism and embryonic development. *Varroa*-parasitised worker pupae at the blue-eye stage showed upregulation of the genes *baz* and *CG9520* (non-determined), whereas non-parasitised worker pupae showed upregulation of the genes *Alh* and *Hr78* (Navajas et al., 2008). Genomic responses to pesticides and pathogens at the transcriptome level can be used to explore host–pathogen interactions (Moritz et al., 2010). Some storage proteins are part of the immune system in various arthropods (Hall et al., 1999). One of these storage proteins is the vitellogenin, which is present with significantly lower levels in those worker bees that suffered infestation during the pupae stage, compared the non-infested controls (Amdam et al., 2004). Moreover, Lourenco et al. (2009) demonstrated a downregulation of four

genes encoding storage proteins (vitellogenin, hexamine 70a and two apolipophorins) as a consequence of activation of the immune system.

Fraczek et al. (2009) described the activity of 19 hydrolases in *Varroa* mites and haemolymph of *A. mellifera carnica*. The enzymes were divided into three subclasses: esterase, protease and glycosidase. The activity of all enzymes was measured in the haemolymph of workers, and the relative activity of most was at the same level in hosts and parasites. Zhang et al. (2010) studied gene expression levels in two honey bee species (*A. mellifera* and *A. cerana*) subject to *V. destructor* infection. Two genes were significantly differentially expressed: the *hex 110* gene, which was upregulated in *A. cerana* but downregulated in *A. mellifera*, and the *Npy-r* gene which was downregulated in both species.

Transcript levels for a pathogen recognition gene increased in larvae exposed to *Varroa* mites ($P < 0.001$) (Gregorc et al., 2011). In this study, significantly higher transcript levels were detected in antimicrobial peptides abacein, defensin1 and hymenoptaecin. Bull et al. (2012) demonstrated the age-related activation of specific immune system pathways (Toll). The young adult workers were found to have more upregulated immune genes (abacein, SP and serpins) shortly after eclosion, while the older individuals had more resistant genes expressed to infection. Lee et al. (2013) investigated the effects of heterozygosity on two components of the honey bee innate immune system: encapsulation and PO activity. The positive effects of genetic diversity on parasite and pathogen resistance in *A. mellifera* were not confirmed, because inbred and outbred workers have similar innate immune system.

Non-immune genes have also been implicated in *Varroa* tolerance. For example, the *dopamine receptor gene* has previously been shown to be required for aversive olfactory learning in honey bees, which is probably necessary for identifying mites within brood cells (Tsurada et al., 2012). Vertebrates and invertebrates have two distinct classes of dopamine receptors: D1-like receptors and D2-like receptors (Neve et al., 2004). There are three distinct dopamine receptors in honey bees: two D1-like receptors (AmDOP1 and AmDOP2) (Blenau et al., 1998; Humphries et al., 2003) and one D2-like receptor (AmDOP3). Each receptor is expressed in the mushroom bodies of the brain of adult workers (Beggs et al., 2005). Dopamine or the dopamine receptor antagonist flupentixol was injected into the haemolymph of workers, and was found to produce specific behaviours. Workers were found to spend less time walking and flying, but at the same time showed an increased amount of grooming behaviour (Mustard et al., 2010).

The QTL confidence interval on chromosome 9 contains the gene "*no receptor potential A*" that encodes a phospholipase C that is essential to vision and olfaction, and also plays a role in the recognition of mites within brood cells (Tsurada et al., 2012). Arechavaleta-Velasco et al. (2012) described 27 genes in the workers, including *Atlastin*, *Ataxin* and *Neurexin1* (*A. mellifera Neurexin1—AmNrx1*). Transcription of the *AmNrx1* gene codes presynaptic proteins that are concentrated in the mushroom bodies of the brain and localised in the *groom1* QTL region. The *groom1* QTL region also contains a sequence for the honey bee orthologue of *Neurexin1*, which influences the growth and maturation of synapses in the brain. The *Neurexin1* gene is very important in the self-grooming behaviour in mice, and has been found to play a role in autism–spectrum disorder and schizophrenia in humans. The genes *Atlastin* and *Ataxin* have potential neurodevelopmental and behavioural effects, and homologues have been implicated in neurological disease in humans.

Although the expression of these genes (except genes affecting behaviour) has been correlated with mite infestation, direct evidence that expression affects tolerance has yet to be presented. Further studies on these genes may further elucidate the functional role of these genes and facilitate genetic improvement using gene-assisted selection.

Discussion

The aim of this review is to describe the state of the research investigating the genetic basis of honey bee tolerance to *Varroa* mites and how this information can be used for breeding purposes. There is a strong evidence to show that resistance to *Varroa* is driven by specific mite-directed hygienic behaviour and decrease in fertility of mites.

It is known that *Varroa* can transmit multiple viruses to their host (Webster and Delaplane, 2001), and previous studies have suggested that mite and virus presence are contributing factors to colony collapse disorder (van Engelsdorp et al., 2009). Morphological deformity and death were positively correlated with the increasing number of mites on individual bees. This is showed by the large number of viral particles transmitted by the mites, which caused many infested bees to die before fully fledged (Bowen-Walker et al., 1999).

The identification of candidate genes and elucidation of their function has been performed with QTL analysis (Behrens et al., 2011), gene expression studies (Evans, 2006) and gene knockdown studies (Campbell et al., 2010). The results described in this review highlight the usefulness of these three methods in determining the genetic basis of the honey bee immune response for *Varroa* infection.

We have found specific gene variants that have a major impact on the mechanisms of behavioural resistance to

Varroa mites. These results suggest that it should be possible to select for resistance traits in bees through a simple genotyping assays in the future. In addition, it is important to identify the genes and specific causal mutations to obtain a better understanding on how these affect innate immunity in bees.

Our review also focused on the results of recent gene expression studies investigating honey bee immune responses. In the future, it will be important to integrate this knowledge with the complex social immunity of honeybees. Navajas et al. (2008) suggested that honey bees exhibit differences in gene expression in the parasitised and non-parasitised pupae. In addition, Bull et al. (2012) using a whole genome microarray in individual workers demonstrated that immune genes were upregulated in worker stage when staying in the hive compared to foragers in response to infection. On the contrary, older workers were more resistant to the pathogen than minor workers.

The innate immune system includes the humoral and the cellular reaction. Part of the cellular immune system is melanisation, encapsulation and reaction to phagocytosis. The humoral immune response involves the activation of intracellular signalling pathways and production of antimicrobial peptides (Erler et al., 2011).

Stanimirovic et al. (2010) published the heritability of grooming behaviour: in the three consecutive generations of queens (x, y and z) examined, highly variable heritability ($h^2yx = 0.49 \pm 0.02$; $h^2zx = 0.18 \pm 0.01$; $h^2zy = 0.16 \pm 0.01$) indicated a strong influence of environmental factors. However, a much higher heritability ($h^2 = 0.71$) was reported by Moretto et al. (1993). Arechavaleta-Velasco et al. (2012) identified a specific gene which coded the grooming behaviour and could be used for the creation of *Varroa* tolerance lines. The heritability of VSH in infested brood cells has been reported to be $h^2 = 0.18$, and towards dead brood cells to be $h^2 = 0.36$ (Boecking et al., 2000). The hygienic behaviour represents the main factor for the sensitive breeding of mite-tolerant European honey bees (Spivak and Reuter, 1998). Similar behavioural responses of Africanised and European Carniolan bees could indicate that hygienic behaviour is not a key trait for tolerance against mites (Aumeier et al., 2000). It has also been found that selection for only hygienic behaviour is not enough in honey bee breeding (Stanimirovic et al., 2008). The heritability of suppressing mite reproduction has been estimated at 0.46 (Harbo and Harris, 1999). In a later study, Harbo and Harris (2005) stated that suppression of mite reproduction is of equal importance to VSH, while, according to Behrens et al. (2011), selection for the suppression of mite reproduction breeding is much easier than for other complex behaviours such as VSH. In addition, the suppression of reproductive success of mites is an important tolerance factor in Africanised honey bees (Rosenkranz, 1999).

Our review provides insights into the new molecular methods used for the study of mite-tolerant mechanisms in honey bees. Microarray analysis of differences in gene expression is a powerful tool in the study of genotypic variance in tolerance of bees and host–pathogen interactions. Genome-wide RNA interference (RNAi) (Stroschein-Stevenson et al., 2009) is also an important method for the identification of genes and proteins that could have effect on *Varroa* control (Garbian et al., 2012). New research highlights the importance of different mechanisms in response to *Varroa* parasitism and suggests that a study focused on the brain should be prioritised for the future.

It was concluded that up to date significant knowledge exists on honey bee immune responses, the expression of immune genes and candidate genes that are instrumental in honey bee tolerance. However, the exact function and effects of most candidate genes are still largely unknown. Therefore, to be able use gene-assisted selection as a useful tool, researchers should be focused on genes that affect behaviours which allow honey bees to defend themselves against *Varroa* mites.

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