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# *Varroa destructor* and its impacts on honey bee biology

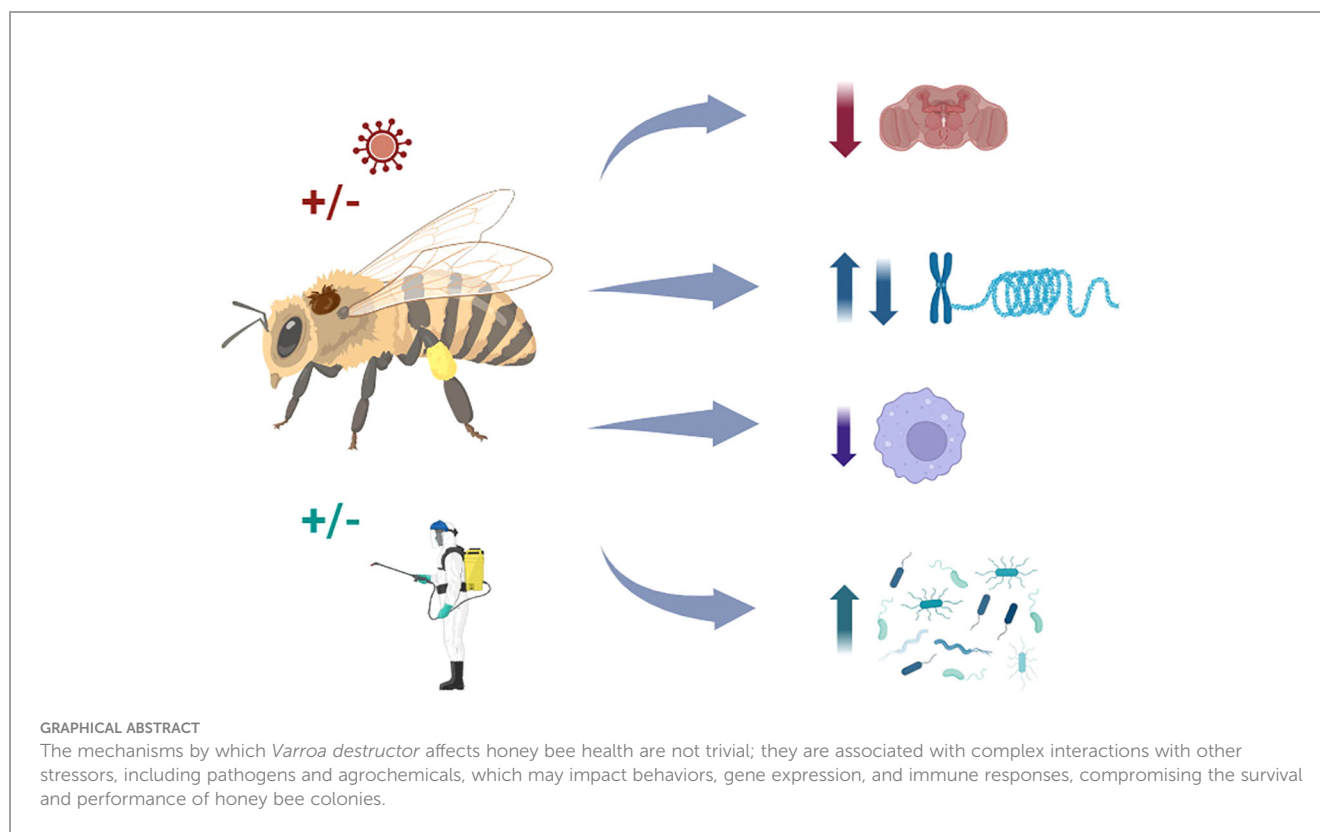
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*Varroa destructor* is considered one of the greatest threats to the health of the honey bee, *A. mellifera*. In recent years, there has been a considerable increase in the number of studies on the mite and its interaction with the bee at a cellular and molecular level. However, these studies have also revealed just how complex the interaction is. A significant factor in the virulence of *V. destructor* is the proteins secreted in its saliva, but only a fraction of these have yet been examined. These proteins can negatively affect the bee's immune system and promote viruses associated with mite parasitism. Initially, studies on parasitized bees concentrated on immune-related genes, but as more genes of the bees have been examined, it is clear that many other aspects of the bee are affected, such as metabolism and neural functioning. Some of those could be responsible for the detrimental changes in certain behaviors of parasitized bees, which compromises the health of the entire colony. Several viruses are associated with *V. destructor* parasitism, but it remains difficult to distinguish the effects of the viruses from those of the mite. Reduced immunity in parasitized bees also opens the possibility of secondary microbial infections, adding complexity to the mite-bee interaction. Further complicating studies is the impact of other factors, like agrochemicals, which can alter how *V. destructor* parasitism affects bee immunity, metabolism, and neural functioning. In addition, differences due to age and sex of the bee being parasitized is a factor that needs to be considered in all studies. While much has been learned in recent years about this complex interaction, the number of unanswered questions only increases.

## KEYWORDS

*Varroa destructor*, varroosis, virus, honey bees, gene expression, behavior, immunity



## 1 Introduction

Among the seven to 11 species of *Apis*, the most important economically managed pollinator worldwide is the western honey bee, *A. mellifera*, followed much less by the Asian honey bee, *A. cerana*, which is primarily found in East Asia (Caron and Connor, 2013). Insect pollination is estimated to increase global food production by 15–30%, which can be up to 6,700% compared to self-pollination (Verma and Partap, 1993; Papa et al., 2022). In the USA, honey bee pollination has a value of approx. 11 billion USD (Khalifa et al., 2021) and approx. €153 billion worldwide (Gallai et al., 2009). Pollination increases plant yield and quality (Papa et al., 2022). Vegetables and fruits are the leading types of crops utilizing insect pollination, followed by edible oil crops, stimulants, nuts, and spices (Gallai et al., 2009). Also, honey bees are essential for pollinating wild plants, helping to maintain biodiversity (Papa et al., 2022).

Starting in the winter of 2006–2007, there has been a notable increase in *A. mellifera* colony losses, particularly in the USA and Europe (vanEngelsdorp et al., 2010). An overwintering loss of colonies of approx. 10% was considered normal, but in the winter of 2006–2007, losses in the USA reached 32% (vanEngelsdorp et al., 2009). Additionally, researchers from other parts of the world, including the Middle East and parts of Asia, reported high rates of colony losses for the same period, in some cases reaching more than 50% (Neumann and Carreck, 2010). More recent losses of colonies have been reported from Spain (36%), Mexico (27.65%), Slovenia (28.9%) (Medina-Flores et al., 2021; Gray et al., 2023), and the USA

(51%) (Bruckner et al., 2023a). These high rates of colony losses have been described as Colony Collapse Disorder (CCD) (vanEngelsdorp et al., 2009). With CCD, most worker bees in a colony disappear and leave behind a queen, plenty of food and a few nurse bees to care for the remaining immature bees and the queen (<https://www.epa.gov/pollinator-protection/colony-collapse-disorder>). CCD is likely due to multiple factors, including arthropod parasites, such as *Varroa destructor* (Gengersch, 2010; Guzman-Novoa et al., 2010), *Acarapis woodi* (Oldroyd, 2007), and *Aethina tumida* (Morawetz et al., 2019), and pathogens, such as RNA viruses, like *Deformed wing virus* (DWV) and *Acute bee paralysis virus* (APBV), microsporidians, such as *Vairimorpha* (*Nosema*) *ceranae*, and bacteria, such as *Mellisococcus plutonius* and *Paenibacillus larvae* (Gengersch, 2010; Hristov et al., 2020). In addition, queen failure (Gengersch, 2010), weak colonies in autumn (Gengersch, 2010; Guzman-Novoa et al., 2010), synthetic acaricides (vanEngelsdorp et al., 2009), deforestation and habitat loss, agrochemicals, and climate change have been reported as contributing factors (Oldroyd, 2007; Khalifa et al., 2021). Among the causes of CCD, *V. destructor* is often cited as the most important. For example, in the winter of 2008–2009 in Canada, *V. destructor* was associated with >85% of colony deaths (Guzman-Novoa et al., 2010) between 2012 and 2015 in the Netherlands, 83% of colonies not treated for *V. destructor* died (van Dooremalen and van Langevelde, 2021), in the winter of 2015–2016 in Austria, losses were 54.6% when *V. destructor* levels were at 30% (Morawetz et al., 2019).

Originally, *Varroa jacobsoni* was described as a brood parasite of *A. mellifera* that had switched host from *A. cerana* (Oldroyd,

1999). However, *V. jacobsoni* was a complex of at least two species, *V. jacobsoni* and a new species, *V. destructor* (Anderson and Trueman, 2000). After switching hosts, *V. destructor* spread relatively rapidly, reaching Europe by the early 1970s, and worldwide by approx. 2000 (Traynor et al., 2020). One reason for its rapid spread is that *A. mellifera* has lower individual behavioral defenses against the mite than its original host, *A. cerana*. These defenses include grooming, hygienic behavior, varroa sensitive hygiene (uncapping and removal of infested brood), entombing of infested brood, and inhibition of mite fertility (Rath, 1999; Traynor et al., 2020; Grindrod and Martin, 2023).

## 2 *Varroa destructor* genetic variants

Both *V. jacobsoni* and *V. destructor* can be divided into several variants. Based on the sequence of the mitochondrial DNA cytochrome oxidase subunit I (*COI/CO-I/COXI*), there were 12 haplotypes of *V. jacobsoni* and four of *V. destructor* in *A. cerana*. In contrast, there were two haplotypes (Korea and Japan-Thailand) of *V. destructor* in *A. mellifera* (Anderson and Trueman, 2000). The Korea haplotype is the most virulent and common worldwide, while the less virulent Japan-Thailand haplotype is limited to Japan, Thailand, French Guyana, Chile, and Brazil (Traynor et al., 2020). However, more haplotypes or sub-haplotypes have been found by analyzing additional mitochondrial sequences. By examining sequence variation in cytochrome B (*CytB*) in addition to *COXI*, Lin et al. (2021) identified two variants for the Korea haplotype in China with *A. mellifera*, and Gajić et al. (2019), found five variants of the Korea haplotype in Serbia with *A. mellifera*. By examining sequence variation in cytochrome c oxidase subunit III (*COX3*), ATP synthase subunit 6 (*ATP6*), *CytB*, and *COXI*, Navajas et al. (2010) found three variants of the Korea haplotype and four variants of the Japan-Thailand haplotype, both in northeast Asia with *A. mellifera*. However, with that same set of genes, Ogihara et al. (2020) did not find any variation within the Korea haplotype in Japan.

The amount of sequence differences between the genes used to define *V. destructor* haplotypes can vary considerably. Navajas et al. (2010) failed to detect any single nucleotide polymorphisms (SNPs) in *CytB* within the Korea haplotype of *V. destructor* parasitizing *A. mellifera* in Asia, and all the variation was detected with *COXI*. For the Korea haplotype in Turkey, the genetic distance between *V. destructor* samples was greatest for *COX3* (0.55%), followed by *CytB* (0.06%), and none for *ATP6* (0.00%) (Koç et al., 2021). For *V. destructor* in Serbia, *COXI* and *CytB* revealed SNPs in 51.8 and 16.3% of the samples, respectively (Gajić et al., 2019). For the Korea haplotype in Japan, Ogihara et al. (2020) found no variation in *ATP6*, *COXIII*, and *CytB* among 15 apiaries. Thus, it appears that *COXI* and *COXIII* are generally better for distinguishing haplotypes/sub-haplotypes. However, an examination of *V. destructor* from Argentina with *COXI*, NADH dehydrogenase subunit 4 (*ND4*), subunit 4 L (*ND4L*), and subunit 5 (*ND5*) showed that the *ND4* was best as it could reveal two Korea sub-haplotypes (Muntaabski et al., 2020). Thus, there may be other

sequences that can provide better differentiation of *V. destructor* haplotypes.

Another approach to determining variation among *V. destructor* is to examine polymorphisms in microsatellites. Analysis of the *V. destructor* transcriptome revealed 27,775 potential microsatellite loci, and among 60 randomly selected microsatellite loci, six were confirmed to be polymorphic in *V. destructor* from five locations in China (Duan et al., 2020). Thus, there are many possible microsatellites in *V. destructor*, but only a fraction may show polymorphisms. For samples of the Korea haplotype from France, Chile, USA, and Philippines, only two of 20 microsatellites revealed variability (Solignac et al., 2005). For samples from Madagascar, three of 11 microsatellites showed polymorphisms, one with two alleles, another with three, and the last one with four alleles (Rasolofoaivao et al., 2017). For samples from China, five of six microsatellites were polymorphic, and four showed polymorphisms with nine to 16 alleles within two Korea sub-haplotypes (Lin et al., 2021). Greater success can be obtained if *V. destructor* microsatellites are chosen that were previously shown to be polymorphic. Dynes et al. (2016) used 10 microsatellites showing polymorphisms in earlier papers and found all showed polymorphism at the apiary and colony level in the USA. The same approach was used to select six microsatellites, all of which were highly polymorphic for Philippine and Vietnam samples belonging to the Korea haplotype (Beaurepaire et al., 2015). However, Strapazon et al. (2009) used some of the same microsatellites as Dynes et al. (2016) and Beaurepaire et al. (2015), and none of the four chosen showed polymorphisms within the Korea or Japan haplotypes in two locations in Brazil.

Genetic variability of *V. destructor* has been compared in a variety of ways, mostly by sequencing mitochondrial genes or microsatellite length polymorphisms. Ultimately, the best approach is full genome sequence comparisons. The genome of *V. destructor* is approx. 565 Mbp, like many other Acari (Cornman et al., 2010). As sequencing technology continues to advance, it should become more feasible to compare complete *V. destructor* genomes (Hasegawa et al., 2021). Even a limited comparison between complete genomes of samples could be useful in identifying better markers, such as sequences with the highest number of SNPs or microsatellites with the most significant length variation. As more markers are developed to distinguish sub-haplotypes, like those within the Korea haplotype, it will also be important to compare their virulence to better understand how that varies within a haplotype. Also, one factor that has not been considered much in these studies is sampling time. It appears that mite population can shift considerably over time. The number of alleles varied over a brood season in Germany when examined with seven microsatellites (Beaurepaire et al., 2015).

## 3 *Varroa destructor* life cycle and virulence

The life cycle of *V. destructor* can be divided into two phases, the dispersal or phoretic phase and the reproductive phase. In the

phoretic phase, *V. destructor* females adhere and parasitize adult bees, which enable them to be transported to worker or drone brood cells where they reproduce, and to spread between colonies on parasitized robber or drifting bees (Boecking and Genersch, 2008). The reproductive phase involves *V. destructor* females entering cells of bee larvae before they are capped (5-day-old instar bee larvae). Once inside the cell, the female mite hides underneath the larva's food until the cell is capped (De Jong et al., 1982). Drone cells appear to be preferred as they were invaded 11.6 times more frequently than worker cells (Boot et al., 1995). During the molt of the bee prepupa (48 h after cell capping), the mother mite, also known as the foundress mite, prepares a feeding site for her daughters on the bee pupa by puncturing the prepupa's cuticle at the sternite of the second abdominal segment of drone pupae or in the mesothorax of worker pupae. Following penetration by the mite's toothed chelicerae and serrated-edged corniculi to form a channel in the bee's cuticle, a pharyngeal pump is used to feed on the bee's internal fluids at 4.5 cycles per second with each feeding event lasting approx. 10 seconds separated by approx. 2 minutes (Li et al., 2019). Bee fluid is extracted from the feeding site by the foundress mite and her progeny throughout the bee's pupal phase (Kanbar and Engels, 2003). Also, the foundress mite establishes a fecal accumulation site on the wall of the cell, where the mating of her offspring also takes place (Donzé and Guerin, 1994).

Approximately 60 to 70 h after the cell is capped, the foundress lays her first egg, which is unfertilized and develops into a haploid male (Rehm and Ritter, 1989). After this, the foundress lays 2 to 5 fertilized eggs at about 30 h intervals between them, and these eggs become females. The developmental period of *V. destructor* from egg to adult is 5.8 days for females and 6.6 days for males. Mating starts approximately 230–280 h post cell capping with female mites reaching sexual maturity 10 to 20 h after the males (Donzé and Guerin, 1994). Males fertilize a sister or sexually mature female mite from a different foundress mite, and the female stores the semen in her spermatheca. A female has to mate at least four times to obtain sufficient spermatozoa to achieve 1.6 to 1.7 reproductive cycles (Ifantidis, 1983). A male can fertilize an average of 3.75 females in a worker cell but 7.5 females in a drone cell since the metamorphosis period is longer in drone cells (Donzé et al., 1996). When workers or drones hatch from their cells, *V. destructor* female progeny leave the cells attached to their hosts. In contrast, progeny males remain in the cell where they starve to death since their mouthparts (chelicerae) are modified to perform sperm transportation into the female receptacle (oviduct II) and thus are unable to feed (Alberti and Hänel, 1986).

A key element of the virulence of *V. destructor* to bees is its saliva. Typically, a damaged cuticle and epidermis of an insect will heal preventing loss of hemolymph, but puncture wounds caused by *V. destructor* remain open (Kanbar and Engels, 2003). There were at least 15 different proteins detected by SDS-PAGE and electroblotting in the saliva of *V. destructor*, and when incubated with hemocytes of the caterpillar, *Lacanobia oleracea*, the saliva damaged the hemocytes and suppressed their ability to extend pseudopods and form aggregates, which are needed for wound healing (Richards et al., 2011). However, a much greater number (356 proteins) were detected in the mite saliva by SDS-PAGE, gel

sectioning and nano-liquid chromatography coupled to mass spectrometry (Zhang and Han, 2019). Saliva was toxic to the larvae of *A. cerana* workers, and some of the proteins in it were potential virulence factors, such as lysophospholipase for membrane destabilization, antimicrobial factors, such as lysozyme for bacterial cell wall degradation, nutrient utilization factors, such as dipeptidyl peptidase III for ingesting host erythrocytes, antioxidant/oxidation–reduction factors, such as thioredoxin peroxidase for hydrogen peroxide breakdown, and detoxification factors, such as sulfotransferase for xenobiotic elimination. Another protein in the mite's saliva is a chitinase (Vd-CHI<sub>sal</sub>) related to chitinases of parasitic arthropods (Becchimanzi et al., 2020). The gene encoding it was highly expressed in mite salivary glands and silencing it reduced mite survival. It may be essential for hydrolysis of the bee's chitin keeping the wound site open and anti-microbial activity attacking the cell wall of any opportunistic bacteria near the wound site. Enzyme activities of cholinesterases, carboxylesterases and phosphatases were also detected in the secretion products of *V. destructor*, presumably in the saliva, that could enhance virulence by causing cell lysis and degrading bee tissues (Dmitryjuk et al., 2014). Perhaps the clearest example of a saliva protein being involved in mite virulence is Varroa toxic protein, which is lethal to *A. cerana* worker larvae and pupae, but not *A. mellifera* worker larvae and pupae. Also it was not toxic to *A. cerana* worker adults and drones (Zhang and Han, 2018). Considering the significant number of proteins, in the saliva, it will be important to conduct more studies involving gene silencing to assess their importance.

Much of the literature about factors secreted by *V. destructor* is related to honey bee-associated viruses. In *V. destructor*, peptides were detected from *Varroa destructor Macula-like virus* (VdMLV), DWV and ABPV (Erban et al., 2015). Lack of detection of non-structural proteins compared with high amounts of structural proteins suggested that the viruses did not replicate in the mite but more likely accumulated in the mite gut from hemolymph feeding. Santillan-Galicia et al. (2008) also did not find that DWV replicated in *V. destructor* since there was no specific antibody binding to DWV in any mite tissues, and DWV was only detected in the mite's midgut lumen in structures resembling fecal pellets. However, the variant DWV-B was able to infect the mite's intestinal epithelium and salivary glands, while variant DWV-A could not infect the mite (Gisder and Genersch, 2021). DWV was detected among the mite's saliva proteins, but other viruses, APBV, *Black queen cell virus* (BQCV), *Chronic bee paralysis virus* (CBPV), *Israeli acute paralysis virus* (IAPV) and *Sacbrood virus* (SBV), were not (Zhang and Han, 2019). However, another study reported SBV and *Kashmir bee virus* (KBV) in mite saliva (Shen et al., 2005). Therefore, at least DWV-B, and perhaps other viruses, can be transmitted in mite saliva.

In addition to transmission in saliva, DWV in bees can be affected by *V. destructor* parasitism. Mite parasitized brood can have overt DWV infections with deformed wings, general paralysis, discoloration, and bloated abdomen compared to covert DWV infections where none of those symptoms appear (Martin and Brettell, 2019). In workers, DWV was found in the thorax and abdomen, but not the head with covert infections, whereas the virus was spread throughout the body in overt infections at elevated levels

(Yue and Genersch, 2005). The saliva of *V. destructor* may be a factor in triggering overt infections as it stimulated the appearance of deformed wings in *A. mellifera* adults in the presence of DWV (Zhang and Han, 2019). Mite parasitism suppressed bee immunity based on reduced expression of genes for antimicrobial peptides and immunity-related enzymes allowing for increased DWV replication (Yang and Cox-Foster, 2005). One explanation for this could be Varroa toxic protein in the saliva, which was not toxic to *A. mellifera*, but increased DWV levels and the subsequent development of deformed-wing symptoms in adults (Zhang and Han, 2018). Another explanation is that *V. destructor* suppresses the immune systems by feeding on the fat body of the bee mechanically removing a tissue that is responsible for immune responses (Galbraith et al., 2015; Ramsey et al., 2019). Mite reproduction was also increased by DWV infection as the virus adversely impacted the bee's immune responses by interfering with NF- $\kappa$ B signaling (Di Prisco et al., 2016). However, proteome changes in the bee during interactions with *V. destructor* and DWV showed that they were mostly due to the mite rather than the virus (Erban et al., 2019). The effects of the mite and DWV were both cooperative and antagonistic. Opposite effects included the mite and DWV activating and suppressing NF- $\kappa$ B signaling, respectively, while cooperative effects included both the mite and DWV increasing p53-induced apoptosis, hyperactivation of the JAK/STAT pathway and disruption of p53-BCL-6 feedback. Another way that *V. destructor* could affect viruses in bees is through hemolymph removal during feeding as loss of increasing volumes of hemolymph from bees increased DWV densities by destabilizing viral immune control (Annoscia et al., 2019). Hemolymph removal favors the extraction of antiviral molecules, triggering greater viral replication. While some of the studies do conflict, it is clear that the relationship between the virulence of the mite and honey bee-associated viruses is more driven by the mite and that possibly both mite saliva proteins and physical removal of hemolymph are involved.

#### 4 Individual honey bee damage by mite parasitism

The above review of the life cycle and virulence of *V. destructor* shows that it is highly damaging to bees both directly and indirectly. Effects on individual bees include decreased body weight, lifespan, water content, immune gene expression, neural processes, learning and behaviors, such as flying and orientation (Noël et al., 2020). One of the most common measurements in studies of the effects of the mite on individual bees is the bee's weight, and thus can be compared between multiple studies. For newly emerged bees without deformed wing symptoms, Bowen-Walker and Gunn (2001) found that non-parasitized bees weighed 5% more than parasitized bees inoculated with one mite per capped brood, but this increased to 7% more than bees with three mites per capped brood. For newly emerged bees with deformed wing symptoms, the impact was greater with 12% more weight for non-parasitized bees than those with one mite per capped brood, and 17% more than those with three mites per capped brood. Similar results were obtained by

Yang and Cox-Foster (2007) where newly emerged non-parasitized bees weighed 5 and 8% more than those with one or three mites per capped brood, respectively, without deformed wing symptoms, and 11, 21 and 27% more with one, three or 10 mites per capped brood with deformed wing symptoms. Annoscia et al. (2012) results without deformed wing symptoms were similar with non-parasitized newly emerged bees weighing 8 and 12% more than those with one or three mites per capped brood, respectively. However, with deformed wing symptoms, the weight of non-parasitized newly emerged bees was 4% and 13% higher than those with one or three mites per capped brood, respectively, indicating that deformed wing symptoms did not greatly affect weight reductions. Other studies have only examined parasitized bee weight without deformed wing symptoms. These include van Dooremalen et al. (2013) with non-parasitized bees at seven days of post emergence having 5% more weight than those with one or two mites per capped brood; Strauss et al. (2016) with non-parasitized bees at emergence having 7 and 15% more weight than those with one or with two to three mites per capped brood, respectively; Morfin et al. (2020a) with non-parasitized bees at emergence having 8% more weight than those with one mite per capped brood; and Yang et al. (2021) with non-parasitized bees at emergence having 17% greater weight than those with two mites per capped brood. A summary of studies of the effect of *V. destructor* parasitism of brood on weight of the emerged adult indicates that weight loss always increased with more mites per capped brood, which was relatively consistent, mostly with non-parasitized bees being 5-8% heavier than those with one mite and 15-20% than those with two to three mites. However, more studies are needed to compare the effect of DWV infections on weight loss, particularly by quantifying the amount of DWV. In contrast to brood parasitism, only Morfin et al. (2020b) examined weight loss where mites were placed on adults, showing that non-parasitized bees weighed 7% more than those with one mite per adult bee at 21 days of parasitism with no deformed wing symptoms.

Another factor commonly measured for the effect of *V. destructor* on individual bees is bee survival, and thus can be compared between studies. The median lifespan of bees without deformed wing symptoms after emergence was 13.6 d and 8.9 d with one or two mites per brood, respectively, compared to 27.6 d for non-parasitized bees (De Jong and De Jong, 1983). The time of 75% cumulative dead after emergence with one mite per capped brood was 18.5 d without deformed wing symptoms and 1.1 d with deformed wing symptoms compared to 20.7 d for non-parasitized bees (Yang and Cox-Foster, 2007). The average lifespan of emerged bees with one and three mites per capped brood was 16 or 8.5 d, respectively, without deformed wing symptoms, and 7.5 or 4 d, respectively, with deformed wing symptoms compared to 19 d for non-parasitized bees (Annoscia et al., 2012). The average lifespan of emerged bees with two mites per capped brood was 8.5 d without deformed wing symptoms compared to 14.4 d for non-parasitized bees (Reyes-Quintana et al., 2019). Similar to studies on weight, there has been little examination of the effects of mite parasitism on adult bee lifespans. Morfin et al. (2020b) did not calculate the average lifespan but showed that 20% of adult bees with one mite per bee survived by 21 days compared to 83% for non-parasitized

adult bees. All these studies show that mite parasitism of brood reduced the lifespan of the bee with much greater effects with DWV symptoms, although the effect on adults appears much more limited. Also, the degree of impact of *V. destructor* parasitism on bee lifespan is much greater than that on weight.

In the above studies, weight reductions due to mite parasitism was hypothesized to be due to hemolymph loss (Bowen-Walker and Gunn, 2001; Annoscia et al., 2012; Morfin et al., 2020a), fat body consumption (Morfin et al., 2020a), DWV symptoms (Bowen-Walker and Gunn, 2001; Morfin et al., 2020a), reduced bee water content (Annoscia et al., 2012; Strauss et al., 2016), and reduced bee protein content (van Dooremalen et al., 2013). Reduced lifespan due to mite parasitism were proposed to be due to piercing of cuticle and membranes (Morfin et al., 2020a), suppression of bee immunity (Yang and Cox-Foster, 2007; Reyes-Quintana et al., 2019; Morfin et al., 2020b), up-regulation of genes associated with cardiac pathology, and down-regulation of genes associated with glycolysis/glucogenesis (Morfin et al., 2020b), and DWV symptoms resulting in bees being unable to feed themselves (Yang and Cox-Foster, 2007; Annoscia et al., 2012; Reyes-Quintana et al., 2019).

## 5 Impacts of *V. destructor* on haemocytes and cellular immunity

Hemolymph is the circulating fluid of insects, composed mostly of water (approx. 20-50% of total water in an insect), but it also contains a variety of chemicals, such as inorganic compounds, low molecular weight organic compounds and proteins, as well as circulating cells called hemocytes (Kanost, 2009). All of these are important for the proper functioning of an insect's metabolism, such as ions to help maintain pH, trehalose as an energy source, glycerol, and sorbitol to lower the freezing point for cold damage protection, and proteins to maintain osmotic pressure, transport lipids, provide food storage, and contribute to the immune response. Also, hemolymph transports nutrients from the digestive system, and removes cellular wastes (Caron and Connor, 2013). The physical loss of hemolymph during *V. destructor* parasitism could negatively affect any of these functions to varying extents. For example, body water content of bees was negatively correlated with the degree of mite parasitism (Bowen-Walker and Gunn, 2001), and total hemolymph basic proteins decreased 34% with one to three mites and 56% with four to six mites per larva (Glinski and Jarosz, 1984).

Insect hemocytes are divided into plasmatocytes, granulocytes and lamellocytes based on their morphology/presumed function with plasmatocytes (a pleiomorphic hemocyte) being the most abundant (Ling and Yu, 2006; Marmaras and Lampropoulou, 2009). Plasmatocytes, granulocytes and lamellocytes are part of the immune response involved in phagocytosis of microbes, nodule formation where multiple hemocytes aggregate to trap microbes, encapsulation where hemocytes attach to the surface of a larger parasite and form a multilayered capsule killing the parasite. In addition, plasmatocytes aggregate to seal wounds preventing hemolymph loss, and granulocytes synthesize an extracellular

matrix that covers tissues exposed to the hemolymph (Strand, 2008). Newly emerged bees with *V. destructor* had up to 90% reduction in the number of normal hemocytes compared to the non-parasitized control, but by 8 to 25 days post-emergence, there were no significance differences from the non-parasitized control (Amdam et al., 2004). Another study of newly emerged bees with *V. destructor* showed approx. 25% reduced hemocyte concentration when exposed to one mite per capped pupa (Morfin et al., 2020a). For nurse bees from colonies parasitized with *V. destructor*, there was an approx. 33% decrease in hemocyte concentration (Belaïd and Doumandji, 2010). For adult worker bees in cages with *V. destructor*, there was an approx. 50% reduction in total hemocyte concentrations by 12 hours of parasitism, although the numbers recovered by 24 and 48 hours (Koleoglu et al., 2018). For drones taken from infested colonies, hemocyte concentrations were decreased by 47%, 13% and 65% for infested larvae, pupae, and adults, respectively (Salem et al., 2006). While all these studies show that parasitism reduces hemocytes in bees, there is a considerable range in the level of reductions, and evidence that the reductions can be transient.

Reductions in hemocyte concentrations by *V. destructor* parasitism as well as down-regulated immune gene expression (Yang and Cox-Foster, 2005; Erban et al., 2019) may help explain the ability of pathogens or potential pathogens to invade the hemolymph of mite parasitized bees. The pathogen, *V. ceranae*, is normally restricted to the bee's digestive system, but its DNA was found in 68% of the hemolymph samples of bees parasitized by *V. destructor* (Glavinić et al., 2014). *Serratia marcescens* was found in the hemolymph of more than 90% of dying worker bees in winter hives and about half of the *V. destructor* in those colonies (Burritt et al., 2016). The bacterium was not generally found on the surface or digestive tract of dying bees, and it was not found in healthy bees. Sequencing the genome showed that this was a novel strain with unique genes related to those of certain bacterial insect pathogens, and there was evidence that the bacterium could be reducing hemocyte populations and permeabilizing some bee organ membranes. Varroa toxic protein could be involved as injection of it into *A. mellifera* larvae resulted in the isolation of *Stenotrophomonas maltophilia* and *Staphylococcus aureus*, while bacterial colonies were not detected from the larval hemolymph with injection only (Balakrishnan et al., 2021). The authors suggested that those species of bacteria were present in the hemolymph of healthy larvae at undetectable levels and Varroa toxic protein stimulated their growth. While none of these studies have shown that the microbes detected are causing diseases in the hemolymph, they do indicate that microbes that normally would be controlled by the bee's cellular immunity now have the opportunity to proliferate and spread in the hemolymph during *V. destructor* parasitism.

## 6 Impacts of *V. destructor* on honey bee tissues

While it is generally assumed that mite uptake of hemolymph was related to consumption of hemocytes as their numbers declined with parasitism, there is evidence that fat body cells are fed upon.

The mite preferred to feed on the ventral rather than the dorsal region of the bee's metasoma providing access to the fat body, and degraded fat body cells were found directly underneath the intersegmental membrane at the feeding site, likely a result of extra-oral digestion by the mite (Ramsey et al., 2018; Ramsey et al., 2019). Marking hemolymph and fat body in honey bees with different fluorescent stains revealed that the guts of mites contained the stain fluorescence of the fat body rather than the hemolymph (Ramsey et al., 2019). Also, mites feeding on the fat body lived longer and produced more eggs than those feeding on the hemolymph. All of this indicated that fat body cells were being fed upon rather than hemolymph following extra-oral digestion by the mite creating dissolved semisolid fat body tissue in the bee. Ramsey et al. (2019) proposed that the diverse symptoms of *V. destructor* parasitism would be consistent with the loss of fat body tissue during feeding as the bee's fat body is important for immunity, detoxification, nutrient storage, and other functions.

There are other bee organs affected by mite feeding, such as the hypopharyngeal gland. The size of the hypopharyngeal gland in parasitized nurse bees was reduced with fewer vacuoles suggesting decreased secretion by the gland, which could be detrimental to brood development and queen nutrition (Pinto et al., 2011). Similarly, newly emerged bees with mite parasitism of the brood showed reduced hypopharyngeal gland size, which could result in bees with less secretion of royal jelly and brood food as well as less secretion of antimicrobial enzymes reducing immunity (Bruckner et al., 2023b). Pinto et al. (2011) proposed that reduced hypopharyngeal gland size was due to direct parasitism and viral infections. Other evidence that DWV may be involved was the smaller hypopharyngeal glands of bees with deformed wings that also had few small vacuoles but many eosinophilic granules, suggesting increased production of serous secretion, more typical of foragers (Power et al., 2021). Power et al. (2021) proposed that this was an impact of DWV on the gland in both symptomatic as well as asymptomatic bees.

Other organs affected by mite parasitism are the mandibular glands and smaller reservoir glands (Zakaria and Abd El-Wahab, 2004), antennal sense organs (Abd El-Wahab et al., 2006), and flight muscles and mid-gut (Power et al., 2021). Mandibular and smaller reservoir glands were smaller in newly emerged bees with mite parasitism and more severe effects were observed with deformed bees, which could be related to inadequate protein levels during development (Zakaria and Abd El-Wahab, 2004). Mandibular glands are needed to produce pheromones for communication among colony members, and so reduced function would negatively affect the entire colony (Zakaria and Abd El-Wahab, 2004). Mite parasitized bees also have fewer sensilla trichodea and smaller antennal flagellum (Abd El-Wahab et al., 2006). Such a reduction in the antennal sense organs could adversely affect the bee's behavior with a reduced sense of touch, smell and taste. In addition, mite parasitized bees showed an incomplete development of flight muscles and an inflammation of the midgut and hemocele (Power et al., 2021). There were increased numbers of inflammatory cells (plasmacytes and granulocytes) and the accumulation of melanin between the midgut villi and the hemocele. These changes were observed in symptomatic and asymptomatic bees infected with

DWV. While ABPV is typically associated with DWV in bees and was also found in all samples with altered morphology, it was believed to be less likely responsible as ABPV does not trigger an immune response in the bee. The inflammation of the midgut could be related to reduced immunity against microbial infections, consistent with the finding of *N. ceranae* in the hemolymph samples of parasitized bees (Glavinić et al., 2014).

Finally, the brain of the bee appears to be negatively affected with *V. destructor* parasitism. The size of the total brain and specific brain regions were reduced by approx. 13% in workers that developed from mite parasitized brood (Lucas et al., 2006). Mite parasitism also dysregulated many brain metabolites, including fatty acids, amino acids, carboxylic acid, and phospholipids, with the greatest changes in linoleic acid, propanoate, glycine, serine, and threonine metabolism (Wu et al., 2017). The result would be reduced brain function negatively affecting the processing of sensory inputs as well as outputs like bee behaviors, which would adversely impact both individuals and colonies. Other evidence for reduced brain function is altered gene expression of parasitized capped brood related to decreased dopamine production and suppression of the prevention of neural degeneration, which could result in greater neuronal apoptosis during aging causing cognitive impairment (Navajas et al., 2008). Mite parasitism of adult bees also down-regulated expression of several neural genes indicating disruption of synaptic function and reduced ability to counteract neurodegeneration (Morfin et al., 2020b; Morfin et al., 2020c). These negative changes in the bee brain during mite parasitism may contribute to the behavioral changes in parasitized bees reviewed below.

## 7 Impacts of *V. destructor* on immune gene expression

One of the highest impacts of *V. destructor* on individual bees is the effect on the humoral immune system. Activation of humoral immunity relies on changes in protein levels regulated by intracellular signaling pathways (Morfin et al., 2021). These signaling pathways include Toll, Immune deficiency (Imd), Janus kinase-signal transducer and activator of transcription (JAK/STAT), and c-Jun N-terminal kinase (JNK). The activation of any of these pathways culminates in the synthesis of antimicrobial compounds, mainly antimicrobial peptides (AMP), defensive enzymes, and complement-like proteins (Morfin et al., 2021).

Several studies have found differential immune gene expression in developing and adult honey bees with mite parasitism, either showing or not showing symptoms of viral infection. For example, Yang and Cox-Foster (2005) found differences in gene expression between bees with no signs of wing deformity and no mites, mite-parasitized bees with normal wing development, and mite-parasitized bees with deformed wings due to DWV infection. When these bees were challenged with *Escherichia coli*, immunosuppression was detected by the downregulation of *hymenoptaecin*, which was not observed in non-parasitized control bees (Supplementary Table 1). Navajas et al. (2008) compared bee gene expression from colonies that were

presumably susceptible or resistant to *V. destructor* and reported differences in transcriptomic profiles as a response to *V. destructor* parasitism during the pupal stage. Of the differentially expressed genes that were identified, 15 genes were upregulated and 17 were downregulated. The differentially expressed genes were linked to biological pathways related to embryonic development (perhaps explaining wing deformity), cell metabolism, and immune responses. Another study also found that *V. destructor* parasitism affected the expression of bee immune-related genes, including the downregulation of *defensin* and *spaetzle* in parasitized honey bee pupae (Khongphinitbunjong et al., 2015). Defensin and hymenoptaecin are AMPs synthesized after the activation of the Toll pathway by the protein *spaetzle* (Rolf and Reynolds, 2009), which suggests that all the proteins regulated by the Toll pathway are suppressed by the mite during bee development. However, in adult bees, Abbo et al. (2017) found an up-regulatory effect of mite parasitism on the humoral immunity genes, *defensin* and *hymenoptaecin*, and the cellular immunity gene, *eater*, indicating that the effects may be quite different between parasitism of developing and adult bees. They also reported a positive correlation between the expression of *defensin* and *hymenoptaecin* with levels of varroa mite parasitism. Barroso-Arévalo et al. (2019) found a negative correlation between mite loads and the downregulation of the defense gene *defensin* and the development gene *dorsal*, both related to the Toll pathway, but a positive correlation with the up-regulation of the humoral immunity regulator gene, *relish*. However, Gregory et al. (2005) found that pupae parasitized with one to four mites had lower expression levels of the immunity genes, *abaecin* and *defensin*, compared to non-parasitized pupae or pupae parasitized with five to six mites, suggesting a non-linear expression pattern with respect to the number of mites parasitizing the bees. However, not all studies have reported immunosuppression during parasitism. Kuster et al. (2014) found immunostimulatory effects of varroa parasitism in developing bees based on the expression of 10 immune related genes (including *defensin-2* and *hymenoptaecin*) at 24, 72, 120, 192, and 240-hours post cell capping (hpc). However, the upregulation of immune-related genes only occurred at some hpc and with  $\geq 3$  mites per cell, indicating an inconsistent effect of *V. destructor* on immune responses in developing bees. Their study found that experimental wounding also increased the expression of immune genes, suggesting that the mechanical injury caused by the mite's feeding is related to changes in immune gene expression. Likewise, Koleoglu et al. (2017) found that the effect of *V. destructor* parasitism and the injection of a saline buffer had similar effects, but in this case resulting in downregulation of the immune-related genes *hymenoptaecin* and *defensin*, in developing and adult honey bees, suggesting that the effects of the mite on immune responses could be, at least in part, related to the wounds caused by the mite or the needle used to inject the buffer. In contrast to all these studies, Aronstein et al. (2012) found no significant effects of the mite on the expression of the immune genes *defensin-1*, *abaecin*, and *hymenoptaecin* studied in parasitized bees at different stages of development.

In addition to cellular immune responses mediated by hemocytes that circulate in the hemolymph of bees, there are a

number of genes related to cellular immunity (Morfin et al., 2021). Besides a reduction in hemocyte numbers, Koleoglu et al. (2018) found that the mite caused a downregulation of *AmPPO*, a prophenoloxidase gene whose expression was directly correlated with hemocyte count. Morfin et al. (2020a) found that a reduction of uncharacterized hemocytes in newly emerged bees parasitized with *V. destructor* during their pupal stage occurred along with 21 upregulated and 45 downregulated differentially expressed genes, that included biological pathways linked to leucocyte transendothelial migration and phagosomes, linking the effect of *V. destructor* on cellular immune responses. Furthermore, Zaobidna et al. (2015) found mostly down-regulatory effects of *V. destructor* parasitism on the expression of *proPO*, and a reduced enzymatic activity of prophenoloxidase in most of the developmental stages of parasitized honey bee workers and drones. The cleavage of prophenoloxidase by serine proteases leads to activation of phenoloxidase, which in turn allows the production of melanin. Melanin and the reactive oxygen and nitrogen species formed during its synthesis are toxic to parasites, bacteria, fungi, and viruses (Morfin et al., 2021). Thus, not only are hemocyte numbers reduced in response to *V. destructor* parasitism, gene expression and enzyme activities related to them are also suppressed. However, it is not yet clear if such reductions are simply a result of fewer hemocytes or whether haemocytes are not functioning normally. Studies on the effects of *V. destructor* on hemocytes need to identify gene expression in the different types of hemocytes as well.

Although it is evident that *V. destructor* alters gene expression in honey bees, there are clearly inconsistencies among the above studies in the way *V. destructor* dysregulates the expression of immune related genes. Differences between the studies could be related to the experimental setup, developing versus adult bees, or the levels of other stressors, like viruses. For example, Zaobidna et al. (2017) observed effects on 14 genes related to the Toll pathway in parasitized workers and drones during different developmental stages, finding significantly increased expression of 10 of the 14 genes, including *defensin-1* and *defensin-2*, in larvae, with a slower increase in drones. However, expression was silenced in later life stages showing that the impact of mite parasitism on bee gene expression was sex and life-stage specific.

## 8 Honey bee transcriptomic responses to *V. destructor*

The effect of mite parasitism on honey bee gene expression in most of the studies above have been limited to three to 14 genes, but transcriptomic studies have allowed the examination of a much broader range of genes shedding light on the complex effects of *V. destructor* on honey bee health. For example, Doublet et al. (2017) identified 88 upregulated genes and 79 downregulated genes in bees parasitized by *V. destructor*, and a gene ontology (GO) analysis identified nutrient reservoir activity linked to the down-regulated genes. Differentially expressed immune related genes identified by Doublet et al. (2017) included *iap2*, *rel*, *tube*, and *defensin-2*, which are part of the Imd and Toll immunity pathways. The involvement



of the aforementioned genes in anti-bacterial and anti-fungal mechanisms has been well defined, but most recently their activation in response to viral infections has also been described (McMenamin et al., 2018). However, Doublet et al. (2017) failed to identify transcripts related to the antiviral defense mechanism, RNAi, and as discussed by the authors, this was possibly due to the transient nature of the transcripts and the technical inability of RNA sequencing to detect them. However, they found that *vitellogenin* (*Vg*) was down-regulated and *malvolio* (*Mvl*) was up-regulated. These genes are involved in behavioral division of labor, indicating a potential effect of *V. destructor* on behavioral social responses (Alaux et al., 2012; Salmela and Sundström, 2018). Similarly, Amdam et al. (2012) showed lower titres of *Vg* protein in parasitized worker bees infested during their pupal stage that could be affecting the onset of foraging behavior in parasitized bees. Zanni et al. (2017) identified 1333 differentially expressed genes in honey bees highly parasitized by *V. destructor* in field and laboratory experiments, and apart from observing higher DWV levels in parasitized bees, they identified dysregulated genes linked to stress responses, immune responses, nervous system function, metabolism, and behavioral maturation. Dysregulated expression of immune genes included up-regulation of *PGRP-2* and *hymenoptaecin* and down-regulation of *UNC93*, and up-regulation of the neural genes for nicotinic acetylcholine receptors (*nAChRa9* and *nAChRb2*), indicating a possible impact of the mite on both immunity and behavioral immune responses (discussed in section 11). Using a combined Omics approach (transcriptomics, proteomic, metabolomic, and functional analysis), Kunc et al. (2023) showed an activation of immune responses and sphingolipid metabolism in parasitized 10-day old worker bees, but an inhibition of olfactory recognition and oxidative stress. Their metabolome analysis indicated a decrease in nutrients and energy stores in parasitized bees, which agrees with the mite's feeding behavior of consuming primarily fat body but also hemolymph (Annoscia et al., 2019; Ramsey et al., 2019). The up-regulated immune response genes by *V. destructor* identified by Kunc et al. (2023) included *apidaecin 1*, *abaecin*, *hymenoptaecin*, *defensin 1*, *RISC-loading complex subunit TARBP2*, *dicer*, *argonaute-2*, *peptidoglycan recognition protein S2*, and *beta-1,3 glucan binding protein*. However, no down-regulated genes linked to immune response. Down-regulated genes were involved in the maintenance of structural integrity and cell development and included *cell wall integrity* and *stress response component 1*, *collagen alpha-2(IV) chain like*, *tubulin alpha chain*, and the detoxification genes *cytochrome P450 6A1* and *cytochrome P450 9e2*. Other down-regulated genes were related to metabolic processes including *pancreatic triacylglycerol lipase-like*, *glucose dehydrogenase*, and *major royal jelly protein 6*, and sensory recognition, such as *carotenoid isomeroxygenase* and *chemosensory protein 6*. Thus, a quite broad range of impacts were observed. These studies showed the complex effects of *V. destructor* on different biological pathways (immune and metabolic pathways) that could be interconnected.

Although *V. destructor* has been considered the main culprit of honey bee colony mortality in the last three decades, most of the conclusions regarding the effect of the mite on honey bee immune

responses are based on the expression of a few genes used as molecular markers (Supplementary Table 1). However, this has started to change as more studies have examined the effect of the mite on the immunity and other biological pathways using high throughput transcriptomic, proteomic, and metabolomic studies (Duay et al., 2002; Navajas et al., 2008; Zanni et al., 2017; Morfin et al., 2019; Morfin et al., 2020a; Morfin et al., 2020b; Kunc et al., 2023). Nevertheless, it is apparent that the response of bees differs based on sex and age (Zaobidna et al., 2017), and many of the studies so far conducted have focused on worker bees, but little is known about the effect of *V. destructor* parasitism on immune mechanisms of queens and drones (Supplementary Table 1). Moreover, studies on relationships between *V. destructor* parasitism, energetic cost of immune responses, and consequences to other metabolic pathways (like neural processes and reproduction) are warranted. The crosstalk between immune and metabolic pathways, known as immunometabolism, is a new area of research in other animal models (i.e. *Mus musculus*) (Lercher et al., 2020), and needs to be further explored in bees. Lastly, there are difficulties in determining the sole effect of *V. destructor* as there are many factors, like viruses and altered neural and metabolic processes, that could be interacting with the effects of varroa mite parasitism. Addressing the sole effects of the mite as well as the synergistic effects from the interaction of the parasite with different stressors (biotic and abiotic) and their influence on different biological processes is not trivial (discussed in section 5). Hopefully the use of high throughput molecular techniques and studies on the description and quantification of biomolecules and their role on different biological pathways and networks will help understand the impact of *V. destructor* on honey bee health at the molecular level, as well as to help develop therapeutic strategies for varroosis control.

## 9 Interaction of *V. destructor* with biotic stressors

In addition to the host-parasite interaction between *V. destructor* and *A. mellifera*, there is also the interaction between the mite and viruses. Several studies have found an increase in viral levels (i.e. DWV and IAPV) in honey bees from *V. destructor*-parasitized colonies, and also an increase in bee mortality in parasitized and infected bees of those colonies, compared to colonies not parasitized by the mite (Dainat et al., 2012; Francis et al., 2013). Zhu et al. (2022) found that colonies that were treated with the synthetic acaricide Apivar<sup>®</sup> (containing the active ingredient amitraz) not only had lower mite loads and lower DWV levels compared to untreated colonies, but they also showed higher tolerance (lower mortality) to insecticides such as imidacloprid, cyhalothrin and oxamyl, indicating that *V. destructor* parasitism may affect tolerance to insecticide exposure. Several studies support the notion that viruses associated with *V. destructor* have detrimental effects on honey bee health, including immune responses. For example, a negative correlation between DWV levels and the cellular immune responses of melanization and encapsulation has been documented in honey bee brood artificially

parasitized with *V. destructor* (Di Prisco et al., 2016). This correlation resulted from a down-regulatory effect of DWV on *Amel102*, a gene involved in cellular immunity, which is regulated by the transcription factor NB-kB of the Toll signaling pathway. Di Prisco et al. (2016) also proposed that DWV increased *V. destructor* fitness because the proportion of reproducing mites increased as DWV levels in the pupae increased, up to a threshold of  $10^8$  DWV genome copies per bee. Conversely, higher DWV levels seemed to negatively impact *V. destructor* reproduction. Furthermore, mites showed twice the fertility rate (40%) when they parasitized larvae that later emerged with evident signs of wing deformity, compared to mites that parasitized asymptomatic bees (22% fertility rate), suggesting that DWV infections might favor *V. destructor* fitness. However, confirmation of the levels of DWV in the bees was lacking, and thus, further studies are needed to confirm the claims made by the authors of the study.

Three known variants of DWV that infect honey bees (DWV-A, DWV-B, and DWV-C) are mechanically vectored by *V. destructor*, but apparently only DWV-B can infect mite tissues (intestinal epithelium and salivary glands), which turns the parasite into a biological vector and a parasite of *V. destructor* (Gisder and Genersch, 2021). However, very few studies have been conducted on the relationship between DWV variants and *V. destructor*, and if the interaction with the vector (*V. destructor*) is affecting the mutation rate of DWV (Regoes et al., 2013). It is possible that variants of DWV will eventually appear that are primarily pathogens of the mite rather than the bee. Variants of *Varroa destructor virus* (VDV; Iflaviridae), including VDV-2, VDV-3, and VDV-5, seem to selectively infect mites but not bees (Levin et al., 2016; Herrero et al., 2019; Chen et al., 2021). Most of these studies have used RNA extraction and sequencing, which is a technique able to identify the presence of the virus but is not capable of determining if the virus is replicating and damaging tissues of either host. However, there are not many techniques available to confirm the infectivity of viruses in *V. destructor* and *A. mellifera*, as there are limited or no commercial cell lines. Identifying negative RNA strands, immunoassays, or *in situ* hybridization could be the first steps to study virus infectivity (Shen et al., 2005; Yue and Genersch, 2005; Shah et al., 2009). Moreover, the interactions between the different viruses may be a confounding factor. For example, Gregorc et al. (2012) found higher and lower DWV and BQCV transcripts, respectively, in mite parasitized bees, indicating a possible competition between viruses, which should be further explored. Moreover, there are less well studied viruses associated with honey bee and/or mite tissues, such as *Bee Macula-like virus* (BeeMLV), and De Miranda et al. (2015) suggested that mites are likely a biological vector of this virus based on the identification of sub-genomic RNA, which allowed the detection of actively replicating virus in bee and mite tissues. Other less studied viruses have been identified in *V. destructor* and bees using high throughput RNA sequencing, like *Moku virus* and *Bee macula virus*, and the implications of these viruses on *V. destructor* and bee health are unknown (Mordecai et al., 2016; Morfin et al., 2022).

*Varroa destructor* seems to interact with pathogens other than viruses, including the microsporidians *Vairimorpha (Nosema)* spp., as it appears that honey bee colonies parasitized by the mite tend to

have higher *Vairimorpha (Nosema)* spp. loads (Bermejo and Fernández, 1997; Mariani et al., 2012; Little et al., 2015). Additionally, van Dooremalen et al. (2018) found that colonies parasitized by *V. destructor* that were exposed to imidacloprid, showed higher *Vairimorpha (Nosema)* spp. levels, but they found no interactions between the stressors on colony size or colony survival. Contrary to these findings, Ostermann (2003) found no effect of *V. destructor* on *Vairimorpha (Nosema) apis* levels, nor an effect of the mite and formic acid treatment on the prevalence of chalkbrood disease (*Ascospheera apis*) in their experimental colonies.

In recent years, there has been an increasing interest in understanding how the gut microbiome impacts bee health, as evidence suggests that it plays an important role in bee metabolism, immunity, and development (Raymann and Moran, 2018). Parasitized bees showed a reduction in the relative abundance of *Bartonella apis* and *Lactobacillus apis*, and an increase of *Lactobacillus helsingborgensis*, *Lactobacillus mellis*, and *Commensalibacter intestini*, in whole bodies compared to non-parasitized bees (Hubert et al., 2017). The effect of *V. destructor* on the bee gut was greater for bacteria than for fungi, such as *V. apis* and *V. ceranae*, or trypanosomes, such as *Lotmaria passim*, which is interesting because *Vairimorpha (Nosema)* spp. and *L. passim* infect the epithelial cells of the bees' gut, and thus, a more pronounced effect on the microbiome would be expected from these pathogens than from *V. destructor*. Marche et al. (2019) also found that the abundance of bacterial community in whole bee bodies was altered by mite parasitism, with increased relative abundance of Gammaproteobacteria, Betaproteobacteria, *Lactobacillus* spp., *Bifidobacterium* spp., and *Brevibacillus laterosporus*.

There are a few reports on the interaction of *V. destructor* parasitism and multicellular organisms attacking bees. For example, a decrease in mite levels in apiaries infested with the small hive beetle (SHB), *Aethina tumida*, was reported, but no effects resulting from the interaction between *V. destructor* and SHB were observed on parameters associated to honey bee health like adult bee population, body mass, brood density, or colony weight (Delaplane et al., 2010). More studies are needed to confirm the nature of the interaction between *V. destructor* and SHB, and the possible consequences on honey bee health.

## 10 Interaction of *V. destructor* with abiotic stressors

Abiotic stressors also interact with *V. destructor* parasitism, impacting honey bee health. Most studies have focused on analyzing the effect of the mite and insecticide exposure on different aspects of bee health, behavior, and neural gene expression. For behavior, negative effects on flight capacity (i.e. bees flying shorter distances) and homing success of forager bees from colonies exposed to neonicotinoid insecticides (i.e. imidacloprid or thiamethoxam) and parasitized by *V. destructor* have been reported (Blanken et al., 2015; Monchanin et al., 2019). Detrimental effects on memory retention and in the expression of neural related genes, like *neurexin (AmNrx-1)*, in honey bees parasitized by *V. destructor* and exposed to sublethal doses of

neonicotinoid insecticides, like imidacloprid and clothianidin, have been described, which could be responsible for the altered behaviors (Morfin et al., 2020b; Morfin et al., 2020c; Schwartz et al., 2021). The complex formed by pre and postsynaptic proteins regulated by neural genes such as neurexin and neuroligin is essential for neurotransmission and linked to learning processes and memory retention in bees (Biswas et al., 2010). Therefore, these effects of insecticide exposure and *V. destructor* parasitism affect the performance of behaviors that are essential for colony survival, such as foraging behavior and navigation. An effect of sublethal doses of clothianidin and *V. destructor* parasitism on the proportion of bees performing intense self-grooming and an effect of both stressors on genes linked to neurological dysfunction was reported by Morfin et al. (2019).

Exposure to imidacloprid and thiamethoxam plus *V. destructor* parasitism also dysregulated immune related genes in bees at different developmental stages, like a down-regulation of *abaecin* and *defensin-1* in white-eyed pupa, and an up-regulation of *PPOact* and *spaetzle* in brown-eyed pupae (Tesovnik et al., 2017; Tesovnik et al., 2019). The mechanism by which neonicotinoid insecticides interact with *V. destructor* seems complex. Annoscia et al. (2020) found that bees exposed to sublethal doses of clothianidin had lower expression levels of immune related genes (*Amel/102* and *dorsal 1*), higher DWV levels, and lower mite fertility (reproducing mites/total mites). Although the immunosuppressive effect of clothianidin could be exacerbating the effects of the other two biotic stressors (DWV and *V. destructor*), the nature of the interactions needs to be investigated.

The combined effects of *V. destructor* parasitism and neonicotinoid insecticide exposure may reduce the body mass of newly emerged bees as well as their subsequent longevity (Straub et al., 2019), and this could be related to the detrimental effect of the parasite and insecticide on hypopharyngeal gland size in workers, and on drone body mass at emergence (Bruckner et al., 2021; Bruckner et al., 2023b). Other studies have found that the interaction between *V. destructor* and neonicotinoid insecticides impact bee metabolism by dysregulating genes linked to the biosynthesis of secondary metabolites and amino acids, as well as genes involved in fat digestion and absorption (Morfin et al., 2020a; Morfin et al., 2020b).

Most of the studies conducted so far have analyzed the effect of *V. destructor* in combination with other stressors on worker bees, but it would be interesting to know more about the synergistic impact of these stressors on the reproductive casts and the possible consequences to colony health and fitness. Additionally, the effect of *V. destructor* in combination with stressors other than insecticides used in agriculture that honey bees are exposed to, like a variety of agrochemicals, such as herbicides, fungicides, and acaricides (including the ones used to treat varroosis), low or high temperatures, drought, etc, warrant investigation to find out if their interaction with the mite impacts honey bee health and colony fitness.

## 11 Honey bee behavioral responses to *V. destructor* parasitism

Relatively little is known about how *V. destructor* affects bee behavior. The best documented behaviors of honey bees in response

to *V. destructor* parasitism are hygienic and grooming behavior, but other behaviors such as foraging and defensive behavior may also be affected by the mite.

Parasitism by *V. destructor* affects honey bee foraging behavior in different ways. Foragers infested by the mite perform longer foraging trips and many do not return to their hives at all. Parasitized bees also show impaired homing and orientation ability at the hive entrance (Kralj and Fuchs, 2006), which may limit the ability of colonies to collect resources. The fact that bees take long foraging trips or do not return to the hive could be an adaptive behavior of the insects to reduce parasitism load in the hive by leaving the hive or could be due to neurological damage caused by the mite, affecting recognition and responsiveness to environmental stimuli. This last hypothesis was tested by Kralj et al. (2007), who found that bees infested with *V. destructor* significantly decreased their responses to a reward stimulus (sucrose syrup) when a habituation test was conducted by repeatedly presenting the stimulus to the bees. The parasitized bees also showed a lower response to a scent stimulus than non-parasitized bees, suggesting that mites may interfere with neural transmission that enables learning and memory retention. Morfin et al. (2020c) also showed a detrimental impact of *V. destructor* on the ability of honey bees to respond to foraging-related stimuli, and possibly other behaviors that require cognitive processing. Studies have also found a down regulatory effect of *V. destructor* on neural related genes, like *AmNrx-1*, *neuroligin* (*Am-Nlg-1*) and *acetylcholinesterase* (*AmAChE-2*) (Morfin et al., 2020b; Morfin et al., 2020c). Likewise, Duay et al. (2002) found that *V. destructor*-parasitized drones showed decreased flight performance and lower ability to mate with queens compared to non-parasitized drones. Neural disorders because of *V. destructor* parasitism could also be due to infections caused by viruses transmitted by the mite. For example, DWV and IAPV affect the expression of neural genes and impair the homing ability and learning processes in honey bees (Iqbal and Mueller, 2007; Li et al., 2013). Therefore, the combination of mite parasitism and viral infections could be detrimental to the foraging activity and fitness of honey bee colonies.

*Varroa destructor* parasitism and reproduction in honey bee brood stimulates hygienic behavior by adult bees. Hygienic worker bees detect and uncap comb cells containing larvae infested with bacteria or fungi, as well as parasitized with *V. destructor* (Guzman-Novoa and Morfin, 2019). A parasitized larva may be removed from its cell (Boecking and Spivak, 1999; Arathi et al., 2000) or the cell may be posteriorly recapped (Hawkins and Martin, 2021), all of which interrupts the mite's life cycle. It is well established that hygienic behavior is a heritable trait (Lapidge et al., 2002; Unger and Guzman-Novoa, 2010) and that some honey bee strains are more hygienic than others (Spivak and Reuter, 1998), particularly those expressing the varroa sensitive hygiene (VSH) trait, that allows them to identify cells infested with reproducing mites (Harbo and Harris, 2009). Worker bees that are hygienic can detect *V. destructor*-parasitized larvae by perceiving with their antennae chemical odorants from the brood such as hydrocarbons and oleic acid (Nazzi et al., 2004; Mondet et al., 2015; McAfee et al., 2018) that elicit hygienic responses in the bees. Some of these

compounds have also been identified from *V. destructor* and DWV (Wagoner et al., 2019), which again, shows that the combination of both pathogens can influence several honey bee behaviors.

Grooming behavior of adult honey bees may also be triggered by *V. destructor* infestations. Parasitized workers use their legs and mandibles to remove mites from their bodies, sometimes biting and injuring them (Boecking and Spivak, 1999). The presence of *V. destructor* as well as the wounds inflicted by the mite while feeding irritate the bees, which elicits grooming responses (Morfin et al., 2019) that are governed by neural processes (Hamiduzzaman et al., 2017; Morfin et al., 2023). Grooming behavior is a heritable trait (Arechavaleta-Velasco et al., 2012; Morfin et al., 2020d), and the expression of this behavior varies among different genotypes and strains of honey bees (Arechavaleta-Velasco and Guzmán-Novoa, 2001; Rinderer et al., 2001; Guzman-Novoa et al., 2012; Bık and Wilde, 2015; Invernizzi et al., 2015). Interestingly, younger daughter mites elicit stronger grooming responses by honey bees than older foundress mites (Kirrane et al., 2012). Perhaps these responses are related with the greater reproductive potential of younger mites or with different chemical signals produced by the mites.

*Varroa destructor* may also influence defensive behaviors of honey bees. De la Mora et al. (2021) measured the stinging response threshold of *V. destructor*-parasitized and non-parasitized worker bees by exposing them to a constant electric stimulus while measuring the time that the bees took to sting a leather patch. Parasitized bees stung significantly faster than non-parasitized bees. The authors concluded that the irritation caused by the parasite affected neural processes that made the bees more sensitive and prone to sting. The implications of these results are that colonies infested with the mite may be better adapted to protect their nests from predators, which would be beneficial for their ecological success, but they would also be more difficult to manage by beekeepers for production purposes.

While it is clear that *V. destructor* affects different honey bee behaviors, these could result in higher or lower mite infestation rates. More research, particularly related to breeding and selection for different behaviors are needed. In addition to better understanding the effects of the mite on bee behaviors, such studies could have practical outcomes for improved health and fitness of honey bee colonies.

## 12 Conclusions

Undoubtedly, *V. destructor* parasitism is one of the main impacts currently affecting *A. mellifera* worldwide. Although advancements have been made to understand the development of *V. destructor* parasitism, there is insufficient knowledge on the effects of the parasite on different immune responses in honey bees and the consequences (or interactions) on biological processes, like metabolic pathways. However, recent studies using more comprehensive methods to investigate the effect of *V. destructor* on molecular processes, like the use of 'omic' tools, can help understand this interaction. Nevertheless, they need to be applied

to bees of different developmental stages and sexes rather than concentrating mostly on newly emerged bees parasitized as brood. They would also be useful to combine with studies showing behavioral changes. Complicating all studies of *V. destructor* parasitism is its interaction in different ways with many biotic and abiotic factors. Such interactions are not trivial, especially those with viruses, as separating the effects of viral diseases (e.g. DWV) and *V. destructor* is challenging. While research on the effect of *V. destructor* on immune pathways, cellular immunity, and metabolic pathways may appear to have limited practical outcomes, it will hopefully lead to the development of therapies and bees that could better fight *V. destructor*, thus reducing economic losses, which is urgently needed.

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/frbee.2023.1272937/full#supplementary-material>

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