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Review

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Understanding the Enemy: A Review of the Genetics, Behavior and Chemical Ecology of *Varroa destructor*, the Parasitic Mite of *Apis mellifera*

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Abstract

Varroa destructor (Mesostigmata: Varroidae) is arguably the most damaging parasitic mite that attacks honey bees worldwide. Since its initial host switch from the Asian honey bee (*Apis cerana*) (Hymenoptera: Apidae) to the Western honey bee *(Apis mellifera*) (Hymenoptera: Apidae), *Varroa* has become a widely successful invasive species, attacking honey bees on almost every continent where apiculture is practiced. Two haplotypes of *V. destructor* (Japanese and Korean) parasitize *A. mellifera*, both of which vector various honey bee-associated viruses. As the population of *Varroa* grows within a colony in the spring and summer, so do the levels of viral infections. Not surprisingly, high *Varroa* parasitization impacts bees at the individual level, causing bees to exhibit lower weight, decreased learning capacity, and shorter lifespan. High levels of *Varroa* infestation can lead to colony-wide varroosis and eventually colony death, especially when no control measures are taken against the mites. *Varroa* has become a successful parasite of *A. mellifera* because of its ability to reproduce within both drone cells and worker cells, which allows populations to expand rapidly. *Varroa* uses several chemical cues to complete its life cycle, many of which remain understudied and should be further explored. Given the growing reports of pesticide resistance by *Varroa* in several countries, a better understanding of the mite's basic biology is needed to find alternative pest management strategies. This review focuses on the genetics, behavior, and chemical ecology of *V. destructor* within *A. mellifera* colonies, and points to areas of research that should be exploited to better control this pervasive honey bee enemy.

Key words: Western honey bee, parasitic mite, host–parasite interaction, chemical ecology, behavioral ecology

Varroa destructor (Mesostigmata: Varroidae) is a cosmopolitan ectoparasitic mite known for its successful infestation of Western honey bee (*Apis mellifera* L. (Hymenoptera: Apidae)) colonies worldwide. The *Varroa* mite is the leading driver of colony mortality in the United States, causing colony collapse and/or death if highly infested colonies are left untreated (Guzmán-Novoa et al. 2010, Kulhanek et al. 2017, Brodschneider et al. 2018, Steinhauer et al. 2018). Since its introduction in the 1980's, *Varroa* has caused significant damage to the U.S. beekeeping industry in terms of colony and economic losses (Brodschneider et al. 2018). The average loss of honey bee colonies in the U.S. was 40.5 and 45.5% in 2015–2016 and 2020–2021, respectively, with both surveys showing *Varroa* mites as the top culprit for colony mortality reported by surveyed beekeepers (Kulhanek et al. 2017, Steinhauer et al. 2021). Similar patterns of high colony losses due to *Varroa* parasitization have been seen around the world. For example, regional surveys in Europe reported an average winter loss of 20.9% in 2016–2017 (Brodschneider et al. 2018) and 16.7% in 2018–2019 (Gray et al. 2020). While colony losses have not been tracked as closely in Latin America as they have in the U.S. or Europe (Requier et al. 2018), In Uruguay, the average winter colony loss was 18.3% in 2013–2014, with 61.5% of the reported losses being caused by parasites and disease (Antunez et al. 2017). Given the negative impact that *Varroa* has caused on Western honey bee populations, multiple lines of integrated pest management have been deployed for *Varroa* control around the world. However, to date, none of the existing management options have been able to fully eliminate *Varroa* from infested colonies, and instead, have only allowed us to maintain infestations below damaging levels (Lee et al. 2015, Kulhanek et al. 2017, Brodschneider et al. 2018, Jack and Ellis 2021).

The overall reduction of colony health and longevity caused by high mite infestation is known as *Varroa* disease or varroosis (Boecking and Benersch 2008). The severity of varroosis-caused symptoms depends on the level of mite infestation and is often associated with a steady and linear increase in a colony's *Varroa* population through the spring and mid-summer (Wegener et al. 2016). Around July, colonies with varroosis show a high brood-to-adult bee ratio and bees exhibit higher expression of phenol oxidase (POX) and glucose oxidase (GOX) enzymes (Wegener et al. 2016). The

high expression of bee immune responses, at least partially, could be explained by increased levels in the viruses vectored by *Varroa* (Wegener et al. 2016). By late August, GOX and POX are expressed at low levels, possibly allowing viral loads to increase (Wegener et al. 2016). In the fall, the weight of overwintering workers is lower in infested colonies compared to uninfested ones, regardless of infestation levels (Aronstein et al. 2012, Wegener et al. 2016). Throughout fall and winter, as the infestation threshold is reached, varroosis can lead to colony collapse. However, the infestation thresholds vary depending on environmental factors (e.g., average precipitation, geographic region, and food availability), genetics (e.g., differences between Africanized and European lineages or the presence of hygienic behavior), as well as their interaction (Dainet et al. 2012, Wegener et al. 2016, Dechatre et al. 2021). For example, the growth of *Varroa* mite populations fluctuates with the weather, with years in which the amount of rainfall is below the annual average showing lower mite growth rates than wetter years (Harris et al. 2003). Furthermore, colonies with higher amounts of honey and brood show higher levels of infestation levels than colonies with lower amounts of honey and brood, likely caused by the increased amount of available brood cells to invade (Lodesani et al. 2002). Interestingly, even though hygienic behavior has been a phenotype selected by beekeepers to control mite loads, this behavior does not always show a significant correlation with lower *Varroa* infestation levels (Arechavaleta-Velasco et al. 2001, Lodesani et al. 2002).

V. destructor feeds on the fat bodies of developing and adult honey bees (Ramsey et al. 2019), all while transmitting several honey bee-associated viruses (Francis et al. 2013, Mondet et al. 2014, Emsen et al. 2015). These include Deformed wing virus (DWV), Acute bee paralysis virus (ABPV), Israeli acute paralysis virus (IAPV), Kashmir bee virus (KBV), and Sacbrood virus (SBV) (Ball 1983, Ball and Allen 1988, Chen et al. 2004, Yue and Genersch 2005, Boecking and Genersch 2008). Because DWV has evolved alongside *A. mellifera*, the virus was found in most hives at covert levels before the introduction of *Varroa* as an invasive parasite of Western honey bees (Wilfert et al. 2016). However, with the spread of *Varroa* the symptoms caused by DWV have become much more prevalent because the mite spreads the virus from bee to bee as it feeds on different hosts. Furthermore, increased time spent by mites on adult bees can increase the chance of DWV spread to bee brood (Prisco et al. 2011, Piou et al. 2016). It is unclear if all honey bee-associated viruses harm and/or replicate within the *Varroa* mite, however. For instance, Posada-Florez et al. (2019a) found that *V. destructor* is a nonpropagative vector of DWV-type A, which means that the virus does not replicate within the mite. More studies are needed to determine if other honey bee-associated viruses vectored by *Varroa* mites can replicate within the mite, as well as the health consequences to the mite from carrying the viruses, which would give insight on its ability to spread pathogens within and among honey bee colonies.

Varroa parasitization causes several physiological problems for bees at the individual and colony levels (for reviews on these topics see Noel et al. 2020 and Traynor et al. 2020). At the individual level, the constant feeding of mites using their sucking-piercing mouthparts causes an open wound on parasitized pupae, resulting in the development of scar tissue and bacterial infections (Kanbar and Engels 2003). *Varroa* feeding also leads to decreased hemocyte concentrations and reduced POX expression, both of which are important for immune function (Koleoglu et al. 2018). Parasitized worker and drone brood also exhibit lower weight at emergence compared with unparasitized brood and are unable to regain the lost weight upon emergence (De Jong et al. 1982, Duay et al. 2003, Van Dooremalen et al. 2013). Workers parasitized by *Varroa* also tend to have smaller hypopharyngeal glands and, when parasitized by three or more mites, typically have smaller mandibular glands (Ayoub et al. 2015). Infested bees also exhibit increased metabolic rates, likely because of their immune response and the mites' consumption of their fat bodies (Ramsey et al. 2019, Aldea and Bozinovic 2020). Large infestations also show decreased learning capacity in workers, particularly in their homing ability, which causes highly parasitized foragers to get disoriented when returning to the hive (Kraji and Fuchs 2006). Moreover, drones within *Varroa* infested colonies weigh less and have significantly lighter testes than drones in noninfested colonies (Omar 2017). At the colony level, high *Varroa* infestation levels cause a large proportion of the worker population to be inactive (Annoscia et al. 2015). Colonies with high mite infestations also have lower success in the retention of newly introduced queens (Rateb et al. 2010).

While many studies have focused on the health, management, and economic impacts of *Varroa* parasitization on *A. mellifera* (see Jack and Ellis 2021), there is still a need for greater understanding of the life history of *V. destructor* within *A. mellifera* colonies. This review is a compilation of influential studies regarding *V. destructor*'s genetics, behavior, and chemical ecology, and points to those aspects of the mite's biology that should be studied further. A growing body of knowledge about the mechanisms of *Varroa* parasitization of *A. mellifera* colonies will help us better understand how this mite has become so successful in attacking colonies and will help inform the future development of improved management strategies against this devastating honey bee enemy.

Varroa Destructor's Initial Host Switch and Genetics

The Asian honey bee, *Apis cerana* (Hymenoptera: Apidae), is the original obligate host of *V. destructor*. It is also the host of another mite species in the same genus, *V. jacobsoni* (Anderson and Trueman 2000). The two *Varroa* species are very similar genetically, sharing 99.7% of their genome in common (Techer et al. 2019). Their morphological similarities require genetic testing using amplified fragment length polymorphism (AFLP) to accurately identify each species (Anderson and Trueman 2000, Roberts et al. 2015, Techer et al. 2019). While *V. jacobsoni* is found mostly within *A. cerana* colonies throughout Asia, in 2008 the mite was found parasitizing worker and drone brood of *A. mellifera* in Papua New Guinea (Roberts et al. 2015). This shows that *V. jacobsoni* can spread to *A. mellifera* colonies, although more studies are needed to fully understand the extent of this novel parasitism. Originally it was believed that *V. jacobsoni* was the mite species that switched hosts and began infesting *A. mellifera* colonies. However, in the year 2000, *V. destructor* was identified as the infesting mite and was separated phylogenetically from *V. jacobsoni* soon after (Anderson and Trueman 2000). Because of this confusing misidentification of mites, the studies referencing *V. jacobsoni* parasitization of *A. mellifera* published before 2000 actually refer to *V. destructor.*

In 1952, a host switching event by *V. destructor* from *A. cerana* to *A. mellifera* colonies in eastern Russia produced the Korean haplotype of this mite (Oldroyd 1999). A second host switching event occurred around 1957, which led to the description of the Japanese haplotype (Oldroyd 1999). While both *V. destructor* haplotypes have spread west from their countries of origin, the Korean haplotype has become dominant (Anderson and Trueman 2000, Guerra Junior et al. 2010, Gajic et al. 2013, Ayan et al. 2017, Kelomey et al. 2017, Octaviano-Salvadé et al. 2017, Hajializadeh et al. 2018, Muntaabski et al. 2020, Ogihara et al. 2020). Several variants of the Korean and Japanese haplotypes have been identified in parts of Asia (Navajas et al. 2010). Even though only the Japanese and Korean haplotypes are known to invade *A. mellifera* colonies, a recent study looking at the mite's

genome suggests a higher genomic diversity among mite populations across the world than previously thought (Techer et al. 2021). In that study, the authors analyzed the genome-wide variation and divergence among *V. destructor* and *V. jacobsoni* females from their original and novel hosts, confirming *Varroa* species identity by aligning single nucleotide polymorphisms (SNPs) within the mitogenomes, along with known reference sequences of the mitochondrial DNA (mtDNA) *COX1* 458-bp standard marker. They found multiple previously undiscovered mitochondrial lineages on the novel hosts of each mite, as well the genetic equivalent of tens of individuals that were involved in the initial host switch. Therefore, contrary to previous beliefs, modest gene flow remains between mites adapted to different hosts. The low genetic diversity of *V. destructor* within *A. mellifera* colonies is thought to be caused by three factors: i) a genetic bottleneck that occurred during the host switching events, ii) the founder effect that occurred after the spread of the two haplotypes into new regions, and iii) the mite's sibling–sibling mating system (Solignac et al. 2005, Navajas et al. 2010, Dynes et al. 2017).

Varroa Destructor's Life Cycle

Reproductive Phase

There are two phases in the life cycle of *V. destructor*: the reproductive phase and the dispersal phase (for a review of Varroa's life cycle see Rosenkranz et al. 2010). To begin the reproductive phase, a gravid adult female mite, referred to as a foundress, invades the cell of a 5th instar bee larva (Figs. 1 and 2). Upon invasion, the foundress is temporarily trapped in the brood food found at the bottom of the cell and remains trapped for up to six hours in workers cells and 20 hr in drone cells before the cell is capped for the bee to initiate pupation (Ifantidis 1988, Aumeier et al. 2002). The capped bee pupa then releases an unknown chemical signal that initiates egg production by the mite (Garrido and Rosenkranz 2003).

Varroa destructor undergoes arrhenotokous parthenogenesis (Rehm and Ritter 1989, Häußermann et al. 2018, 2020). Because of its haploid–diploid sex determining system, the first egg laid by the foundress mite (approximately 60 hr postcapping) is unfertilized and develops into a male (Ifantidis 1983). The rest of the eggs, which are laid every 24–36 hr, are fertilized and develop into females (Rehm and Ritter 1989, Martin 1994). A foundress typically produces four to five offspring in a worker cell and five to six offspring in a drone cell (Ifantidis 1983, Martin 1995). However, the average production of mature daughters in single-infested cells is 1.8 and 3.0 for worker and drone cells, respectively (Donze et al. 1996, Martin 1998). A feeding site is established by the foundress along with a fecal accumulation site on the cell wall (Donze and Guerin 1994). Feeding by the mites occurs every 1.6 hr and competition for food is common at the feeding site (Donze and Guerin 1994). Based on the amount of excretion and fecal matter produced, it is estimated that mites can consume up to 1 µl of host fluids per day (Posada-Florez et al. 2019b). The development of mite offspring takes approximately 5.5 d within worker cells and 7.5 d within drone cells (Ifantidis 1983, Martin 1994).

After maturation, mating among mites occurs at the fecal accumulation site and is bimodal, being either three or six minutes in duration (Donze et al. 1996). Mite mating is activated by volatile compounds released by the female (Ziegelmann et al. 2013) and females who mature first usually mate more times. Because mating events are often interrupted, it is thought that several mating events are required for proper fertilization. This may explain why females that mate only once typically do not become fertilized (Donze et al. 1996). Successful mating is also not possible sometimes due to the lack of a mature male within the cell. In fact, an estimated average of 17% of worker cells and 23% of drone cells is thought to contain no mature male mites at all (Donze et al. 1996).

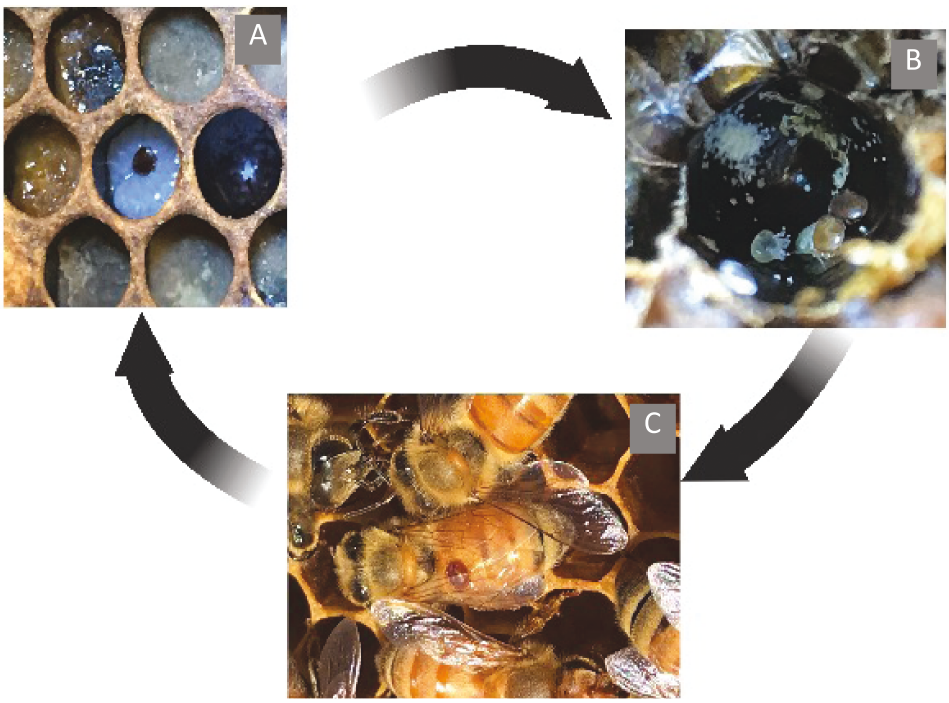

Fig. 1. A simplified diagram showing the life cycle of *Varroa destructor*. During the reproductive phase, a gravid female mite enters the cell of a worker or drone larva before it being capped (A). Once the cell is capped, the foundress mite produces a son and several daughters who undergo sibling–sibling mating, all while feeding on the bee pupa (B). Upon the emergence of the adult bee, the mites leave the cell and begin the dispersal phase (C), during which newly gravid females get transported by bees to reach a bee brood cell to invade, starting the cycle again.

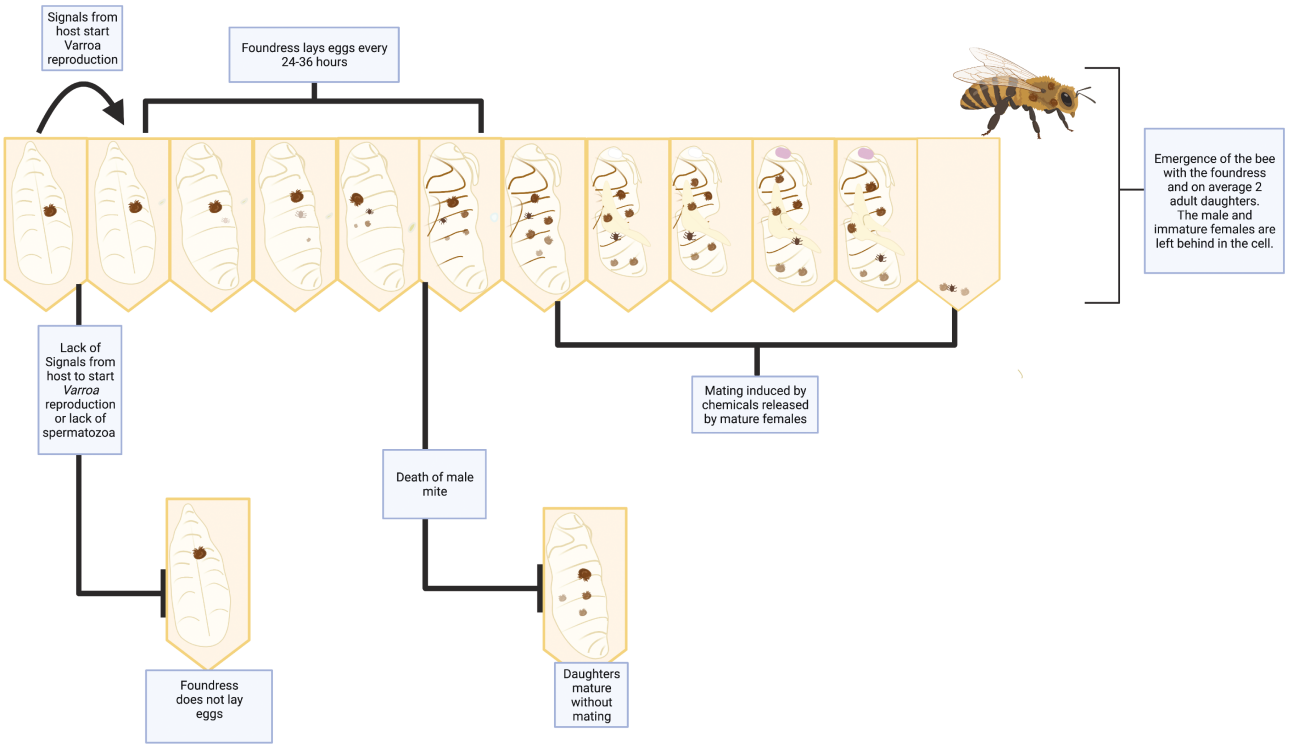

Fig. 2. Reproductive cycle of *Varroa destructor* after a gravid female foundress invades a developing honey bee cell. Once the adult bee completes pupation and emerges from its cell, the mites that developed therein also exit to begin the dispersal phase.

Varroa mites typically invade a cell individually, leading to the offspring of one foundress to mate with one another. However, multiple foundresses can invade the same cell, which leads to mating between offspring from different mothers (Beaurepaire et al. 2019). When more than one male is present in the cell, female mites exhibit polyandry (Donze et al. 1996). After fertilization, a female's full spermatheca generally contains up to 35 spermatozoa (Donze et al. 1996). Since *Varroa* foundresses generally lay between 4 and 7 eggs per invasion, and one egg is a haploid male, it could be estimated that one *Varroa* foundress can invade a bee cell up to five times in her lifespan if her spermatheca is full of spermatozoa (Ifantidis 1983, Donze et al. 1996). However, the actual number of times that a foundress can invade cells varies and is likely to be between 1.5 and 3 (Fries and Rosenkranz 1996, Martin and Kemp 1997). Once the female mites are fully mated, they can begin the dispersal phase upon the emergence of the adult bee host.

Dispersal Phase

The second component of the mite's life cycle was recently renamed the 'dispersal' phase instead of the 'phoretic' phase. This is because we now know that feeding by the mite does occur on adult bees as they get transported to potential sites for invasion, whereas in a truly phoretic species, the host is only used as a mode of transport and no feeding occurs (Ramsey et al. 2019). The dispersal phase starts when the adult bee emerges from a mite-invaded cell (Fig. 1). The foundress and her adult daughters attach themselves to the bee before it exits, while the male and all immature females are left behind and die soon after (Donze et al. 1996). After leaving the cell, the female mites switch from riding on the original newly emerged bee to riding on a nurse bee (Kuenen and Calderone 1997). Mites are not often found walking directly on the comb (Kuenen and Calderone 1997). The number of times that a mite switches from one bee host to the next, as well as the duration of each transport event is still unknown.

The most likely spot to find mites on adult bees is between sternites on the ventral side of the abdomen. Interestingly, mites show a preference for attaching on the left side of the bee's abdomen for reasons still undetermined (Delfinado-Baker et al. 1992, Fernández et al. 1993). *Varroa* mites exhibit a preference for dispersing atop nurse bees, likely because nurses give mites the access to brood cells (Xie et al. 2016). However, mite preference for adult hosts can change during the year. For instance, when there are high *Varroa* loads within a colony, mites can be more frequently found atop foragers (DeGrandi-Hoffman et al. 2016).

Mites can also gain access to new colonies during the dispersal phase (Fig. 3). This can happen through robbing and drifting behaviors performed by the bees, as well as through beekeeping practices (Peck et al. 2016, Peck and Seeley 2019). For example, as a colony's mite population increases to the point that it begins to collapse, robbing by foragers from other colonies is common. Mites can also jump hosts from robbing bees to other workers and infest a new colony (Fig. 3). They can also attach to foragers of their original colony and, in the event those foragers accidentally drift, mites gain access to other colonies (Peck and Seeley 2019). Additionally, mites can be introduced into foreign colonies by beekeepers when they transfer supplies and bees between hives (Fig. 3). Studies have also found mites on drones collected at drone congregation areas, or 'DCAs' (Mortensen et al. 2018, Galindo-Cardona et al. 2020). Moreover, Galindo-Cardona et al. (2020) showed that drones returning from mating flights could drift into neighboring colonies, spreading mites throughout the apiary. However, it has not been confirmed that mites can access new colonies after getting infested at DCAs, and thus, more information on how adult drones and *Varroa* mites interact is needed to further understand how drones play a role in the spread of *Varroa* within and among colonies.

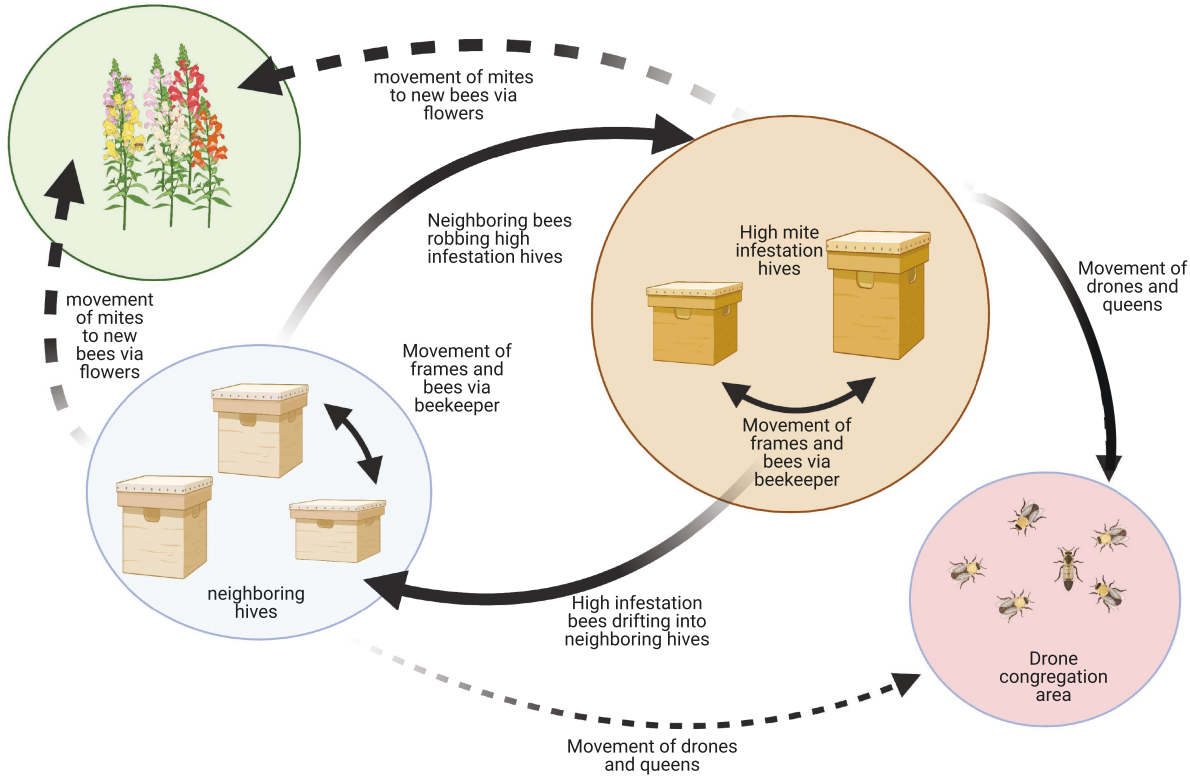

Fig. 3. Diagram depicting the potential ways in which *Varroa* mites can move between honey bee colonies. Solid arrows represent those paths that have been confirmed by previous studies and dashed arrows represent suspected (yet mostly understudied) methods of how *Varroa* mites spread between hives.

Additionally, the bees' own behavior may contribute to the mite's success during the dispersal phase. For example, Rivera-Marchand et al. (2012) found that commercial honey bees of Italian maternal descent habituated faster to mite attempts at 'catching a ride' on adult bees than mites in colonies of Africanized maternal descent. Finally, there is some evidence that mites can spread to new colonies via flowers (Peck et al. 2016), but more studies are needed to understand how likely this is to occur.

Behavior and Chemical Ecology

Chemical-Producing Organs in Varroa

Varroa destructor lacks visual organs and relies on chemical cues for orientation within the confined darkness of a honey bee hive. The mite uses the front pair of legs as sensory organs (Diller et al. 2006, Nganso et al. 2020). Each front leg has a sensory pit organ with nine sensillae within and nine sensillae around the organ, respectively (Liu 1988). This pit organ has been compared to the Haller's organ in ticks (Diller et al. 2006) and has been shown to react to compounds of honey bee brood pheromone (Eliash et al. 2014). The mite also has chemoreceptive sensillae on the palptarsi of the front two legs, which are responsive to brood chemicals such as methyl oleate and methyl palmitate, as well as alarm pheromones such as 2-heptanone (Liu and Peng 1990, Light et al. 2020).

Host Preference

There is a clear host preference exhibited by *Varroa* during both the reproductive and the dispersal phases. It is thought that mites detect cuticle compounds in bee brood to choose their host and to make their way around the hive depending on what phase of the life cycle they are in. During the dispersal phase, mites show a preference for attaching onto nurse bees over foragers or adult drones (Kraus 1994, Kuenen and Calderone 1997, Pernal et al. 2005, Del Piccolo et al. 2010, Xie et al. 2016). *Varroa*'s preference for attaching to nurse bees during the dispersal phase is thought to be mediated by chemical cues. Foragers exhibit shorter straight-chain cuticular hydrocarbons (CHC) profiles compared to nurses, which have higher amounts of longer straight-chain CHCs (Del Piccolo et al. 2010). The cuticle of foragers also has higher amounts of (Z)-8-heptadecene, which acts as a repellent to mites (Del Piccolo et al. 2010). As the population of *V. destructor* increases in a colony, the distinct preference of mites for attaching onto nurses over foragers decreases (Cervo et al. 2014, Xie et al. 2016). This could be explained by the fact that the CHC profiles of bee foragers and nurses are more similar in colonies that have high mite loads compared to those with low mite loads (Cervo et al. 2014, Xie et al. 2016). The increased presence of mites on foragers could be a way for *Varroa* to spread to new colonies. This change in host preference during the dispersal phase does not come without a cost, however, as mites that attach to foragers have increased infertility rates and lower fitness after invading a cell (Xie et al. 2016). It is still unclear if the increased infertility is caused by a decreased nutritional state in the forager, the host age of the mite, or other factors (Xie et al. 2016). Interestingly, Lin et al. (2018) showed that *V. destructor* did not exhibit different larval host preferences within *A. mellifera* and *A. cerana* colonies. From that study, we could infer that *Varroa*'s host preferences during the dispersal phase within *A. cerana* also does not differ from mites within *A. mellifera* colonies. However, there is an overall lack of studies focusing on the host preferences of *V. destructor* during the dispersal phase within *A. cerana* colonies, and thus more studies are needed to understand this important life phase of the mite.

The decision-making mechanisms through which *Varroa* mites invade bee cells to begin the reproductive phase are not fully understood. Within Asian honey bee colonies, *V. destructor* can only successfully reproduce in drone cells because bees remove parasitized worker larvae, which interrupts *Varroa* reproduction in worker cells (Lin et al. 2018). However, the development of parasitized worker larvae is not normally interrupted in Western honey bee colonies, allowing the mite to successfully reproduce in both worker and drone cells. Nevertheless, *Varroa* displays a clear preference for invading cells of drone larvae over worker larvae in *A. mellifera* colonies (Fuchs 1990, Boot et al. 1995). This preference was originally attributed to differences in CHC profiles between drone and worker larvae (Le Conte et al. 1989, 1990; Trouiller et al. 1991; Rickli et al. 1992, 1994; Del Piccolo et al. 2010; Cervo et al. 2014). Several compounds within the honey bee brood pheromone have been identified as kairomones for *V. destructor*. Of these compounds, the most active is methyl palmitate, which is found in higher amounts in drone brood compared to worker brood (Le Conte et al. 1989, Trouiller et al. 1992).

In the past, methyl palmitate and other brood pheromones were used to explain the mite's preference for invading drone brood over worker brood. In fact, for some time it was believed that methyl palmitate could be exploited as a potential method for mite management. Continued studies showed contradicting results, however, as methyl palmitate and other cuticular compounds did not always elicit clear host choices by mites between the drone and worker brood (Boot 1994, Calderone and Lin 2001, Nazzi et al. 2001, Pernal et al. 2005). A study also revealed that topical application of methyl palmitate onto brood cell caps resulted in bee larval death at high doses (Boot 1994). Furthermore, even though drone and worker larvae produce different amounts of brood pheromone (Trouiller et al. 1992), choice tests showed that *Varroa* is equally attracted to drone and worker cuticle extracts (Calderone and Lin 2001), showing that these compounds likely do not help the mite in choosing between bee larval types. Instead, these compounds may simply help the mites determine if a cell is empty or contains a larva (Boot 1994, Calderone and Lin 2001). Other compounds within larval cells have also been studied as possible mite attractants. For example, a particular compound in the brood food, 2-hydroxyhexanioc acid, elicits a behavioral response from *Varroa* (Nazzi et al. 2001, 2004). However, this attraction does not differ between brood types (Calderone and Lin 2001). Therefore, while mites have the ability to detect bee larvae through chemical cues, olfactory stimuli alone may not be enough to elicit the invasion of a specific type of bee cell (Kraus 1994).

The distance between a bee larva and the top of the cell's opening may also be a key factor in cell invasion by *Varroa*. Once this distance is 7.0–7.5 mm, the cell becomes attractive to mites and invasion begins (Goetz and Koeniger 1993, Beetsma et al. 1999). If this distance is achieved earlier, the attractive period is longer (Boot et al. 1995). Comb that is older is also more attractive to mites, possibly because the cells are smaller from use and thus the distance from a larva to the top of a cell is shorter sooner (Piccirillo and Jong 2004). This suggests that beekeepers should remove older comb or only use it for honey supers to reduce mite invasion. Interestingly, small cell comb, which was once suggested as a possible mechanical management system to reduce *Varroa* populations (Martin and Kryger 2002, McMullan and Brown 2006), has now been deemed ineffective in reducing *Varroa* loads compared to normal comb (Seeley and Griffin 2011) and can actually elicit a higher chance of cell invasion. Thus, small cell comb should be discontinued as a mite treatment option (Berry et al. 2010, Coffey et al. 2010).

The visitation rates of larvae by nurse bees could also be a factor in *Varroa*'s cell invasion process. Drone larvae are attractive to mites for around 40 hr before capping, while worker larvae are attractive for only 20 hr before capping (Boot et al. 1992). This extended period of mite attraction toward drone larvae could contribute to the preference of *Varroa* for invading drone cells. Drone larvae also have roughly 2.5 times higher visitation rates by nurse bees than worker larvae (Calderone and Kuenen 2003, Reams et al. unpublished data). Brood that has a higher visitation rate is likely exposed to *Varroa* mites more often and thus has a higher chance of being invaded. More studies are needed to understand how nurse visitation rates influence *Varroa* cell invasion, however.

Cell Invasion

The cell invasion rate of *Varroa* within a colony throughout the year varies and is heavily influenced by the presence of adult and larval bees, as well as the choices made by other mites. As the number of available brood cells to be invaded in the spring increases, so does the *Varroa* invasion rate (Boot et al. 1994a, Martin 1998). This increase is caused by the higher number of larvae that are at the right developmental stage for invasion. As the population of adult bees increases, *Varroa* invasion rate decreases (Boot et al. 1994b). This brood-to-adult bee ratio is important for studying *Varroa* invasion: as the population of adult bees increases, the chance that a mite is exposed to brood cells goes down, thus decreasing invasion and vice versa. As the number of larvae in the 5th instar increases, the invasion of *Varroa* should increase, since this is the appropriate time period for mite invasion.

The invasion of *Varroa* into brood cells can be looked at in multiple ways: the invasion rate of both larval types, the drone cell preference, and the worker cell acceptance. The invasion rates of drone and worker larvae are calculated by the number of mites per cell (Fuchs 1990). Drone cell invasion rates are usually higher than worker cell invasion rates (Fuchs 1990, Boot et al. 1995). Drone cell preference is the drone cell infestation rate divided by the worker cell infestation rate (Fuchs 1990). As the ratio of drone cells to worker cells increases, the drone cell preference decreases (Fuchs 1990, 1992). This means that with less drone brood, drone cells are preferred, and thus, drone cell preference is not constant but fluctuates throughout the year (Fuchs 1990, 1992). Drone cell acceptance also depends on the population of mites within the entire colony. As the mite population increases (typically over the spring and summer) the preference for drone cells decreases (Fuchs 1990).

Worker cell acceptance is the threshold at which *Varroa* will begin invading worker cells (Fuchs 1992). This threshold is measured as the total number of mites within the hive, which is around 300. After this threshold is reached, worker cell acceptance begins to increase (Fuchs 1992). This is because after the threshold is reached, there are so many mites within the colony that it becomes more optimal for a mite to invade a worker cell as the sole foundress than to invade a drone cell that already contains mites. This shows that both drone and worker cell invasion can fluctuate throughout the year and as the mite population changes. Interestingly, while *Varroa* prefers to invade drone and worker cells, they can also be found invading queen cells. In fact, queen cell invasion can range from 1 to 9.1%, depending on the presence or absence of brood (Harizanis 1991). Mites that invade queen cells cannot complete their reproductive cycle, but do not typically affect the grafting success of queen cells (Harizanis 1991).

Multiple mites can invade the same cell and this occurs more often with higher mite infestation rates (Martin 1995, Floris et al. 2020). This may be advantageous to the overall mite population within a

hive because it increases the chance of outbreeding. However, mites that invade the same cell are likely to be related, so the offspring may not achieve an increased genetic diversity from outbreeding (Beaurepaire et al. 2019). Multiple invasions also have a negative impact on the mite. As the number of invasions per cell increases, fewer eggs are laid per mite and offspring mortality increases (Martin 1995). Interestingly, even after a successful invasion of a brood cell, female mite infertility is relatively high. Low fertility is caused by several factors including male mortality, such as by crushing or dislodging by the pupa, which leads to unfertilized mites and an 'unsuccessful' mite invasion (Martin 1997, 2001; Nganso et al. 2020). Male mortality tends to be higher during the winter (Martin 2001) and can lead to mature females leaving the cell without mating (Martin et al. 1997, Häußermann et al. 2020). The failure of a foundress to lay eggs within the brood cell can also be caused by delayed oviposition or by a low number of spermatozoa stored in her spermatheca (Harris and Harbo 1999).

Egg Laying and Mating

Semiochemicals are also involved in *V. destructor*'s egg laying behavior within a brood cell. The capping of the cell triggers oocyte activation in the foundress, which is caused by a signal lasting about 14 hr postcapping (Garrido et al. 2000, Garrido and Rosenkranz 2003, Rosenkranz and Garrido 2004). *Varroa* mating occurs within the cell and is also started by chemical cues. The male mite detects six chemicals emitted from the newly mature females, which lead to mating: oleic acid, palmitic acid, stearic acid, ethyl palmitate, ethyl oleate, and ethyl stearate (Ziegelmann et al. 2013). Once a younger female mite matures, the male stops mating with the older female and mates with the newly mature female (Ziegelmann et al. 2013).

Camouflage

Varroa mites rely on chemical ecology to remain hidden inside a honey bee hive and uses passive chemical camouflage to stay undetected by the bees. The CHCs of bee pupae have a high alkene-to-methyl alkane ratio, while the CHCs in adult bees have a low alkene-to-methyl alkane ratio (Kather et al. 2015). This means that the mite has to change its chemical profile with every pupa-to-adult or adult-to-pupa host switch. The mite needs direct contact with the bee's cuticle to make this change, which takes between three and nine hours. This has been shown to occur with dead mites as well as live mites, meaning that the change in CHC profiles is passive (Kather et al. 2015). There are also some chemicals in the hive that have possible negative or repulsive reactions on *V. destructor*. For example, (Z)-8-heptadecene has been shown to reduce mite reproduction when applied to larval cells (Milani et al. 2004), resulting in a significant reduction in the number of possibly mated daughters. This reduction could disrupt the *Varroa* population within a colony, but more studies on this aspect of *Varroa*'s chemical ecology are still needed.

Concluding Remarks

Varroa destructor is the biggest enemy currently faced by the beekeeping industry worldwide. The swift spread of the *Varroa* mite has caused detrimental impacts on *A. mellifera* populations, from substantial viral spread to massive colony losses around the world. The genetics of the mite is important for understanding and developing novel methods of chemical control against the mites. Recent studies suggest that the genetic diversity of *V. destructor* has been historically underestimated and thus, more studies are needed to measure the mite's genetic structure in all the countries where it is present. While the life cycle of the mite is generally well understood, there is still a lack of behavioral studies on the dispersal phase. A better understanding of the dispersal phase would shed light on the decision-making process of cell invasion by *Varroa*. Moreover, the mite's reliance on chemical communication for mating and host selection is an important component of the mite's behavior that begs to be studied further. Determining how chemical ecology is involved in mite host choice during the reproductive phase would be a substantial step for future mite control methods. In conclusion, the behavioral ecology of the *Varroa* mite needs to be fully understood before we are able to truly understand and control this devastating honey bee parasite on a global scale.

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Author Contributions

T.R.: Conceptualization, investigation, methodology, visualization, writing - original draft, writing – review and editing. J.R.: Conceptualization, funding acquisition, methodology, resources, supervision, writing - original draft, writing – review and editing.

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