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**Review article**

# *Varroa* resistance in *Apis cerana*: a review

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**Abstract** – *Varroa* is a major world-wide pest to Western honey bees (*Apis mellifera*), causing huge ongoing losses of colonies every year. Conversely, the Eastern honey bee (*Apis cerana*) is less vulnerable to the mite having existed alongside it over a long evolutionary period. Research conducted during the 1980s and 1990s, shortly after *Varroa* had spread across the globe, concluded that the Eastern honey bee was less vulnerable because it displayed higher levels of grooming behaviour, brood removal behaviour and mite infertility than its Western counterpart. However, this review on these *Varroa* resistance traits in *A. cerana* indicates that there is surprisingly little evidence for these conclusions. This review explores this evidence and discusses the potential flaws in the studies and the gaps that still remain in our knowledge of *Varroa* resistance traits in *A. cerana.*

***Apis cerana* / *Varroa* resistance / grooming / brood removal / *Varroa* infertility**

## 1. INTRODUCTION

*Varroa* is a genus of ectoparasitic mite which parasitises honey bee colonies across the world. Female *Varroa* mites live on the body of adult honey bees and reproduce in the brood cells alongside the developing honey bee pupae. *Varroa* garnered attention approximately 70 years ago when the now infamous species *Varroa destructor* jumped from its original host, the Eastern honey bee (*Apis cerana*) to the Western honey bee (*Apis mellifera*) (Oldroyd 1999). This jump was actually a collection of independent species jumps that were made possible because *A. mellifera* populations were moved into regions in the native range of *A. cerana* (Roberts et al. 2015; Rosenkranz et al. 2010). Once the parasite switched host, it was accidentally traded worldwide with its Western honey bee hosts, excluding the island of Newfoundland (Shutler et al. 2014), causing widespread colony losses particularly in regions within the Northern hemisphere. To date, *Varroa* is still a major pest in the Northern hemisphere and financial burden to Western honey bee apiculture (Rosenkranz et al. 2010). Eastern honey bees, on the other hand, suffer fewer negative effects of the parasitisation and generally lack the need for human intervention (Lin et al. 2016). Indeed, *A. cerana* can be described as resistant to the mite, which is defined here as the ability of a *Varroa* infested colony to survive long term, without control methods administered by humans, within a given environment (Grindrod and Martin 2021).

Over the decades since *Varroa* spread outside Asia, *Varroa* resistance has been increasingly observed in western honey bees. Firstly, resistance developed within the Cape and Savannah honey bees in Africa (Allsopp 2006; Nganso et al. 2018) and the Africanised bees of South America (Moretto et al. 1991). Resistance

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developed fairly quickly in these areas, and this is thought to be because of the lack of chemical control and the more naturalised bee keeping style (Grindrod and Martin 2021). Indeed, this is also true of European honey bees in Cuba, a country which is believed to contain the largest population of resistant European honey bees (Luis et al. 2022). However, resistance has begun to be observed in Europe and the USA (Oddie et al. 2018; Martin et al. 2019; Hawkins and Martin 2021; Grindrod and Martin 2021). Research on these populations suggests that *Varroa* resistance is the product of a number of resistance traits that regulate the populations of mites within the colony. These traits include brood removal behaviour, which is the removal of dead or diseased or *Varroa*-infested pupae, grooming behaviour in which bees remove mites from themselves or other individuals, recapping behaviour in which infested cells are opened and resealed and finally mite infertility where mites are incapable of producing viable offspring. Three of these traits brood removal, grooming and mite infertility are the same as those previously reported in *A. cerana*. The fourth behaviour, recapping, has not been studied in *A. cerana* as it was more recently discovered by Oddie et al. (2017). Data, from *A. cerana*, on all four of these traits would therefore be beneficial comparison and be a valuable asset in understanding *Varroa* resistance in *A. mellifera.* Recently, an evidence-driven framework was constructed to suggest how these traits may interlink and allow for the development of resistance in *A. mellifera* (Grindrod and Martin 2021). However, it is not possible to see if the same occurs in *A. cerana* as there exists relatively little published data, and the data that does exist is from studies with small sample sizes and outdated knowledge including the absence of the identification of *Varroa destructor* (Anderson and Trueman 2000).

Henceforth, our understanding of the relationship between *Varroa* and *A. cerana* and consequently resistance traits is limited and often based on assumptions, which are then used to make further assumptions about *A. mellifera*. With the continual advancement of *Varroa* research methodologies, it seems prudent that the relationship between *Varroa* and *A. cerana* is re-evaluated. In this review, the major areas of research into *Varroa* resistance traits, grooming, brood removal and mite infertility, in *A. cerana* are outlined and discussed to identify gaps and provide suggestions for future research.

## 2. GROOMING

Grooming behaviour is often included in the suite of behaviours used in defence against *Varroa.* It entails adult bees removing mites either from themselves (auto-grooming) or from other adult bees (allo-grooming) using their legs and mandibles (Pritchard 2016). The removal and possible injury of the mites is thought to control the size of the phoretic mite population and thus the overall colony infestation (Moosbeckhofer 1992). However, grooming behaviour is notoriously hard to measure accurately as it relies either on indirect measurements such as mite damage or from potentially subjective direct observations. Despite this, a single study by Peng et al. (1987) (cited almost 300 times in web of science, accessed 17/02/22) appears to have led to the acceptance of *A. cerana* as the superior groomer over *A. mellifera* and from this the assumption that grooming is a considerable factor of *Varroa* resistance. Certainly, at first glance, the results are very enticing as the 99.6% removal of mites by *A. cerana* vastly overshadows the 0.3% removal by *A. mellifera* seemingly solving the mystery of why *A. cerana* are more resistant in one shot. There are, however, a number of reasons to be highly sceptical of the results.

### 2.1. The issue of mite source

Firstly, Peng et al. (1987) used mites from *A. mellifera* colonies on *A. cerana* adults. This is likely to have unintentionally exaggerated the results because *A. cerana* respond much more strongly to mites sourced from *A. mellifera* colonies compared to their own species (Büchler et al. 1992; Fries et al. 1996; Rath 1991a; Rosenkranz et al. 1993a, b). This

heightened response could possibly occur because the mites are a different species (*V. destructor*) and/or because the mites have mimicked the original hosts (*Apis mellifera*) cuticular hydrocarbons (Kather et al. 2015; Le Conte et al. 2015; Martin et al. 2001) which can rapidly be detected as foreign by the new *A. cerana* hosts (Fries et al. 1996; Rahman et al. 2016; Rath 1991a).

### 2.2. Limitations to direct observation methods

The results may also have been spuriously elevated because, to assess grooming ability, Peng et al. (1987) attempted to directly observe the adult bees undertaking the behaviour. Naturally, this approach is prone to inaccuracy because it is difficult to follow individual mites and to be sure of their fate (Fries et al. 1996). As a result, the authors erroneously considered both the movement of mites from one bee to another and the disappearance of mites to the observer as a successful removal. Indeed, the potential inflation of the results was highlighted nearer the time in a review by Boecking et al. (1993). They also indicated that a constant removal rate as high as 99% would mean that *A. cerana* colonies would be devoid of mites during periods when drone brood is absent, which is not the case. Nonetheless, despite these shortfalls, the Peng et al. (1987) article is still highly cited with 19 citations in 2021 (web of science, accessed 17 Feb. 22).

Since its publication, only three other studies have sought to repeat or re-evaluate these results (Table I). Büchler et al. (1992) also utilised the direct observation method and used a mix of phoretic and brood mites for *A. mellifera* colonies. They did improve the methodology by using phoretic *A. cerana* mites for the *A. cerana* colonies; however, they chose to source these mites from different *A. cerana* colonies. This may still affect the results because *Varroa* mites can mimic the colonies cuticular hydrocarbon profile down to the level of each colony (Kather et al. 2015). Despite this their results appear more realistic, in terms of the earlier criticism by Boecking et al. (1993), with 75% removal rather than 99% for *A. cerana.* They also found a much greater result for *A. mellifera* at 48% removal, but both figures need to be interpreted with care as they were based on small sample sizes of 36 and 25 mites, respectively.

### 2.3. Mite damage as a proxy for grooming ability

To avoid the issues with direct observation experiments Fries et al. (1996) used mite damage

**Table I** Details of previous studies conducted on the grooming behaviour in *A. cerana*

| Author | *Varroa* source | *A. cerana* grooming (%) | *A. mellifera* grooming (%) | Observation time | Hive type | How grooming is assessed? |
|---|---|---|---|---|---|---|
| Peng et al. (1987) | *A. mellifera* — brood and phoretic | 99.6 ($n=270$) | 0.3 ($n=270$) | Up to 2 h | Observation hive | Direct observation |
| Büchler et al. (1992) | *A. cerana* phoretic, *A. mellifera* — phoretic and brood | 75 ($n=36$) | 48 ($n=25$) | 10 min | Observation hive | Direct observation |
| Fries et al. (1996) | *A. mellifera* phoretic | 29.6 ($n=115$) | 12.3 ($n=65$) | 6 h | Full size Langstroth hives | No. of damaged mites |
| Rath (1991a) | *A. mellifera* phoretic | 61.7* | 2.8* | 48 h | Cage experiment | No. of dead mites |

*Sample size could not be ascertained

as a proxy for grooming success. During grooming, mites can endure damage to their idiosoma and legs caused by the bee's mandibles (Rosenkranz et al. 1997; Ruttner and Hänel 1992). Using this as a proxy allowed the experiment to be conducted in a normal, full-size hive compared to the smaller observation hives used in previous studies. Fries et al. (1996) found that, over a 6-h period, 29.6% of introduced mites were damaged by *A. cerana* and 12.3% by *A. mellifera*. The result for *A. cerana* may again have been impacted by the use of *A. mellifera* mites although the difference between the species is notably smaller than the results of both Peng et al. (1987) and Büchler et al. (1992). The smaller difference could be the result of using an indirect method; however, this is not supported by Rath (1991a). They recorded the number of mites, sourced from *A. mellifera*, which died and were injured when introduced to adult bees in a cage experiment. Furthermore, Peng et al. (1987) also found a large difference between the two species, 61.7% of introduced mites died on *A. cerana* in 48 h, whereas only 2.8% died on *A. mellifera.* Of those that died, they found that 83% from *A. cerana* had injuries whilst none of the dead mites from *A. mellifera* showed any sign of injury.

### 2.4. The uncertainty caused by using a proxy

Measuring grooming indirectly brings its own level of uncertainty to the results because grooming is not the single cause of damage to mites. Mites may also be damaged when infested brood cells are cleaned out (Boecking and Drescher 1991) or by other hive predators such as ants (Bienefeld et al. 1999; Davis et al. 2007) or wax moth (Szabo and Walker 1995). Care also needs to be taken when observing damage to the idiosoma to prevent regular dorsal dimples, a developmental defect, from being confused for grooming induced damage (Davis 2009; Rosenkranz et al. 1997). Also, as with the observation methods, Fries et al. (1996) noted that the artificial introduction of mites into a colony substantially increased the initial mite drop. Additionally, the presence of emerging brood increases the mite fall and mite damage (Hoffman 1995; Lobb and Martin 1997; Martin and Kemp 1997; Rosenkranz et al. 1997) thus adding to the variability of measurements. It is also difficult to conclude whether the damage occurred pre or post mortem; for example, Fries et al. (1996) found that in an *A. mellifera* colony, 26.4% of naturally fallen dead mites (killed by freezing the combs) had damage but only 9.1% of naturally fallen live mites were damaged, suggesting that either bees injure dead mites or that the injury caused by bees leads to the death of mites.

### 2.5. Summary

It is widely believed that *A. cerana* perform grooming behaviour to a higher extent than *A. mellifera* and subsequently that this behaviour plays a large role in the resistance of *A. cerana* to *Varroa*. However, this belief is based on only four studies with a disproportionate amount of belief placed on a single, highly cited, study by Peng et al. (1987) that may have falsely elevated results due to flaws in the methodology. Additionally, there are many discrepancies between the methodologies used by the four studies. Grooming is also highly variable both within and between colonies due to the season (Büchler 1993; Mondragón et al. 2005; Moosbeckhofer 1997; Russo et al. 2020), environmental conditions (Currie and Tahmasbi 2008), presence of emerging brood (Hoffman 1995; Lobb and Martin 1997; Martin and Kemp 1997; Rosenkranz et al. 1997) and levels of brood removal behaviour (Kirrane et al. 2018). This variability means that many measurements are required to increase the accuracy of results. Thus, the existing four studies do not provide enough data to accurately suggest the magnitude of the role that grooming plays in *Varroa* resistance in *A. cerana*.

Indeed, despite the larger number of studies on grooming in *A. mellifera*, the results have been highly variable. In some instances, resistant colonies have been found to groom to significantly more (Mendoza et al. 2020) and some studies found a negative correlation between mite damage and infestation rate (Arechavaleta-Velasco and Guzmán-Novoa 2001; Mondragón

et al. 2005; Moosbeckhofer 1992; Ruttner and Hänel 1992). Conversely, many others have found the opposite with no significant difference between resistant and susceptible populations suggesting that grooming does not significantly contribute to resistant behaviour (Aumeier 2001; Kovačić et al. 2018; Kruitwagen et al. 2017; Locke and Fries 2011; Nganso et al. 2017; Oddie et al. 2018). Certainly, when the impact of reduced mite fertility is considered, grooming behaviour is not necessary to explain *Varroa* resistance in *A. mellifera* (Locke and Fries 2011; Oddie et al. 2017) or in *A. cerana* in which there is complete infertility of mites in the worker brood (Fries et al. 1994). This is why grooming was not included in the framework proposed by Grindrod and Martin (2021).

## 3. BROOD REMOVAL

Brood removal is the archetypal hygienic behaviour in which adult bees uncap and remove dead, diseased or parasitised pupae. Whilst it is used in response to *Varroa* infestation, it was first described as a response to American foulbrood (Rothenbuhler 1964) and then to chalkbrood (Gilliam et al. 1983). Such brood diseases usually result in the death of the pupae and in turn the release of potent death pheromones such as oleic acid (McAfee et al. 2018). As a consequence of this and the hazards involved in inoculating pupae with diseases, hygienic behaviour has typically been measured using methods that cause the death of the pupae including freeze-killed brood (FKB) and pin-killed brood (PKB) methods (Spivak and Downey 1998).

### 3.1. The results and limitations of FKB methodology

*A. cerana* respond well to FKB; they remove fairly high levels of FKB, 82% in 24 h (Rath and dresecher 1990) and also remove it faster than *A. mellifera* (Lin et al. 2016; Shakeel et al. 2020). However, *A. cerana* colonies can be highly susceptible to the brood diseases sac brood (Abrol 2000; Ai et al. 2012; Hassanyar et al. 2019; Ma 2014; Vung et al. 2020) and American foul brood (Chen et al. 2000). This suggests that high FKB results do not necessarily equate to a high hygienic response to disease which may be because disease transmission can be enhanced by hygienic behaviours particularly due to removing already deceased brood containing highly infective elements.

Additionally, whilst FKB and PKB can offer some insight into the general hygienic capabilities of a colony, they have so far failed to correlate with the results of *Varroa*-infested brood removal (Boecking and Drescher 1992; Danka et al. 2013; Grindrod and Martin 2021; Leclerq et al. 2018; Martin et al. 2019). Arguably, this is because *Varroa* rarely kills the developing brood, and thus, the cues used by workers to detect a *Varroa* infestation are different (Mondet et al. 2021; Spivak 1996).

### 3.2. Artificial mite infestation experiments

Ultimately, due to their observed natural resistance to the mite, *A. cerana* are generally believed to express a higher level of brood removal behaviour than *A. mellifera.* However, despite a plethora of anecdotal evidence, this literature search only found three studies that measured the ability of *A. cerana* to remove cells artificially or naturally infested with *Varroa*. The first of these papers by Rath and Drescher (1990) found very high removal rates, 97.4% and 91.9%, of *A. cerana* worker cells artificially infested with live and dead ethanol-washed mites respectively, which indicated the ethanol wash had little, if any, effect. The mites used were again sourced from *A. mellifera* colonies which, as with grooming, will artificially increase the removal response (Boot et al. 1999; Rosenkranz et al. 1993a, b) due to different chemical profiles. One could argue that the scent of previous hosts would be negated by the ethanol wash of the dead mites; however, even when washed with ethanol, cuticular hydrocarbons, potentially from the original host, are very likely to remain on the mite (da Silva Cunha et al. 2021). Boot et al.

(1999) found that *A. cerana* however removed 84% of mite (sourced from *A. mellifera*)-infested worker brood cells over 10 days, which whilst lower is still a high result for the removal of *Varroa* brood in comparison to resistant western honey bees (Grindrod and Martin 2021). Although, a separate experiment by Boot et al. (1999) comparing both species showed that *A. mellifera* and *A. cerana* removed a similar percentage of *Varroa*-infested cells over 4 days, 32% ($n = 104$) and 29% ($n = 131$) respectively.

Additionally, the results of Rath and Drescher (1990) may also be somewhat artificially inflated as the results include the cells in which mites had disappeared from as well as fully emptied cells. Whilst workers do seem to be able to remove dead mites and re-seal cells (Rosenkranz et al. 1993a, b) live mites pose more of a challenge to remove and can also exit cells of their own volition whilst the cell is left open. This uncertainty means that the "disappearance" of live and dead mites should ideally be reported as a separate statistic as exemplified by Rosenkranz et al. (1993a, b) and Boot et al. (1999) (Table II). Live mites also "disappear" in *A. mellifera* colonies (De Guzman et al. 2016).

### 3.3. Observations of natural mite infestation

Boot et al. (1999) noted, albeit without numerical evidence, a low removal response in naturally infested colonies, but this may be because of the low infestation rates and thus low levels of stimulus. Conversely, low natural responses may also be the result of the fact that *A. cerana* mites avoid reproducing in worker brood and thus do not produce the cues necessary to be detected (Mondet et al. 2021). This may also explain why unlike Rath and Drescher (1990) who found an immediate high removal response, Boot et al. (1999) noticed that the removal response of *A. mellifera* mites was delayed by a couple of days, as time may be needed for reproduction to produce the cues. Accordingly, in *A. mellifera*, peak removal has been shown to occur roughly 3–5 days post capping (Harris 2007; De Guzman et al. 2016). Although, if a low removal response is due to a lack of reproduction, then it is not easy to explain why Rosenkranz et al. (1993a, b) found a low removal response of 8% in *A. cerana* when mites were transferred within the same colony (intracolonial) but a high removal response of 50% with mites from a different *A. cerana* colony (intercolonial). Mites from another *A. cerana* colony would be likely to avoid reproducing in worker brood to the same degree.

*Cells resealed without mite was not treated as a separate statistic and raw data was unobtainable.

### 3.4. The social apoptosis phenomenon

Indeed, other studies have suggested that brood removal may be stimulated by damage to the pupae rather than scents from the mite. Page et al. (2016) and Lin et al. (2018) discovered that the worker pupae of *A. cerana* in Thailand and China were more susceptible to wounding and infestation by *V. destructor* of the Korean haplotype than *A. mellifera* pupae. The increased susceptibility meant that *A. cerana* pupae were more likely to be developmentally delayed and die, which would simultaneously prevent successful mite reproduction and provide a signal to worker bees for removal (Lin et al. 2018). As a result, they termed the phenomenon social apoptosis. In support of this, Zhang et al. (2018) discovered a protein in the saliva of mites called *Varroa* toxic protein, or VTP, which was extremely toxic to *A. cerana* worker brood but not *A. mellifera*. However, whilst these results are promising, they seemingly lack support from previous mite reproduction studies in which an enhanced death rate of worker brood was not observed, or at least not recorded (Koeniger et al. 1981, 1983; Rath 1991a; Rosenkranz et al. 1993b). Although, in the majority of these previous infertility studies, *V. jacobsoni* was the infesting mite and may differ to *Varroa destructor* in terms of the impact of wounding towards its host. It may also be different with other haplotypes of *V. destructor* (Lin et al. 2018) again highlighting the need for more research.

**Table II** Details on the studies conducted on *Varroa*-infested worker brood removal behaviour in *A. cerana*

| Study | Emptied cells (%) | Cells resealed without mite (%) | *Number* | No. Colonies | Control cells removed (%) | *Number* | Observation time (days) | Mite source/ status |
|---|---|---|---|---|---|---|---|---|
| Rath and Drescher (1990) | 97%* | | 105 | Not stated | 13% | 107 | 5 | *A. mellifera* brood |
| | 92%* | | 148 | Not stated | 12% | 149 | 5 | Dead *A. mellifera* mites (ethanol washed) |
| Boot et al. (1999) | 84% | 7% | 127 | 10 | 4% | 122 | 10 | *A. mellifera* phoretic |
| | 29% | 27% | 131 | 10 | – | – | 4 | *A. mellifera* phoretic |
| | 0% | 0% | 13 | 10 | – | – | 4 | *A. cerana* (natural infestation) |
| Rosenkranz et al. (1993a) | 8% | 40% | 26 | 5 | 10% | 62 | 5 | *A. cerana* (intracolonial transfer) |
| | 50% | 20% | 74 | 5 | – | | 5 | *A. cerana* (intercolonial transfer) |
| | 62% | 30% | 29 | 5 | – | | 5 | *A. mellifera* |
| | 40% | 5% | 46 | 5 | – | | 5 | Dead *A. cerana* mites (ethanol washed) |

### 3.5. Summary

The removal of *Varroa* infested brood is thought to be the cornerstone for resistance in honey bee populations (Grindrod and Martin 2021). Unfortunately, however, there remains a lot missing in our understanding of brood removal behaviour in *A. cerana*. Firstly, as with grooming, there is very little data concerning the removal of artificially infested cells which combined with the high variability of the behaviour means we do not have a reliable indicator of its relevance to resistance. Additionally, as with grooming behaviour, the methodology used to collect the data varies between studies. Indeed live mites are often sourced from *A. mellifera* colonies for use on *A. cerana*, and sometimes, dead mites are used. There are additional data on the removal of FKB; however, as *Varroa* does not usually kill, the brood FKB ability does not tend to correlate with the ability to remove infested brood. As a final note, there is a distinct lack of clarity concerning the phenomenon entitled social apoptosis, in particular, its prevalence and whether it occurs with both mite species and all the haplotypes.

## 4. MITE INFERTILITY

### 4.1. Infertility of Varroa in Apis cerana worker brood under natural conditions

The definition of mite infertility can include *Varroa* females producing no eggs at all or

*Varroa* females failing to produce viable, i.e. fully matured and mated, female daughters. The former definition, also known as strict or complete infertility, was used in the studies conducted in the 80 s and 90 s and thus applies to the data reviewed here. Infertility was first reported as a characteristic of the natural infestation of *Varroa* mites on *A. cerana* worker brood in Sri Lanka and Java (Koeniger et al. 1981, 1983) and has since been reported in Vietnam (Boot et al. 1997), Papua New Guinea, Java, Irian Jaya (Anderson 1994), India (Rosenkranz et al. 1993b; Twearson et al. 1992). De Jong (1988) did note some rare incidences of reproduction under natural circumstances in worker brood cells in South Korea. Similarly, a recent study by Wang et al. (2020) that investigated 185 *A. cerana* colonies of six populations in China and Thailand found only one incidence of reproduction in a *Varroa* mite naturally infesting *Apis cerana* worker brood in the south of Thailand, and this incidence was not a successful attempt.

### 4.2. The potential causes of Varroa infertility in worker brood

Whilst this infertility is fairly well-documented, the exact cause remains elusive. Research by Grindrod and Martin (2021) on *A. mellifera* has suggested that a cause of infertility is simply the disruption of reproduction due to brood removal. They suggest that continual high levels of targeted brood removal could cause mites to avoid worker brood in favour of drone brood that is not removed in *A. mellifera* (Grindrod and Martin 2021) or *A. cerana* (Harris 2008). In speculation, this may have occurred in *A. cerana* with the resultant separate evolution of *Varroa* reproduction and worker pupal development leading to a loss of synchrony in the cycles and thus infertility of the mites. This loss of synchrony could include the loss of specific oogenesis triggers from the pupal host which are normally acquired by the mite when feeding. Although, these triggers could also be lost via selective pressure from mites.

Alternatively, infertility may be a factor of the mites not the pupae. This was suggested by the work of Boot et al. (1999) that showed that mites from an *A. cerana* colony will not reproduce in the worker brood of another *A. cerana* or *A. mellifera* colony if transferred but that mites from an *A. mellifera* colony will. Rath (1991b) also found it was possible to get *Varroa* mites from *A. mellifera* to reproduce on *A. cerana* worker brood in a lab setting. Boot et al. (1999) propose that the loss of fertility was the result of *A. cerana* removing reproducing mites more frequently and thus inadvertently selecting for non-reproducing mites. Indeed, a bias toward the removal of reproductive mites is possible because mite reproduction may be required to produce a stimulus that the bees can detect (Mondet et al. 2021).

### 4.3. The fertility of Varroa on *A. cerana* worker brood during artificial infestation

Importantly, due to the time period of the Boot et al. (1999) study, the mites in question were identified as *Varroa jacobsoni*. However, at the time in Vietnam, it appears that it was actually *Varroa destructor* of the Korea and Vietnam haplotypes that were parasitising *Apis mellifera* and *Apis cerana* respectively (Fuchs et al. 2000). Therefore, Boot et al. (1999) showed that it is possible for *V. destructor* of the Korea haplotype, not *V. jacobsoni*, to reproduce on the worker brood of *A. cerana*. Since then, this observation has been repeated by Li et al. (2019) who discovered that, in China, *Varroa* of the Korea haplotype sourced from *A. mellifera* colonies could reproduce in the worker brood of both honey bee species, albeit at a higher fecundity in *A. mellifera*. They also found that much like the Vietnam haplotype, the China haplotype from *A. cerana* colonies was completely sterile in the worker brood of *A. mellifera* and *A. cerana* colonies Li et al. (2019).

In contrast, Le conte et al. (2015) found that *Varroa destructor* from *Apis cerana* could

reproduce in the worker brood of *both* honey bee species when transferred. However, whilst this study also took place in China, the haplotype of *V. destructor* infesting *A. cerana* was the Korean haplotype 3 and not the China haplotype. The Korean haplotype 2 from *Apis mellifera* also reproduced on both honey bee species. Although, it is worth noting that the sample sizes in this study were very small with only 6 to 10 mites transferred per swap.

Finally, the equal fertility of the Korean haplotype on *A. cerana* and *A. mellifera* worker brood in China was also observed by Lin et al. (2018). However, this fertility was facilitated by the removal of adult bees during the experiment. When adult bees were present a high proportion of the infested pupae were removed which Lin et al. (2018) suggest was due to the developmental delays caused by *Varroa*.

### 4.4. The fertility of *Varroa jacobsoni* parasitising *Apis mellifera*

In the absence of competition with *V. destructor*, in Papua New Guinea (PNG), Roberts et al. (2015) found that it is possible for *V. jacobsoni* to overtime develop the ability to reproduce in both the drone and worker brood of *A. mellifera* colonies. Initially, these attempts to reproduce were directed at the drone brood and were largely unsuccessful (Anderson 1994). However, by 2008 V. *jacobsoni*, was reproducing in high numbers on both drone and worker brood in PNG (Anderson 2008), and later, Roberts et al. (2015) discovered that there had been two independent host shifts of *V. jacobsoni* onto *A. mellifera*. Thus, it does appear that the infertility of *V. jacobsoni* on *A. cerana* is a product of their relationship that may be reversible if the barriers to reproduction are removed. And in speculation, if these barriers are created in *A. mellifera* populations, then perhaps the same infertility of mites in worker brood can be established. Although it is important to note that in PNG deformed wing virus is also absent which will alter the relationship between the mite and the honey bee host (Roberts et al. 2020).

### 4.5. Summary

Mite infertility is the most strongly supported trait in *A. cerana*; however, there is no consensus on its origin or how it is maintained. Mite infertility in worker brood appears to play a large role in resistance and is believed to be the main reason why *A. cerana* do not succumb to the mite. Given that some studies have found that it is possible to get *V. destructor* to reproduce on *A. cerana*, it seems worthwhile to explore this relationship further as it may allude to the cause of the infertility of *V. jacobsoni*. For example, whether it is the result of brood removal in general or the result of selection by removing reproducing mites and/or the lack of cues in the pupal feed. Additionally, it would be beneficial to understand the differences, if any, between the *Varroa* species and haplotypes and their ability to reproduce on different honey bee species and castes (Figure 1).

## 5. THE GENETICS OF RESISTANCE

In *Apis mellifera*, there has been a considerable amount of research into the genetic basis of resistance traits; a major goal of this research is to identify molecular markers associated with the traits that could be used to aid the breeding of resistant bees (reviewed in Mondet et al. 2020). Thus far, olfactory sensitivity has generally been highlighted as an integral process for resistance traits (Mondet et al. 2020). Despite this, there is low agreement in the candidate genes isolated by different studies. This is likely due to a number of factors including the variation in the presentation of behavioural traits, the complexity of the underlying genetics and the different sub-species utilised in these studies (Mondet et al. 2020; Traynor et al. 2020).

As one might expect, the research into the genetic basis of resistance traits is comparatively lacking in *Apis cerana* although efforts have been increasing as the ‘omics methods have become more accessible and affordable (Diao et al. 2018; Ji et al. 2014a, b, 2015; Ling et al. 2015). Thus far, Ji et al. (2014a, b, 2015) and Ling et al. (2015)

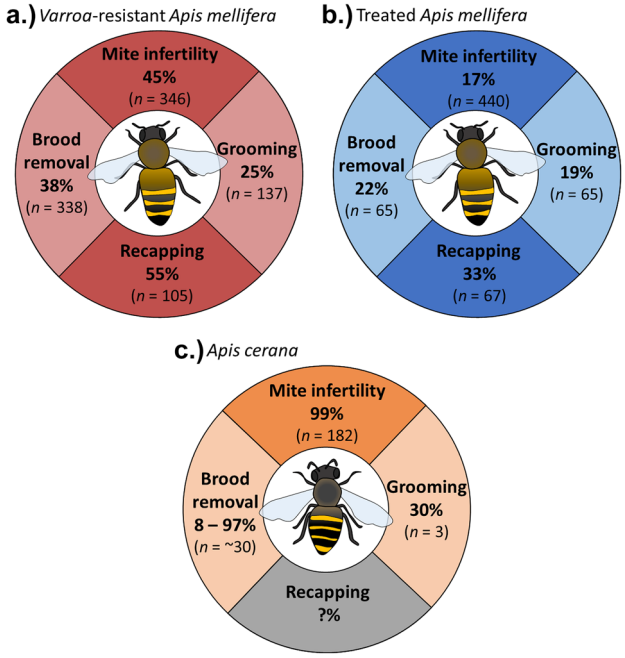


**Figure 1.** Summary of resistance traits displayed in **a** *Varroa*-resistant *A. mellifera*, **b** *Varroa*-treated *A. mellifera* and **c** *A. cerana*. *n* = the number of colonies studied. Data for mite infertility, brood removal and recapping in **a** and **b** is taken from Grindrod and Martin (2021); for studies used to calculate grooming in **a**. and **b**, see supplementary data. Data for **c** comes from this study. All grooming averages are based on results using the mite damage proxy

have discovered an upregulation in genes related to olfactory sensitivity in *A. cerana* after mite introduction. This suggests that olfactory sensitivity may play a key role in expression of the resistant traits in both *A. mellifera* and *A. cerana.* However, genome comparisons by Diao et al. (2018) discovered that the *A. cerana* genome contains fewer genes encoding odorant binding proteins and olfactory receptors than the *A. mellifera* genome. How that translates to the expression of resistance traits olfactory sensitivity is not clear, but it does highlight the need for more research in this area in order to make meaningful comparisons between *A. mellifera* and *A. cerana.*

## 6. CONCLUSION

Here, the main areas of *Varroa-A. cerana* research have been presented, and gaps in the research have been highlighted and discussed. Ultimately, what has become clear is that our assumptions about the ability of *A. cerana* to perform the resistance traits grooming and brood removal are largely based on only a small number of decade old studies, often using small sample sizes. This is problematic because there is considerable natural variation in the displaying of resistance traits between colonies. Some variation exists naturally within and between populations, but it is amplified by many other factors including the seasons, environmental conditions, mite infestation levels and the methodology used.

Additionally, given its more recent discovery, there are also no published data on the presence of recapping behaviour in *A. cerana* colonies. This leaves gaps in our understanding of the relationship between *A. cerana* and *Varroa*, and it is difficult to relate this to the trajectory of *A. mellifera* and *Varroa*'s relationship. Thus, there is a need to complete new research to ascertain the level of grooming, infested brood removal, recapping and

mite infertility displayed in *A. cerana* populations across different regions. Those data could provide important evidence to either support or rebuke the framework of *Varroa* resistance acquisition presented in Grindrod and Martin (2021). Such data could also be backed by further exploration of the genetic basis of resistance traits in both *A. mellifera* and *A. cerana*. Ultimately, achieving a full understanding of the development of resistance in *Apis cerana* will help inform efforts into developing and maintaining resistance in *Apis mellifera* populations thus reducing colony losses and dependency on acaricides.

## SUPPLEMENTARY INFORMATION

The online version contains supplementary material available at https://doi.org/10.1007/s13592-022-00977-8.

## AUTHOR CONTRIBUTION

I. G. conceived this research and collected, analysed and interpreted the data; I. G. wrote the paper with revisions from S. J. M; both authors read and approved the final manuscript.

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## AVAILABILITY OF DATA AND MATERIALS

Summary of grooming data used in Figure 1 is available in the supplementary data file.

## CODE AVAILABILITY

Not applicable.

## DECLARATIONS

**Ethics approval** Ethical approval was not needed for this research.

**Consent to participate** Not applicable.

**Consent for publication** Not applicable.

**Competing interests** The authors declare no competing interests.



## REFERENCES

Abrol DP (2000) Beekeeping with *Apis cerana* in Jammu and Kashmir: present status and future prospects. Bee World 81(3):149–152

Ai H, Yan X, Han R (2012) Occurrence and prevalence of seven bee viruses in *Apis mellifera* and *Apis cerana* apiaries in China. J Invertebr Pathol 109(1):160–164. https://doi.org/10.1016/j.jip.2011.10.006

Allsopp M (2006) Analysis of *Varroa destructor* infestation of southern African honeybee populations. (Mres thesis). University of Pretoria, Pretoria

Anderson DL (1994) Non-reproduction of *Varroa jacobsoni* in *Apis mellifera* colonies in Papua New Guinea and Indonesia. Apidologie 25(4):412–421. https://doi.org/10.1051/apido:19940408

Anderson DL (2008) Surveillance of parasites and diseases of honey bees in Papua New Guinea and Indonesia. CSIRO Report 1–41

Anderson DL, Trueman JW (2000) *Varroa jacobsoni* (Acari: Varroidae) is more than one species. Exp Appl Acarol 24(3):165–189

Arechavaleta-Velasco ME, Guzmán-Novoa E (2001) Relative effect of four characteristics that restrain the population growth of the mite *Varroa destructor* in honey bee (*Apis mellifera*) colonies. Apidologie 32(2):157–174. https://doi.org/10.1051/apido:2001121

Aumeier P (2001) Bioassay for grooming effectiveness towards Varroa destructor mites in Africanized and Carniolan honey bees. Apidologie 32(1):81–90. https://doi.org/10.1051/apido:2001113

Bienefeld K, Zautke F, Pronin D, Mazeed A (1999) Recording the proportion of damaged *Varroa jacobsoni* Oud. In the debris of honey bee colonies

(*Apis mellifera*). Apidologie 30:249–256. https://doi.org/10.1051/apido:19990401

Boecking O, Drescher W (1991) Response of *Apis mellifera* L colonies infested with *Varroa jacobsoni* Oud. Apidologie 22(3):237–241. https://doi.org/10.1051/apido:19910308

Boecking O, Drescher W (1992) The removal response of *Apis mellifera* L. colonies to brood in wax and plastic cells after artificial and natural infestation with *Varroa jacobsoni* Oud. And to freeze-killed brood. Exp Appl Acarol 16(4):321–329. https://doi.org/10.1007/BF01218574

Boecking O, Rath W, Drescher W (1993) Behavioural strategies of *Apis mellifera* and *Apis cerana* against *Varroa jacobsoni*. Int J Acarol 19(2):173–177. https://doi.org/10.1080/01647959308683977

Boot WJ, Calis JN, Beetsma J, Hai DM, Lan NK, Toan TV, Trung LQ, Minh NJ (1999) Natural selection of *Varroa jacobsoni* explains the different reproductive strategies in colonies of *Apis cerana* and *Apis mellifera*. Exp Appl Acarol 23:133–144. https://doi.org/10.1023/A:1006050527004

Boot WJ, Tan NQ, Dien PC, Huan LV, Dung NV, Long LT, Beetsma J (1997) Reproductive success of *Varroa jacobsoni* in brood of its original host, *Apis cerana*, in comparison to that of its new host, *A. mellifera* (Hymenoptera: Apidae). Bull Entomo Res 87(2):119–126. https://doi.org/10.1017/S0007485300027255

Büchler R (1993) Rate of damaged mites in natural mite fall with regard to seasonal effects and infestation development. Apidologie 24:492–493

Büchler R, Drescher W, Tornier I (1992) Grooming behaviour of *Apis cerana*, *Apis mellifera* and *Apis dorsata* and its effect on the parasitic mites *Varroa jacobsoni* and *Tropilaelaps clareae*. Exp Appl Acarol 16:313–319. https://doi.org/10.1007/BF01218573

Chen Y-W, Wang C-H, An J, Kai-Kuang H (2000) Susceptibility of the Asian honey bee, *Apis cerana*, to American foulbrood, *Paenibacillus larvae larvae.* J Apic Res 39(3–4). https://doi.org/10.1080/00218839.2000.11101038

Currie RW, Tahmasbi GH (2008) The ability of high- and low-grooming lines of honey bees to remove the parasitic mite *Varroa destructor* is affected by environmental conditions. Can J Zool 86:1059–1067. https://doi.org/10.1139/Z08-083

Danka RG, Harris JW, Villa JD, Dodds GE (2013) Varying congruence of hygienic responses to *Varroa destructor* and freeze-killed brood among different types of honey bees. Apidologie 44(4):447–457. https://doi.org/10.1007/s13592-013-0195-8

Da Silva Cunha DA, Menezes RST, Cardoso CAL, Antonialli WF Jr (2021) Is it possible to obtain the chemical profile from ethanol-preserved specimens? The hydrocarbon and fatty acid composition of the social wasp *Polybia paulista* (hymenoptera: vespidae: epiponini). Environ Entomol 50(3):580–588. https://doi.org/10.1093/ee/nvab010

Davis AR (2009) Regular dorsal dimples on *Varroa destructor* – damage symptoms or developmental origin? Apidologie 40:151–162. https://doi.org/10.1051/apido/2009001

Davis AR, Bikey D, Mirakhur A, Dyck D (2007) Direct encounters and effect of vapours from three ant species (Formicidae) on *Varroa destructor* mites in laboratory trials. J Apic Res 46(4):282–290. https://doi.org/10.1080/00218839.2007.11101409

De Guzman LI, Rinderer TE, Frake AM, Kirrane MJ (2016) Brood removal influences fall of Varroa destructor in honey bee colonies. J Apic Res 54(3):216–225. https://doi.org/10.1080/00218839.2015.1117294

De Jong D (1988) *Varroa jacobsoni* does reproduce in worker brood cells of *Apis cerana* in South Korea. Apidologie 19(3). https://doi.org/10.1051/apido:19880303

Diao Q, Sun L, Zheng H, Zeng Z, Wang S, Xu S, Wu J (2018) Genomic and transcriptomic analysis of the Asian honey bee *Apis cerana* provides novel insights into honey bee biology. Sci Res 8:822. https://doi.org/10.1038/s41598-017-17338-6

Fries I, Camazine S, Sneyd J (1994) Population dynamics of *Varroa jacobsoni*: a model and a review. Bee World 75(1):5–28. https://doi.org/10.1080/0005772X.1994.11099190

Fries I, Huazen W, Wei S, Jin C-S (1996) Grooming behavior and damaged mites (*Varroa jacobsoni*) in *Apis cerana cerana* and *Apis mellifera ligustica*. Apidologie 27(1):3–11. https://doi.org/10.1051/apido:19960101

Fuchs S, Long LT, Anderson D (2000) A scientific note the genetic distinctness of *Varroa* mites on *Apis mellifera* L. and on *Apis cerana* Fabr. in North Vietnam. Apidologie, 31:459–460. https://doi.org/10.1051/apido:2000138

Gilliam M, Taber S III, Richardson G (1983) Hygienic behaviour of honey bees in relation to chalkbrood disease. Apidologie 14(1):29–39

Grindrod I, Martin SJ (2021) Parallel evolution of *Varroa* resistance in honey bees: a common mechanism across continents? Proc Royal Soc B 288(1956). https://doi.org/10.1098/rspb.2021.1375

Harris JW (2007) Bees with *Varroa* sensitive hygiene preferentially remove mite infested pupae aged ≤ five days post capping. J Apic Res 46(3):134–139. https://doi.org/10.3896/IBRA.1.46.3.02

Harris JW (2008) Effect of brood type on *Varroa*-sensitive hygiene by worker honey bees (Hymenoptera: Apidae). Ann Entomol Soc Am 101(6):1137–1144. https://doi.org/10.1603/0013-8746-101.6.1137

Hassanyar AK, Huang A, Li Z, Rizwan M, Mehmood FR, Qasim M, Su S (2019) Prevalence of bee viruses in *Apis cerana cerana* populations from different locations in the Fujian Province of China. Microbiology Open 8(9):e00830. https://doi.org/10.1002/mbo3.830

Hawkins GP, Martin SJ (2021) Elevated recapping behaviour and reduced *Varroa destructor* reproduction in natural *Varroa* resistant *Apis mellifera* honey bees from the UK. Apidologie 52:647–657. https://doi.org/10.1007/s13592-021-00852-y

Hoffman S (1995) Registration of damaged Varroa mites in small colonies for the assessment of grooming behaviour. Apidologie 26(4):322–324

Ji T, Shen F, Liu Z, Yin L, Shen J, Liang Q, Luo YX (2015) Comparative proteomic analysis reveals mite (*Varroa destructor*) resistance-related proteins in eastern honeybees (*Apis cerana*). Genet Mol Res 14(3):10103–10118. https://doi.org/10.4238/2015.August.21.18

Ji T, Yin L, Liu Z, Liang Q, Luo Y, Shen J, Shen F (2014a) Transcriptional responses in eastern honey bees (*Apis cerana*) infected with mites. Varroa Destructor Genet Mol Res 13(4):8888–8900. https://doi.org/10.4238/2014.October.31.4

Ji T, Yin L, Liu Z, Shen F, Shen J (2014b) High-throughput sequencing identification of genes involved with *Varroa destructor* resistance in the eastern honeybee. Apis Cerana Genet Mol Res 13(4):9086–9096. https://doi.org/10.4238/2014.October.31.24

Kather R, Drijfout F, Martin SJ (2015) Evidence for colony-specific differences in chemical mimicry in the parasitic mite *Varroa destructor*. Chemoecology 25(4):215–222. https://doi.org/10.1007/s00049-015-0191-8

Kirrane MJ, de Guzman LI, Whelan PM, Frake AM, Rinderer TE (2018) Evaluations of the removal of *Varroa destructor* in Russian honey bee colonies that display different levels of *Varroa* sensitive hygienic activities. J Insect Behav 31:283–297. https://doi.org/10.1007/s10905-018-9672-2

Koeniger N, Koeniger G, Wijayagunasekara NHP (1981) Beobachtungen über die anpassung von *Varroa jacobsoni* an inhren natürlichen wirt *Apis cerana* in Sri Lanka. Apidologie 12(1):37–40. https://doi.org/10.1051/APIDO:19810103

Koeniger N, Koeniger G, Delfinado-Baker M (1983) Observations on mites of the Asian honey bee species (*Apis cerana, Apis dorsata, Apis florea*). Apidologie 14(3):197–204. https://doi.org/10.1051/apido:19830305

Kovačić M, Puškadija Z, Dražić MM (2018) Grooming behavior in relation to *Varroa* (*Varroa destructor*) infestation level of Carniolan honey bee colonies (*Apis mellifera carnica*). J Cent Eur Agric 19(4):959–964. https://doi.org/10.5513/JCEA01/19.4.2329

Kruitwagen A, van Langevelde F, van Dooremalen C, Blacquière T (2017) Naturally selected honey bee (*Apis mellifera*) colonies resistant to *Varroa destructor* do not groom more intensively. J Apic Res 56(4):354–365. https://doi.org/10.1080/00218839.2017.1329797

Le Conte Y, Huang ZY, Roux M, Zeng ZJ, Christidès J-P, Bagnères A-G (2015) *Varroa destructor* changes its cuticular hydrocarbons to mimic new hosts. Biol Lett 11:20150233. https://doi.org/10.1098/rsbl.2015.0233

Leclercq G, Francis F, Gengler N, Blacquière T (2018) Bioassays to quantify hygienic behavior in honey bee (*Apis mellifera* L.) colonies: a review. J Apic Res 57(5):663–673. https://doi.org/10.1080/00218839.2018.1494916

Li W, Wang C, Huang ZY, Chen Y, Han R (2019) Reproduction of distinct Varroa destructor genotypes on honey bee worker brood. Insects 10:372. https://doi.org/10.3390/insects10110372

Lin Z, Page P, Li L, Qin Y, Zhang Y, Hu F, Neumann P, Zheng H, Dietemann V (2016) Go east for better honey bee health: *Apis cerana* is faster at hygienic behavior than *A. mellifera*. PLoS ONE 11(9):e0162647. https://doi.org/10.1371/journal.pone.0162647

Lin Z, Qin Y, Page P, Wang S, Li L, Wen Z, ... Dietemann V (2018) Reproduction of parasitic mites *Varroa destructor* in original and new honeybee hosts. Ecol Evol 8(4):2135-2145. https://doi.org/10.1002/ece3.3802

Ling Y, Ting Ji, GuanHua L, DeFang N (2015) Sequence analysis of OBP18 gene and its expression changes upon Varroa destructor resistance in the Chinese honey bee, Apis cerana cerana (Hymrnoptera; Apidae). Southwest China Journal of Agricultural Sciences 28(6):2820–2824

Lobb N, Martin SJ (1997) Mortality of *Varroa jacobsoni* Oudemans during or soon after the emergence of worker and drone honeybees *Apis mellifera* L. Apidologie 28(6). https://doi.org/10.1051/apido:19970604

Locke B, Fries I (2011) Characteristics of honey bee colonies (*Apis mellifera*) in Sweden surviving *Varroa destructor* infestation. Apidologie 42:533–542. https://doi.org/10.1007/s13592-011-0029-5

Luis AR, Grindrod I, Webb G, Piñeiro AP, Martin SJ (2022) Recapping and mite removal behaviour in Cuba: home to the world's largest population of *Varroa*-resistant European honeybees. Sci Rep 12:15597. https://doi.org/10.1038/s41598-022-19871-5

Ma M (2014) New insights of Sacbrood virus. Virologica Sinica 29(6):410–413. https://doi.org/10.1007/s12250-014-3540-9

Martin SJ, Hawkins G, Brettell LE, Reece N, Correia-Oliveira M, Allsopp M (2019) *Varroa destructor* reproduction and cell re-capping in mite-resistant *Apis mellifera* populations. Apidologie 51:369–381. https://doi.org/10.1007/s13592-019-00721-9

Martin SJ, Kemp D (1997) Average number of reproductive cycles performed by *Varroa jacobsoni* in honey bee (*Apis mellifera*) colonies. J Apic Res 36(3–4):113–123. https://doi.org/10.1080/00218839.1997.11100937

Martin C, Salvy M, Provost E, Bagnères A-G, Roux M, Crauser D, Clement J, Le Conte Y (2001) Variations in chemical mimicry by the ectoparasitic mite *Varroa jacobsoni* according to the developmental

stage of the host honey-bee *Apis mellifera*. Insect Biochem Mol Biol 31(4–5):365–379. https://doi.org/10.1016/S0965-1748(00)00130-2

McAfee A, Chapman A, Iovinella I, Gallagher-Kurtzke Y, Collins TF, Higo H, ... Foster LJ (2018) A death pheromone, oleic acid, triggers hygienic behavior in honey bees (*Apis mellifera* L.). Sci Rep 8(1):5719. https://doi.org/10.1038/s41598-018-24054-2

Mendoza Y, Tomasco IH, Antúnez K, Castelli L, Branchiccela B, Santos E, Invernizzi C (2020) Unravelling honey bee–*Varroa destructor* interaction: multiple factors involved in differential resistance between two Uruguayan populations. Vet Sci 7(3):116. https://doi.org/10.3390/vetsci7030116

Mondet F, Beaurepaire A, McAfee A, Locke B, Alaux C, Blanchard S, Le Conte Y (2020) Honey bee survival mechanisms against the parasite *Varroa destructor*: a systematic review of phenotypic and genomic research efforts. Int J Parasitol 50:433–447. https://doi.org/10.1016/j.ijpara.2020.03.005

Mondet F, Blanchard S, Barthes N, Beslay D, Bordier C, Costagliola G, Le Conte Y (2021) Chemical detection triggers honey bee defense against a destructive parasitic threat. Nat Chem Biol 17:524–530. https://doi.org/10.1038/s41589-020-00720-3

Mondragón L, Spivak M, Vandame R (2005) A multifactorial study of the resistance of honeybees *Apis mellifera* to the mite *Varroa destructor* over one year in Mexico. Apidologie 36:345–358. https://doi.org/10.1051/apido:2005022

Moosbeckhofer R (1992) Beobachtungen zum auftreten beschädigter Varroamilben im natürlichen totenfall bei völkern von *Apis mellifera carnica*. Apidologie 23(6):523–531

Moosbeckhofer R (1997) Observations on reproduction rate of *Varroa jacobsoni* and the occurrence of mutilated mites in *Apis mellifera carnica* colonies. Apidologie 28:193–195

Moretto G, Gonçalves LS, De Jong D, Bichuette MZ (1991) The effects of climate and bee race on Varroa jacobsoni Oud infestations in Brazil. Apidologie 22(3):197–203. https://doi.org/10.1051/apido:19910303

Nganso BT, Fombong AT, Yusuf AA, Pirk CWW, Stuhl C, Torto B (2017) Hygienic and grooming behaviors in African and European honeybees—new damage categories in *Varroa destructor*. PLoS ONE 12(6):e0179329. https://doi.org/10.1371/journal.pone.0179329

Nganso BT, Fombong AT, Yusuf AA, Pirk CWW, Stuhl C, Torto B (2018) Low fertility, fecundity and numbers of mated female offspring explain the lower reproductive success of the parasitic mite *Varroa destructor* in African honeybees. Parasitology 145(12):1633–1639. https://doi.org/10.1017/S0031182018000616

Oddie MAY, Buchler R, Dahle B, Kovacic M, Le Conte Y, Locke B, Neumann P (2018) Rapid parallel evolution overcomes global honey bee parasite. Sci Rep 8(1):7704. https://doi.org/10.1038/s41598-018-26001-7

Oddie MAY, Dahle B, Neumann P (2017) Norwegian honey bees surviving *Varroa destructor* mite infestations by means of natural selection. PeerJ 5:e3956. https://doi.org/10.7717/peerj.3956

Oldroyd BP (1999) Coevolution while you wait: *Varroa jacobsoni*, a new parasite of western honey bees. Trends Ecol Evol 14(8):312–315

Page P, Lin Z, Buawangpong N, Zheng H, Hu F, Neumann P, Chantawannakul P, Dietemann V (2016) Social apoptosis in honey bee superorganisms. Sci Rep 6:27210. https://doi.org/10.1038/srep27210

Peng Y, Fang Y, Xu S, Ge L (1987) The resistance mechanism of the Asian honey bee, *Apis cerana* Fabr., to an ectoparasitic mite *Varroa jacobsoni* Oudemans. J Invertebr Pathol 49:54–60. https://doi.org/10.1016/0022-2011(87)90125-X

Pritchard DJ (2016) Grooming by honey bees as a component of *Varroa* resistant behavior. J Apic Res 55(1):38–48. https://doi.org/10.1080/00218839.2016.1196016

Rahman S, Hajong SR, Gévar J, Lenoir A, Darrouzet E (2016) Cuticular hydrocarbon compounds in worker castes and their role in nestmate recognition in *Apis cerana indica*. J Chem Ecol 42:444–451. https://doi.org/10.1007/s10886-016-0700-4

Rath W, Drescher W (1990) Response of *Apis cerana* Fabr towards brood infested with *Varroa jacobsoni* Oud and infestation rate of colonies in Thailand. Apidologie 21:311–321. https://doi.org/10.1051/apido:19900406

Rath W (1991a) Investigations on the parasitic mites *Varroa jacobsoni* Oud. and *Tropilaelaps clareae* (Delfinado & Baker) and their hosts *Apis cerana* Fabr., *Apis dorsata* Fabr. and *Apis mellifera* L. PhD thesis, Rheinischen Friedrich-Wilhelms-Universitat, Bonn, Germany

Rath W (1991b) Laboratory culture of the mites *Varroa jacobsoni* and *Tropilaelaps clareae*. Exp Appl Acarol 10:289–293. https://doi.org/10.1007/BF01198657

Roberts JMK, Anderson DL, Tay WT (2015) Multiple host shifts by the emerging honeybee parasite. Varroa Jacobsoni Mol Biol 24(10):2379–2391. https://doi.org/10.1111/mec.13185

Roberts JMK, Simbiken N, Dale C, Armstrong J, Anderson DL (2020) Tolerance of honey bees to *Varroa* mite in the absence of deformed wing virus. Viruses 12(5):575. https://doi.org/10.3390/v12050575

Rosenkranz P, Aumeier P, Ziegelmann B (2010) Biology and control of *Varroa destructor*. J Invertebr Pathol 103(1):S96-119. https://doi.org/10.1016/j.jip.2009.07.016

Rosenkranz P, Fries I, Boecking O, Stürmer M (1997) Damaged *Varroa* mites in the debris of honey bee (*Apis mellifera* L) colonies with and without hatching brood. Apidologie 28:427–437. https://doi.org/10.1051/apido:19970609

Rosenkranz P, Tewarson NC, Rachinsky A, Strambi A, Strambi C, Engels W (1993a) Juvenile hormone titer and reproduction of *Varroa jacobsoni* in capped brood stages of *Apis cerana indica* in comparison to *Apis mellifera ligustica*. Apidologie 24:375–382. https://doi.org/10.1051/apido:19930403

Rosenkranz P, Tewarson NC, Singh A, Engels W (1993b) Differential hygienic behaviour towards *Varroa jacobsoni* in capped worker brood of *Apis cerana* depends on alien scent adhering to the mites. J Apic Res 32(2):89–93. https://doi.org/10.1080/00218839.1993.11101292

Rothenbuhler WC (1964) Behaviour genetics of nest cleaning in honey bees. I. Responses of four inbred lines to disease-killed brood. Anim Behav 12(4):578–583. https://doi.org/10.1016/0003-3472(64)90082-X

Russo RM, Liendo MC, Landi L, Pietronave H, Merke J, Fain H, Scannapieco AC (2020) Grooming behavior in naturally *Varroa*-resistant *Apis mellifera* colonies From North-Central Argentina. Front Ecol Evol 8:590281. https://doi.org/10.3389/fevo.2020.590281

Ruttner F, Hänel H (1992) Active defense against *Varroa* mites in a Carniolan strain of honey bee (*Apis mellifera carnica* Pollmann). Apidologie 23(2):173–187. https://doi.org/10.1051/apido:19920210

Shakeel M, Ali H, Ahmad S (2020) Comparison of hygienic behaviour of exotic honey bee *Apis mellifera* L. and indigenous honey bee *Apis cerana* of Pakistan. Sociobiology 67(1):74–79. https://doi.org/10.13102/sociobiology.v67i1.4503

Shutler D, Head K, Burgher-MacLellan KL, Colwell MJ, Levitt AL, Ostiguy N (2014) Honey bee *Apis mellifera* parasites in the absence of *Nosema ceranae* fungi and *Varroa destructor* mites. PLoS ONE 9(6):e98599. https://doi.org/10.1371/journal.pone.0098599

Spivak M (1996) Honey bee hygienic behaviour and defense against *Varroa jacobsoni*. Apidologie 27:245–260. https://doi.org/10.1051/apido:19960407

Spivak M, Downey LD (1998) Field assays for hygienic behavior in honey bees (Hymenoptera: Apidae). J Econ Entomol 91(1):64–70

Szabo TI, Walker CRT (1995) Damages to dead *Varroa jacobsoni* caused by the larvae of *Galleria mellonella*. Am Bee J 135:421–422

Traynor KS, Mondet F, de Miranda JR, Techer M, Kowallik V, Oddie MAY, McAfee A (2020) *Varroa destructor*: a complex parasite crippling honey bees worldwide. Trends Parasitol 20(7):592–606. https://doi.org/10.1016/j.pt.2020.04.004

Twearson N, Singh A, Engels W (1992) Reproduction of *Varroa jacobsoni* in colonies of *Apis cerana* indica under natural and experimental conditions. Apidologie 23(2):161–171. https://doi.org/10.1051/APIDO:19920209

Vung NN, Choi YS, Kim I (2020) High resistance to Sacbrood virus disease in *Apis cerana* (Hymenoptera: Apidae) colonies selected for superior brood viability and hygienic behavior. Apidologie 51(1):61-74. https://doi.org/10.1007/s13592-019-00708-6

Wang S, Lin Z, Chen G, Page P, Hu F, Niu Q, ... Dietemann V (2020) Reproduction of ectoparasitic mites in a coevolved system: *Varroa* spp.—Eastern honey bees, *Apis cerana*. Ecol Evol 10(24). https://doi.org/10.1002/ece3.7038

Zhang Y, Han R (2018) A saliva protein of Varroa mites contributes to the toxicity towards Apis cerana and the DWV elevation in A. mellifera. Sci Rep 8(1):3387. https://doi.org/10.1038/s41598-018-21736-9