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Behavioral defenses of honey bees against *Varroa jacobsoni* Oud.

Otto Boecking^a, Marla Spivak^{b,*}

^a Institut für Landwirtschaftliche Zoologie und Bienenkunde der Universität,
Melweg 42, 53127 Bonn, Germany

^b Department of Entomology, 219 Hodson Hall, University of Minnesota,
St. Paul, MN 55108, USA

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Abstract – Two behaviors of honey bees, hygienic behavior and grooming, are mechanisms of defense against brood diseases and parasitic mites. Studies have shown that *Apis mellifera* colonies remove worker brood infested with *Varroa jacobsoni* mites from the nest (hygienic behavior), and groom the mites off other adult bees, but to a limited extent compared to the original host of *V. jacobsoni*, *A. cerana*. Research is reviewed on hygienic and grooming behaviors with respect to their potential as mechanisms of resistance to *V. jacobsoni*. Studies related to hygienic behavior include the removal of experimentally infested and naturally infested brood, measurements of heritability, the uncapping and recapping of cells containing infested pupae, and the detection of infested brood. Studies on grooming include the process by which a groomer detects and damages a mite found on itself or on another adult bee, how the behavior is quantified, and problems with these methods of quantification. Finally, unresolved questions concerning grooming and the effects of hygienic and non-hygienic behaviors on limiting the population growth of *V. jacobsoni* are discussed. © Inra/DIB/AGIB/Elsevier, Paris

Apis mellifera / *Apis cerana* / *Varroa jacobsoni* / hygienic behavior / grooming behavior / resistance / tolerance

1. INTRODUCTION

Beekeeping with *Apis mellifera* L. bees is endangered worldwide by the ectoparasitic mite *Varroa jacobsoni* Oud. [22, 71].

Phoretic mites infest new colonies by attaching to drifting and robbing bees. The mite feeds on the hemolymph of adult bees and brood, and reproduces in the brood. The daily amount of hemolymph that a mite con-

* Correspondence and reprints
E-mail: spiva001@tc.umn.edu

sumes from a bee (0.25 μL according to Moritz [86]) probably does not have a negative effect on an otherwise healthy bee. Bees infested with multiple mites during their metamorphosis show degenerated fat bodies, underdeveloped hypopharyngeal glands and a shortened lifespan [7, 34, 110, 111]. In addition, secondary infections (viruses and bacteria) can be transferred by the mite or triggered in the bees' body and are the primary cause of bee mortality in colonies severely infested with *V. jacobsoni* [1, 4, 5, 23, 55, 56, 59, 94, 95, 113].

Because *A. mellifera* colonies die from varroosis within a few years if the mite population growth is not regulated by the beekeeper [101, 104] and chemical control has its problems and limitations [72, 136], it is of common interest to breed bees for a higher resistance to this mite. Selection and breeding are long-term solutions to the present crisis in apiculture. Szabo [124] stated that this solution "... is the most complex, time consuming, and economically significant research for the beekeeping industry". Using the known defense mechanisms of *A. cerana* Fabr. as a model, we review the present knowledge about behavioral defense mechanisms of the honey bee *A. mellifera* to the mite *V. jacobsoni*. These individual defensive behaviors might form a behavioral complex that contributes to overall resistance by the bees.

2. MECHANISMS TO REDUCE DISEASES AND PARASITES

Feral and domesticated honey bee colonies have evolved elaborate defense mechanisms to protect both themselves and their food from pathogen and parasite invasion. The defense mechanisms of individual bees serve to minimize the threat for the whole colony.

Constitutional defense mechanisms, such as the chitinous cuticle, which serves as a barrier between internal and external envi-

ronments, and the intestinal microflora of the bee gut, can protect each individual bee against infectious diseases [35, 42, 44, 45, 57, 75]. Cellular defense mechanisms (hemocytes) and humoral reactions (enzyme and antimicrobial factors) can contribute to resistance towards infections [29, 57, 76, 77, 134, 137]. The proventricular valve enables the bees to filter ingested spores, which serves as a mechanism of physiological resistance to diseases [35, 122]. These individual responses, coupled with the bees' short life-span and rapid replacement with healthy individuals, can limit the spread of infections between bees within a colony.

Of interest to this article are the behavioral defenses that limit the spread of diseases and parasites. Hygienic and grooming behaviors are examples of such behavioral defenses. Hygienic behavior is the main mechanism by which *A. mellifera* resists the brood diseases American foulbrood (AFB) (*Paenibacillus larvae larvae*) [108, 122] and chalkbrood (*Ascosphaera apis*) [43, 44, 117]. Hygienic honey bee workers have the ability to detect diseased brood, uncup the wax covering over the brood cells and remove the infected larvae or pupae. The impact of hygienic behavior on the spread of infective diseases is maximized if it takes place before the causative organism reaches the infectious sporulating stage [118, 119, 139].

Hygienic behavior of worker honey bees is determined largely by two behavioral components, the uncapping and the removal of dead brood. Few colonies (10 % or less) in nature demonstrate hygienic behavior [89, 116, 117]. However, colonies can be readily selected for the behavior using a field assay [119], or by direct inoculation with the pathogen [44, 108] or mites [13, 114].

Grooming behavior enables individuals and groups of bees within a colony to remove dust and pollen from their bodies, to disperse pheromones, and to remove ectoparasites. Grooming behavior involves

biting and licking with the mouthparts and movements of the mesothoracic legs. Antennae are carefully cleaned by the antenna cleaner on the prothoracic legs [21, 58, 138]. Many self-grooming (auto-grooming) activities of bees can be seen on flowers, in flight, during pollen collection, and in the hive. Social grooming (allo-grooming) between bees can be observed within the hive. Allo-grooming may be elicited by a grooming dance, first described in *A. mellifera* by Haydak [52]. A bee performing this dance is groomed by a nestmate with the mesothoracic legs [74]. Grooming is an important mechanism of protection against the invasion of tracheal mites (*Acarapis woodi*) into the tracheal tubes in bees known to have genetically based resistance to this parasite [31, 64, 93].

3. DEFENSE MECHANISMS OF *A. CERANA* TO *V. JACOBSONI*

The original host of *V. jacobsoni*, *A. cerana*, lives in equilibrium with this ectoparasitic mite. The balanced host–parasite relationship between *A. cerana* and *V. jacobsoni* can be explained by the limited reproduction of the mite on this bee species and behavioral defense mechanisms of the bees against the mites.

The removal of mite-infested worker brood, or hygienic behavior, is one defense mechanism of *A. cerana* towards *V. jacobsoni* [91, 92, 99]. In some cases, *A. cerana* workers open the cappings of mite-infested brood without removing the bee brood. The mites then leave the opened cells or are removed by the bees, and the brood cells are subsequently resealed with a new wax cap [96, 100, 105, 106, 127]. Investigations by Rosenkranz et al. [106] indicated that the number of infested pupae that are removed from experimentally infested cells may be lower than previously reported [91, 92, 100] because the source of mites used for experimental infestation influences the

removal behavior of the bees. When mites from other colonies (either intraspecific *A. cerana*, or interspecific *A. mellifera*) were introduced into the brood cells, the removal rates of mite-infested brood were higher (about 60 %) than when the introduced mites were collected from within the test colony itself (about 10 %).

A. cerana workers do not remove mite-infested drone brood owing to the thick cell capping over the drone cell, a structure unique to *A. koschevnikovi* and this species [96, 100]. Drones which are infested with multiple mites become weakened and are not able to open their cell caps from the inside as they normally do at the time of emergence. They die together with the mites inside such cells because the worker bees do not open these cells from outside, thus creating ‘mite-traps’ in the brood nest [61, 96, 97]. Initial infestation rates with two mother mites per cell can reduce the emergence rate of the infested drones to less than 30 % [97]. Recently Boecking (unpublished data) found that *A. cerana* bees sometimes additionally close the central pore in the drone cell cap of such infested pupae from outside with wax material, entombing the mites within the brood nest. Consequently the successful reproduction of *V. jacobsoni* in *A. cerana* drone brood is limited, resulting in low overall rates of parasitism.

Grooming behavior towards phoretic mites is another known defense mechanism of the Asian bee towards the ectoparasitic mite [28, 39, 91, 92, 96, 126]. Auto- and allo-grooming behavior of *A. cerana* workers is dependent on the ability of the workers to detect the mite and successfully groom it from the bee’s body. The bees can readily be observed grabbing and crushing mites in their mandibles. If a bee fails to groom herself, she may perform the grooming dance by rapidly shaking her abdomen. The dance elicits allo-grooming by nestmates [91]. Thus, mites are disturbed and sometimes killed by the grooming behavior of *A. cerana* workers.

Investigations by Fries et al. [39] indicate that this behavior may be less effective in combating mites than previously reported [28, 91, 92]. Using *A. cerana* and *A. mellifera* bees in cage experiments, observation hives and full-size colonies, the authors found that the proportion of live mites that had visible damage as a consequence of grooming was about 30 % in *A. cerana* compared to 12.5 % in *A. mellifera* colonies. Fries et al. [39] considered only instances of successful grooming in which the mites were damaged, in contrast to Peng [91] and Büchler [28], who included the movement of mites from one bee to another bee, and the observer losing sight of the mites as grooming events.

V. jacobsoni mites are specifically adapted to their host, having distinct attachment sites on the bees' bodies which make it difficult for the bees to successfully groom the mites [33, 96, 98]. If not groomed off, the mites can survive on the bees for long periods without reproduction (several months see Rath [96]) and can disperse to other colonies while attached to drifting bees. Infestation levels of up to several hundred adult female mites in *A. cerana* colonies demonstrate that *V. jacobsoni* does survive in the colonies and is well adapted to its original host [2, 96, 98, 100, 106, 127, 140].

It should be emphasized that, in principle, grooming and hygienic behaviors of *A. cerana* towards *V. jacobsoni* are factors which contribute to a balanced host–parasite relationship. However, because the mite does not reproduce, or has very limited reproduction in worker brood of *A. cerana*, grooming and removal behaviors may not be the most important mechanisms of resistance. Based on simulation studies on the population growth of the mite, Fries et al. [38] argued that the limited reproduction of *V. jacobsoni* on seasonally occurring drone brood in *A. cerana* is sufficient to explain the bees' tolerance of the mite.

4. DEFENSE MECHANISMS OF *A. MELLIFERA* TO *V. JACOBSONI*

When the host–parasite relationship between *V. jacobsoni* and *A. cerana* is used as a model to search for natural resistance to the mite by *A. mellifera*, it is evident that the defense mechanisms of the Asian honey bees (grooming and hygienic behavior) against the mite are also present in European and North American honey bees.

4.1. Hygienic behavior

A. mellifera also removes mite-infested pupae from capped brood cells but to a limited extent compared to *A. cerana* [6, 11, 13, 14, 18, 91, 113]. *A. mellifera* also removes brood infested with *Tropilaelaps clareae* Delfinado & Baker [19, 102]. In most published investigations, brood cells experimentally infested with living *V. jacobsoni* were used to quantify this behavior; data on the removal of naturally infested brood are scant. Africanized bees in Mexico removed 32 % of brood naturally infested with mites compared to 8 % by European bees under the same local conditions [132]. *A. m. intermissa* bees in Tunisia removed on average 15.5 % of the pupae in naturally infested cells (Ritter and Boecking, unpublished) and *A. m. carnica* colonies removed 16.6 % [14]. The mean percentage removal of brood experimentally infested with one living mite per cell by 76 colonies not pre-selected for hygienic behavior was 23.5 ± 18.2 (tested three times during 1997) [17]. Only 9.2 % of these colonies removed more than 50 % of the infested brood. *A. m. ligustica* colonies that had been pre-selected for hygienic behavior in the US (28 colonies total, 1994–1997) removed an average of 52.1 % (± 25.6) of the experimentally infested pupae, compared to 17.4 % (± 14.7) in colonies selected for non-hygienic behavior (19 colonies, 1994–1997) [114, 119].

In contrast to diseased brood, mite-infested larvae and pupae do not necessarily

die. Opened brood cells containing healthy pupae can indicate part of the process of removal behavior [30]. In some cases the caps of mite-infested brood are opened and then closed again by the bees with a new wax cap without eliminating the bee brood. In those cases, the mites may leave these cells by the temporary opening in the capping [6, 12] as observed in *A. cerana*. As a result of opening and closing the cell cap by the bee, a distinct change in the silk/wax-structure of the inner cell cap can be observed [16]. Careful examination of 643 brood cells experimentally infested with one living mite revealed that 69 (10.7 %) of the cell caps showed clear signs that the worker bees had opened and closed those cells at least once during the 10 days of the investigation without eliminating the brood. Although the introduced mite could have left the cell while it was opened, the mite was missing in only 4 (5.8 %) of these cells [17]. Video recordings by Boecking [12] also confirmed that the removal of mite-infested brood did not always involve a strict behavioral sequence of detecting, uncapping and then removing the parasitized brood. In some cases, the cappings of the mite-infested brood cells were opened by the bees then sealed again after some time (e.g. after 150 min). Later, these same cells were uncapped again and the pupae were then removed. In most cases, the mites did not leave the brood cells until the bees had already removed most of the larval/pupal body. Individually marked bees were seen repeatedly detecting and uncapping mite-infested cells. Other bees repeatedly closed such opened brood cells although the mites were still present in these cells [12]. Using infra-red video recordings Thakur et al. [128, 129] also observed a few individuals repeatedly uncapping and removing mite-infested brood cells. These observations indicate that some bees specialize in these different behavioral traits. Early studies on hygienic behavior suggested that hygienic bees were genetic specialists; mixed groups of hygienic and non-hygienic workers expressed the

hygienic phenotype only when the hygienic worker subgroup was large enough to take care of all the uncapping and removing tasks [131]. Experimental colonies containing groups of bees from different patrilines ($n = 4$) uncapped more dead brood cells (killed by freezing) compared to pure supersister groups [63].

The expression of hygienic behavior is known to be strongly influenced by environmental factors. For example, weak colonies, or a lack of incoming nectar have been shown to reduce the removal response to mite-infested and dead brood cells, respectively [15, 79, 114, 117]. Early experiments on hygienic behavior [130] concluded that young hygienic bees will remove all diseased brood regardless of nectar availability, but bees older than about 4 weeks remove the larvae only during a nectar flow. Recent experiments (Spivak, unpublished) on two hygienic colonies composed of bees of 11 different age cohorts (ranging from 3–33 days) indicated that the mean ages of the bees that uncapped and removed freeze-killed brood were 15 and 16 days, respectively ($n = 242$ and 299 bees). The mean ages of the foragers in the same colonies were significantly older, 22 and 20 days, respectively ($n = 79$ and 66 bees; t -tests $P < 0.01$). This experiment indicated that hygienic bees are middle-aged; they have brood-rearing experience but have not necessarily begun foraging.

Recently Boecking and Drescher [17] found the heritability value based on the mother–daughter regression for the removal of brood experimentally infested with one living mite per cell was $h^2 = 0.18 \pm 0.27$ (SD). The value for the removal of dead brood (killed using the pin-killed brood assay) was $h^2 = 0.36 \pm 0.30$. A statistical analysis revealed that the repeatability of the measurements was $w = 0.24$ (four test repetitions, 97 colonies, 11 bee yards, 297 records) for the removal of mite-infested brood, and $w = 0.46$ (six test repetitions, 114 colonies, 11 bee yards, 421 records) for

the removal of dead brood. These values emphasize that environmental effects strongly affect the expression of the hygienic behavior in the experimental bee populations used. Moreover, the results demonstrate that the rate of removal of mite-infested or dead brood within a particular colony even under the same environmental conditions is not always consistent between assays [54, 103, 115]. Quantitative genetic studies on hygienic behavior using a laboratory bioassay also showed only moderate estimates for genetic variance, with $h^2 = 0.14$ for uncapping and $h^2 = 0.02$ for removing dead brood [73]. In contrast, Harbo and Harris [50] calculated the heritability of hygienic behavior, based on the removal of freeze-killed brood, to be $h^2 = 0.65 \pm 0.61$.

There is a positive correlation between the rate of removal of mite-infested brood and dead brood (freeze-killed or pin-killed). Colonies pre-selected for high hygienic behavior on the basis of the freeze-killed brood assay [115], removed significantly more brood cells experimentally infested with mites compared to colonies selected for low hygienic behavior [114, 119].

Honey bees are able to detect even a single cell containing an abnormal, dead or diseased larva or pupa within a healthy brood nest [36, 112]. Mechanical and chemical stimuli, including brood pheromones, enable bees to recognize and distinguish healthy brood within a colony [60, 78]. However, it is not known how the bees determine that a particular larva or pupa is dead or infested with a mite under a wax-capped brood cell. Ritter and Boecking (unpublished data) found the removal of brood experimentally infested with mites that transferred or triggered a secondary infection of acute paralysis virus (APV) was higher (on average 56.7 %) compared to brood infested with mites collected from sources without viruses (on average 12.2 %). The signals that enable the bees to detect mite-infested live brood might be different from the signals used to detect brood that has been killed by diseases or viruses.

Preliminary experiments indicated that in contrast to dead mites (frozen, washed in alcohol or collected from hive debris) and other introduced, non-living particles (tin foil globules, eucalyptus seeds, filter paper), only live mites in brood cells elicit a removal response by the bees. Cells infested with more than one mite elicit a stronger removal response than cells infested with one mite [16, 114]. Rosenkranz et al. [106] demonstrated that the removal of mites by *A. cerana* depends on the alien scent adhering to the mite, which is not the case for *A. mellifera* [3, 16]. These results imply that the odor of the mites itself is probably not an important cue to *A. mellifera*.

It is not clear what cues bees use to detect a brood cell infested with a mite. It has been hypothesized that bees may use acoustical signals to detect infested brood [112]; however, checking for movements released through the capping of brood cells containing live, dead (pin-killed) or mite-infested pupae using a laser interferometer could not support this hypothesis (Kirchner and Boecking, unpublished data). Gramacho et al. [46, 47] showed that the average body temperature of dead pupae (pin-killed) inside the brood cell under brood chamber conditions was significantly lower (0.3–0.7 °C) than the temperature of live pupae. However, the use of an infra-red thermographic system, which allowed a continuous measurement of capped brood combs without physical contact, revealed no differences in temperatures released through the capping of brood cells containing live, dead (pin-killed) and mite-infested pupae (B. Görgens, unpublished data). Since the temperature above the sealed cell should be perceived by worker bees, these data imply that differences in temperature inside the brood cell might not be the cue bees use to detect dead brood.

Pupae treated with hemolymph or body fluid seem to be a strong stimulus for the bees to open cells and to remove the treated pupae. Even colonies pre-selected as non-

hygienic can be induced to express hygienic behavior using hemolymph or body fluid ([115], Gørgens, unpublished data; Gramacho, pers. comm.). Bees may use olfactory cues to detect diseased or parasitized brood in the nest. Preliminary field and laboratory experiments, using proboscis-extension reflex (PER) conditioning (see methods in Bitterman [10]) indicated that individual bees collected from a colony selected for hygienic behavior have lower response thresholds than bees collected from a non-hygienic colony to olfactory stimuli associated with chalkbrood-killed pupae [70]. These results suggest that the differences between individual hygienic and non-hygienic bees lie in their responsiveness to olfactory stimuli associated with diseased brood. However, no published research has revealed the exact nature of the cues that enable hygienic bees to detect mite-infested brood cells.

Mite removal behavior may theoretically limit the population growth of *V. jacobsoni* in three ways [38]: first, immature mites which have begun to develop in brood cells are killed, decreasing the average number of offspring per mother mite. Second, removal of female mites extends the phoretic period of those mites which survive the removal process. Third, mite removal increases the mortality of mother mites.

Experiments on the effects of removal behavior on the survivorship of single mites [11, 12, 15] revealed that most of the adult female mites that escaped the brood cells after removal of the infested brood could invade other brood cells again. In one experiment 104 individually color-marked mites escaped from brood cells during the removal of infested brood by the bees; 61.3 % of these mites invaded new brood cells, and 14.6 % became phoretic on adult bees; 24.6 % were found on the hive bottom board and 10.9 % of those were killed by the bees.

The overall effect of hygienic behavior on population growth of the mites within a colony is still unknown. However, Spivak

and Reuter [120, 121] demonstrated that colonies bred for hygienic behavior (based on rate of removal of freeze-killed brood) had fewer mites than commercial colonies not selected for the behavior after 1 year without mite treatment. The same colonies also had lower incidences of chalkbrood and AFB, and produced more honey than the commercial colonies. These results demonstrate the benefits of selecting bee colonies for hygienic behavior.

4.2. Grooming behavior

Grooming of phoretic mites by *A. mellifera* is not as pronounced as it is in *A. cerana*. This difference was first pointed out by Peng et al. [91] and later confirmed by other researchers [18, 25, 28, 39, 53, 81, 83, 84, 87, 96, 107, 109, 135]. It is evident that *A. mellifera* bees are able to kill mites during grooming activities, since damaged mites still showing movement were found in the debris on the bottom board of the colonies [13, 15, 48, 53, 107, 108]. In these studies, care was taken to ensure that no other insect or scavenger could enter the hive debris to cause the mite damage. The main type of damage to the mite caused by successful grooming is amputation or mutilation of one or more legs. Injuries to the mites' idiosoma or gnathosoma are relatively rare [67, 107]. Using video-technique, *A. mellifera* bees can sometimes be observed grabbing and crushing mites in their mandibles. As on *A. cerana*, the mite chooses distinct attachment sites on the bodies of *A. mellifera* at the 3rd and 4th ventro-lateral tergites of the abdomen, and have a significant preference for the left side of the host, which may demonstrate an adaptation of the mite to grooming [15, 18, 20, 33, 96, 98].

Using infra-red video recordings Thakur et al. [128, 129] confirmed that bees used active grooming defenses against the mites including grabbing and crushing mites in their mandibles. They observed a few indi-

viduals performing repeated grooming activities. General studies of grooming behavior (independent of its effect on mites) have shown that some bees in *A. mellifera* are grooming specialists; the behavior is genetically determined and age specific [31, 40, 62, 93, 133]. Moore et al. [80] observed one highly specialized grooming honey bee as it aged from 4–31 days. This bee groomed other bees 84 % of the time she was observed, and never developed into a forager.

It is not known how the bees that groom detect mites on the bee body. Observation of behavioral patterns of groomers (Boecking, pers. obs. of *A. cerana* and *A. mellifera*) indicate that they begin to allo-groom other infested adults after they perceive the grooming dance. Occasionally bees were observed grooming one bee after another in rapid succession. During grooming, the receiving bees hold their wings perpendicular to the body axis and the grooming bees work on those body parts that can not be reached by auto-grooming. However, the mites are not necessarily located in the places where the bee grooms. Mites have been observed to leave the bee while the bee was still being allo-groomed, indicating that the groomers may not be able to detect the mite itself (Boecking, pers. obs.).

Grooming behavior is thought to be a heritable trait. F1 colonies bred from colonies that demonstrated high or low mite grooming ability showed the same ability as their parent colony [84, 85]. Nevertheless, precise heritability estimates have not been calculated for this trait. As with hygienic behavior, the expression of grooming behavior is known to be strongly influenced by environmental factors [69]. Moosbeckhofer [82] observed low numbers of damaged mites in March (11 %) but significantly higher numbers in June (42 %) in the same colonies. Consequently, the proportion of damaged mites within a particular colony, even under the same environmental conditions, is not always consistent between collections.

The grooming potential of a colony can be measured by counting the number of live and damaged mites that drop from mite-infested bees onto protected sheets (inserts) placed under the brood nest. Observing adult bees that have been inoculated with mites in observation hives provides information about the behavioral patterns of groomers and infested bees, but is not a good measure of the overall grooming ability of the colony. Data from cage experiments and from laboratory bioassays can reveal that a particular genetic line or race of bee reacts more strongly to mites (shaking and biting the mites) compared to others bees, but it is difficult to correlate these data with the behavior of a whole colony [3, 53, 54, 125]. Investigations by Rosenkranz et al. [107] showed that the number of damaged mites can reach relatively high numbers even in colonies that had not been pre-selected for this behavioral trait. Mites were collected from protected inserts every 12 h over 12 days in eight *A. m. carnica* colonies. Approximately 46 % of the dead mites ($n = 3917$) and approximately 11 % of the live mites ($n = 889$) on the inserts were damaged. A portion of the live undamaged mites that fell onto the bottom board were able to reproduce after they were subsequently introduced into freshly capped brood cells.

Although counting the number of damaged mites on an insert within the colonies indicates the grooming potential of the colony, the mites may have been damaged by means other than grooming. Mites that are already dead may be damaged secondarily, and immature mites might be damaged during the removal of infested brood (hygienic behavior). Wax moth larvae may also damage mites [124]. In addition, the number of mites falling onto the bottom board is highly correlated with the presence and stage of brood [37, 65]. Lobb and Martin [66] found a strong correlation between levels of falling mites and the emergence of brood. It was estimated that around half of the falling mites originated from mites that died within the sealed cell; the other half

died shortly after bee emergence. The number of mites falling from worker brood was two to three times higher than from drone brood. Damaged mites that originated from the emerging brood should not be attributed to the grooming potential of a bee colony, although it is difficult to distinguish between the two.

Boecking and Drescher [17] compared the number of damaged mites found on the bottom boards to the estimated mite population that was phoretic on adult bees. In 113 colonies containing no brood, the average percentage of damaged mites captured on the inserts on three successive days was 21.5, 15.6 and 11.7 %. In total, 337 mites were captured. In contrast, 84 136 mites were collected after a chemical treatment of the bees following the last mite collection, averaging 744.6 mites/colony (range 91–2 157 mites/colony). The treatment with an effective acaricide gave an estimate of the mite population that was phoretic on the bees during this investigation. Comparing the number of damaged mites that dropped onto the bottom boards to the number of mites phoretic on the bees, the relative frequency of damaged mites in these colonies on the 3 days was 0.08, 0.06 and 0.03 %.

It is still controversial whether the number of damaged mites on the bottom board is a useful criterion for successful grooming behavior. Because the percentage of damaged mites may vary widely from colony to colony and because of the difficulties in measuring successful grooming, it is not clear if grooming behavior is an effective mechanism of defense against mites [8, 9, 65, 107, 125]. Nevertheless, model simulations showed that control of the mite population depends on the frequency and efficiency of the bees' ability to groom and that a moderate increase in the mite death rate could theoretically help a bee population become genetically resistant to parasitism by *V. jacobsoni* [68].

5. CONSIDERATIONS AND UNRESOLVED QUESTIONS

Promoting natural resistance to *V. jacobsoni* through selection and breeding is in its infancy today. Despite the extensive research on grooming and hygienic behaviors, it is still unclear to what extent they are effective mechanisms of defense against the mites. It is commonly thought that bees that vigorously shake and bite the mites are the most efficient groomers. However, this disturbance by the bees may cause the mites to invade brood cells more readily. This possibility remains to be tested. Likewise, it is thought that colonies that rapidly uncap and remove diseased and parasitized brood are more resistant to diseases and mites. It has been shown that the spread of infective diseases is minimized if hygienic bees remove the diseased brood before the causative organism reaches its infectious sporulating stage [139]. However, Spivak and Gilliam [117] considered the possibility that non-hygienic behavior in honey bees could benefit a colony. If bees leave diseased brood under a capped cell, they may avoid contact with the pathogens in the cell. It is not clear if non-hygienic bees actively avoid diseased brood, or if they do not respond to it. Bees from pre-selected non-hygienic colonies tended to recap partially uncapped cells that contained freeze-killed brood [117]. *A. mellifera* bees also recapped mite-infested brood cells that had been previously uncapped by other bees [12]. In *A. cerana*, this non-removal behavior is much more marked (Boecking, unpublished data); they uncap and remove worker brood cells infested with *V. jacobsoni*, but tend not to remove drone brood infested with multiple mites or with bacteria disease. The bees close the pore of the drone cell cap with wax from the outside. The non-removal of infested drone brood and closure of the central pore by the workers isolates the mites and infectious diseased cells in the brood nest.

When *A. mellifera* does remove mite-infested brood, it is not clear at what point the bees should perform this behavior to most effectively limit the population growth of *V. jacobsoni*. To negatively affect the potential average number of offspring per mother mite, removal should begin after the foundress has laid her full clutch of eggs in the brood cell. At this point, the bees would destroy the offspring during removal of the pupae, and the foundress would have to enter the phoretic stage again before she could invade a new brood cell. In highly infested colonies, it may not be advantageous for the bees to remove all mite-infested worker brood, which could substantially reduce the adult population of the colony.

Hygienic behavior of honey bees may be a generalized adaptation for cell reuse and thus, may present a conflict between risking infection by removing diseased brood and the need to clear out comb space for the queen to lay eggs [117]. *A. cerana* can use another strategy to avoid mites; they tend to abscond from the nest when under high disease or parasite pressure. Predators (such as wax moths) then destroy the abandoned combs, ridding them of the infectious material.

Lines of *A. mellifera* demonstrate different degrees of susceptibility to *V. jacobsoni* [24, 26, 27, 32, 48, 51, 90]. When colonies are initiated with equal numbers of mites, the most resistant colonies can be identified as those that have the fewest mites at the end of the test period [8, 25, 51, 90]. However, numerical data on just the population growth or decline of the mites during the experimental period do not yield information on the mechanisms that influenced these trends. In breeding bees for mite resistance, it is critical to determine the exact nature of the traits that confer resistance, and to ensure they are measurable and heritable (e.g. [49, 50, 51]).

The main genetic parameters which influences the bees' expression of hygienic behavior are maternal and heterotic effects

which dominate additive gene effects [54]. In contrast, grooming behavior is mainly influenced by additive gene effects, such that a combination of specific maternal and paternal bee lines is necessary for the breeding of mite-resistant stock. Such a combination that favors hygienic behavior will not likewise favor grooming ability and vice versa [54]. It has been documented that colonies that show a high degree of removal of mite-infested brood are not necessarily better groomers [114].

High genetic variability and heterosis do not seem to enhance resistance to *V. jacobsoni*, although these factors do increase colony productivity [41, 88]. In a 2-year hybrid queen breeding program, characteristics related to disease resistance and in particular to resistance to *V. jacobsoni* (infestation level, duration of the postcapping brood phase, hygienic behavior and number of damaged mites), line-mixed colonies showed a consistently lower performance compared to the separate lines. This result is contrary to the theoretical expectation of enhanced resistance to diseases with increased genetic variation between workers [41].

In conclusion, bee breeding strategies for increased resistance to *V. jacobsoni* should be based on knowledge of the mechanisms of resistance, their potential for decreasing mite population growth, and their mode of inheritance. At the same time, the ability of the mite to adapt to changes in their host should be taken into consideration.

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Résumé – Les comportements de défense des abeilles mellifères contre *Varroa jacobsoni*.

Les abeilles mellifères présentent deux types de comportements qui sont des mécanismes de défense contre les maladies du couvain et les acariens parasites. Des études ont montré que des colonies d'*Apis mellifera* L. éliminaient du nid le couvain d'ouvrières infesté par l'acarien *Varroa jacobsoni* (comportement hygiénique) et débarassaient les abeilles adultes des acariens (comportement de toilette), mais dans une moindre mesure que l'hôte d'origine, *Apis cerana* Fabr. En Allemagne des colonies d'*A. m. carnica* ($n = 76$), non pré-sélectionnées pour le comportement hygiénique, ont éliminé en moyenne 23,5 % ($\pm 18,2$ %) du couvain infesté expérimentalement avec un acarien par cellule. Aux États-Unis des colonies d'*A. m. ligustica* sélectionnées pour leur comportement hygiénique (28 colonies au total, 1994–1997) ont éliminé en moyenne 52,1 % ($\pm 25,6$ %) des nymphes infestées expérimentalement contre 17,4 % ($\pm 14,7$ %) chez les colonies non sélectionnées (19 colonies, 1994–1997). Les observations concernant l'élimination par *A. m. carnica* du couvain infesté montrent que certaines abeilles peuvent désoperculer et d'autres refermer l'opercule de cire sur des cellules de couvain infestées avant que le couvain ne soit finalement désoperculé et éliminé ou, dans certains cas, laissé tel quel. Des observations préliminaires indiquent une certaine spécialisation des abeilles pour ces tâches. La plupart des fondatrices d'acariens peuvent s'échapper au cours du processus d'élimination du couvain et envahir à nouveau d'autres cellules ou devenir phorétiques sur des abeilles adultes ; un petit pourcentage peut être mutilé par les abeilles lors du toilettage. Les acariens immatures qui ont commencé à se développer sont tués pendant le processus d'élimination du couvain, ce qui contribue à diminuer le nombre moyen de descendants par acarien femelle. L'héritabilité de l'élimination du couvain infesté a été estimée à $h^2 = 0,18$ ($\pm 0,27$) chez *A. m. carnica* ; ceci montre que l'envi-

ronnement agit fortement sur l'expression de ce comportement.

On ne sait pas quels signaux utilisent les abeilles pour détecter le couvain infesté par *V. jacobsoni*. On a émis l'hypothèse que les abeilles hygiéniques avaient un seuil de réponse plus bas que les abeilles non hygiéniques aux signaux olfactifs associés au couvain parasité (ou anormal pour une autre raison).

A. mellifera n'a pas un comportement de toilettage aussi marqué qu'*A. cerana*. L'observation d'abeilles engagées dans le toilettage suggère qu'elles sont peut-être incapables de détecter l'acarien proprement dit sur le corps de l'abeille qu'elles toilettent, puisqu'on a observé des abeilles toiletter alors qu'il n'y avait pas d'acariens et qu'on a vu des acariens quitter l'abeille pendant le toilettage. L'héritabilité du comportement de toilettage n'a pas été estimé. On pense pourtant que son expression est contrôlée par des effets additifs de gènes et qu'il est fortement influencé, comme le comportement hygiénique, par les conditions du milieu. Le potentiel de toilettage est généralement mesuré en dénombrant les acariens morts et mutilés tombés sur des feuilles collantes placées sous le nid à couvain. Pourtant des acariens mutilés ne sont pas toujours le résultat d'un toilettage efficace. Des acariens morts (dans les cellules de couvain d'ouvrières ou sur les adultes) peuvent avoir été mutilés dans un second temps et les larves de fausse-teigne, les fourmis ou d'autres nécrophages sont capables d'infliger des mutilations aux acariens.

En comparant le nombre d'acariens mutilés au nombre d'acariens phorétiques dans 113 colonies, on a trouvé une fréquence relative d'acariens mutilés inférieure à 0,09 %. On ne sait pas si le nombre d'acariens mutilés trouvés sur le plancher est un critère utile pour évaluer l'efficacité du comportement de toilettage.

L'influence globale des comportements hygiénique et de toilettage sur la limitation de la croissance de la population et la survie des acariens dans les colonies d'*A. melli-*

fera reste incertaine. Au cours du toilettage les acariens sont secoués vigoureusement et mordus. On n'a pas testé si cela facilitait l'envahissement d'autres cellules par les acariens. Sélectionner des colonies pour le comportement hygiénique diminue la loque américaine et le couvain plâtré et peut aider à limiter les populations d'acariens. Mais les coûts et les bénéfices de l'élimination du couvain parasité et malade (comportement hygiénique) par rapport au maintien de ce couvain dans des cellules operculées (comportement non hygiénique) n'ont pas été totalement explorés. Lorsqu'on sélectionne des abeilles pour la résistance aux acariens, il est fondamental de déterminer la nature exacte des caractères qui confèrent la résistance et de s'assurer qu'ils sont mesurables et héréditaires. © Inra/DIB/AGIB/Elsevier, Paris

***Apis mellifera* / *Apis cerana* / *Varroa jacobsoni* / comportement hygiénique / comportement de toilettage / résistance / tolérance**

Zusammenfassung – Abwehrverhalten von Honigbienen gegen *Varroa jacobsoni*
Oud. Honigbienen besitzen zwei verschiedene Verhaltensweisen zur Abwehr von Brutkrankheiten und parasitischen Milben, das hygienische Verhalten und das Putzverhalten. Untersuchungen haben gezeigt, daß Völker von *Apis mellifera* mit *Varroa jacobsoni* befallene Arbeiterinnenbrut aus dem Brutnest (hygienisches Verhalten) und Milben von anderen Bienenarbeiterinnen entfernen können. Allerdings erfolgt dies in weit geringerem Ausmaß als bei dem Ursprungswirt von *V. jacobsoni*, *A. cerana*. Nicht vorselektierte Völker von *A. m. carnica* ($n = 76$) in Deutschland entfernten im Mittel 23.5 % (± 18.2) von mit jeweils einer Milbe künstlich infizierten Brutzellen. Dahingegen entfernten in den USA auf hygienisches Verhalten selektierte Völker von *A. m. ligustica* im Mittel 52.1 % (± 25.6 %, insgesamt 28 Völker; 1994–1997) der experimentell infizierten Puppen. Im

Vergleich hierzu entfernten die auf geringes hygienische Verhalten selektierten Völker 17.4 % (± 14.7 ; 19 Völker, 1994–1997). Beobachtungen der Entfernung von milbeninfizierter Brut durch *A. m. carnica* deuten darauf hin, daß nach Öffnung des Deckels der infizierten Zellen durch einige der Arbeiterinnen andere ihn wieder schließen können, bevor die Brutzelle endgültig entdeckt und die Brut entfernt wird oder sie in einigen Fällen auch intakt gelassen wird. Einige Beobachtungen deuten weiter auf einen gewissen Grad von Spezialisierungen der Arbeiterinnen für diese Aufgaben hin. Die meisten Milbenweibchen können während des Entdeckelungsvorganges entkommen und wieder in andere Zellen eindringen oder auf Bienenarbeiterinnen aufsitzen; ein geringer Prozentsatz wird durch das Putzverhalten der Bienen verletzt. Unausgewachsene Milbennachkommen werden während des Entfernens der Brut getötet, hierdurch vermindert sich die mittlere Anzahl von Nachkommen pro Milbenweibchen. Die Erblichkeit des Entfernens von befallener Brut bei *A. m. carnica* beträgt $h^2 = 10.18$ (± 0.27). Dies zeigt an, daß die Ausprägung dieses Verhaltens stark durch Umgebungsfaktoren beeinflusst wird (aber siehe Harbo [51]). Es ist nicht bekannt, auf Grund welcher Anzeichen die Bienen mit *V. jacobsoni* befallene Brutzellen entdecken. Es wird allerdings die Hypothese vertreten, daß hygienische Bienen eine verringerte Ansprechschwelle für die mit parasitierter oder anderswie unnormaler Brut zusammenhängenden geruchlichen Reize haben. Das Putzverhalten ist bei *A. mellifera* nicht so ausgeprägt wie bei *A. cerana*. Da beobachtet wurde, daß sie auch an Stellen putzen, an denen keine Milbe vorhanden war, oder daß die Milben während des Putzens die geputzte Biene verlassen konnten, wird angenommen, daß die Bienen die Milben auf dem Körper der von ihnen geputzten Biene nicht direkt wahrnehmen können. Die Heritabilität für das Putzverhalten wurde bisher nicht bestimmt, man kann aber annehmen, daß dieses wie das hygienische Verhalten durch additive Geneffekte bestimmt

und stark durch Umgebungsfaktoren beeinflusst wird. Das Putzpotential wird üblicherweise durch Auszählen von verletzten Milben auf klebrigen Bodeneinlagen in den Bienenbeuten bestimmt. Allerdings müssen verletzte Milben nicht immer das Ergebnis von erfolgreichem Putzverhalten sein. Es können auch Milben, die in Brutzellen oder auf Arbeiterinnen bereits zuvor gestorben waren, erst später verletzt worden sein. Zusätzlich können auch Wachsmottenlarven, Ameisen und andere Aasfresser die Milben nachträglich verletzen. In einem Vergleich von verletzten Milben und der Anzahl von Milben auf den Arbeiterinnen betrug der Anteil verletzter Milben weniger als 0.09 %. Es ist daher nicht klar, ob der Anteil verletzter Milben auf Bodeneinlagen zur Bestimmung eines erfolgreichen Putzverhalten geeignet ist. Es bleibt weiterhin unklar, welchen Gesamtbeitrag das hygienische Verhalten und das Putzverhalten zu einer Begrenzung des Populationswachstums der Milben in Völkern von *A. mellifera* leisten können. Es wurde bisher nicht untersucht, ob das heftige Beißen und Schütteln während des Putzverhaltens die Milben dazu bewegt kann, eher die Brutzellen zu befallen. Die Zucht von Bienenvölkern auf verstärktes hygienisches Verhalten kann den Schaden durch Amerikanische Faulbrut und Kalkbrut mindern, und es kann helfen die Milbenpopulation zu verringern. Allerdings wurden Nutzen und Kosten der Entfernung parasitierter oder erkrankter Brut (hygienisches Verhalten) gegenüber dem Belassen dieser Brut unter dem Zelldeckel (nicht-hygienisches Verhalten) bislang nicht vollständig aufgeklärt. In der Zucht von milbenresistenten Bienen ist es von grundlegender Bedeutung, die Natur der verantwortlichen Verhaltensweisen aufzuklären und sicherzustellen, daß diese meßbar und erblich sind. © Inra/DIB/AGIB/Elsevier, Paris

***Apis mellifera* / *Apis cerana* / *Varroa jacobsoni* / hygienisches Verhalten / Putzverhalten / Resistenz / Toleranz**

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