

Differential infestation of honey bee, *Apis mellifera*, worker and queen brood by the parasitic mite *Varroa destructor*

Nicholas W. CALDERONE*, Sisi LIN, Lodewyk P.S. KUENEN**

Department of Entomology, Cornell University, Ithaca, NY 14853, USA

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Abstract – We examined the distribution of *Varroa destructor* on worker and queen brood in colonies of *A. mellifera*. With both worker and queen hosts present, the mite prevalence value for worker hosts was $75.0 \pm 4.0\%$ (lsmean \pm SE), compared to $5.1 \pm 4.0\%$ for queen hosts ($P < 0.0001$). We also examined the response of mites to cuticular extracts of 5th instar worker and queen larvae using arrestment bioassays. In binary-choice tests at 0.5 larval equivalents (Leq), worker extract arrested $84.79 \pm 4.98\%$ of the mites, while queen extract arrested $15.21 \pm 4.98\%$ ($P < 0.0001$). At 0.8 Leq, worker extract arrested $89.75 \pm 4.98\%$, while queen extract arrested $10.25 \pm 4.98\%$ ($P < 0.0001$). We also measured the repellent activity of royal jelly extract in a repellent bioassay. Royal jelly extract repelled $78.5 \pm 2.6\%$ of mites at 5 mg royal jelly equivalents (Rjeq); $85.6 \pm 3.7\%$ at 10 mg Rjeq; and $89.2 \pm 3.8\%$ at 20 mg Rjeq. The response at each dose was greater than the $10.5 \pm 2.9\%$ mites repelled by solvent controls ($P < 0.0001$). Our findings suggest that the low incidence of mites in queen brood is due, in part, to the repellent activity of royal jelly, and possibly to intrinsic differences between larval chemistries.

Apis mellifera / *Varroa jacobsoni* / *Varroa destructor* / parasitic mite / royal jelly

1. INTRODUCTION

Varroa destructor Anderson and Trueman (Anderson and Trueman, 2000) is a parasitic mite of the honey bee species *Apis cerana* Fabr. and *A. mellifera* L. In colonies of *A. cerana*, reproduction occurs almost exclusively on drone brood (Koeniger et al., 1983; De Jong, 1988; Tewarson et al., 1992; Rosenkranz et al., 1993; Anderson, 1994; Anderson and Sukarsih, 1996; Boot et al., 1997). In colonies of *A. mellifera*, mites reproduce on both worker and drone brood (reviewed in De Jong et al., 1982; De Jong, 1990; Boot et al., 1997; Beetsma et al., 1999), but not on queen brood (Romaniuk et al., 1988; Rehm and Ritter, 1989).

The reproductive success of a mite on *A. mellifera* is positively correlated with the duration of its host's capped stage, which is longest for drones, intermediate for workers, and shortest for queens (Jay, 1961; reviewed in Martin, 1994). Not surprisingly, mite levels tend to be higher in drone brood than worker brood, with average differences ranging from 5–9 fold (reviewed in Fries et al., 1994; Calderone and Kuenen, 2001). Mites are rarely found in queen cells (Harizanis, 1991) which have a capped stage of only 8.0–8.5 days (Jay, 1961), less than the minimum time mites require for reproduction (Romaniuk et al., 1988; Rehm and Ritter, 1989).

* Correspondence and reprints

E-mail: nwc4@cornell.edu

** Current address: USDA-ARS-HCRL, 2021 South Peach Avenue, Fresno, CA 93727, USA.

Several proximate mechanisms have been proposed to account for the heterogeneous distribution of mites among immature host types. According to one explanation (herein, the chemical hypothesis), differences in mite levels are a result of differences in host chemistries. Le Conte et al. (1989) reported that mites are attracted to a blend of three aliphatic esters (methyl and ethyl palmitate and methyl linolenate) that are produced in greater quantities and over a longer period of time (Trouiller et al., 1991, 1992) by drone larvae than worker larvae. Other compounds are also involved in the host acquisition process (Rickli et al., 1992, 1994; Boot, 1994; Donzé et al., 1998; Calderone and Lin, 2001). Alternatively, the observed bias may be a result of differences in the physical characteristics of worker and drone, larval-cell complexes (De Ruijter and Calis, 1988; De Jong and Morse, 1988; Goetz and Koeniger, 1993; Boot et al., 1995; Kuenen and Calderone, 2000; Calderone and Kuenen, 2001). In contrast, little is known about the mite's interaction with queen brood, although queen larvae produce the lowest levels of the putative attractants proposed by the chemical hypothesis (Trouiller et al., 1994), and the highest levels of methyl oleate, a putative repellent (Le Conte 1990, reported in Trouiller et al., 1994).

Here, we compare the incidence of mites on worker and queen brood, something documented in only one population of *A. m. macedonica* in Greece (Harizanis, 1991). We seek to determine if mites infesting bees in the US exhibit similar behavior. We also examine the responses of mites in bioassays to cuticular extracts of 5th instar worker and queen larvae and to extracts of royal jelly to determine if those substances provide a mite with chemical cues to its host's identity.

2. MATERIALS AND METHODS

2.1. Experiment 1. Mite prevalence values and parasite load scores in worker and queen cells

A naturally-mated queen was caged in a colony for 12 h on an empty worker comb; then, the worker comb was placed in the brood nest of an unrelated,

mite-infested colony. After 2.5 days, the queen in that colony was confined to a cage and placed on the bottom board to create a condition conducive to rearing queen cells. A day later, when experimental worker larvae were still < 24 h old, 45 of them were transferred to beeswax queen rearing cells affixed to wooden bars using standard grafting techniques (Laidlaw, 1989). Unlike Harizanis (1991), who reared queens on a separate frame placed in between a comb of open brood and one with pollen and honey, we embedded the queen cells in the face of the comb containing the remaining worker larvae to create a situation similar to that found when colonies supercede, rear emergency replacement queens, or swarm.

After the experimental brood was capped, it was removed from the colony and immediately examined for mites. This allowed us to determine the number of mites that entered the cells independent of subsequent mite reproduction. We examined approximately 100 worker cells selected at random and all queen cells that were reared to the capped stage. Both mite prevalence values (MPV = the proportion of hosts infested with one or more mites) and parasite load scores (PLS = the number of mites infesting a host) (after Margolis et al., 1982) were determined. We replicated this procedure 12 times using 12 unrelated sources of brood that were reared in 12 unrelated queen-rearing colonies. All colonies were established from queens obtained from commercial sources in non-Africanized regions of the US.

Each queen-rearing colony consisted of approximately 40 full-depth combs of bees and, at the start of experiment 1, a normal compliment of open and sealed brood.

Analysis

Mite prevalence values were transformed with the square root function to equalize variances and then analyzed using a randomized block design (Proc Mixed – SAS, 1996) with caste modeled as a fixed effect and colony and the colony by caste interaction as random effects. Parasite load scores were compared using the Wilcoxon two-sample test (SAS, 1988) because their variances could not be equalized.

We used correlation analyses (SAS, 1988) to examine the relationship between mite prevalence values and colony-average parasite load scores for worker brood. We conducted a separate analysis for queen brood. We also used correlation analyses to examine the relationship between: (1) mite prevalence values for queens and workers, and (2) colony-average parasite load scores for queens and workers.

2.2. Experiment 2. Effect of alternate hosts on mite prevalence values and parasite load scores in queen cells

We conducted a second round of queen rearing immediately after all of the brood in the queen-rearing colonies had been capped. In this experiment, the comb with the source of worker larvae was discarded; therefore, the queen larvae were the only immature hosts available. After the queens cells were capped, they were removed and immediately examined for mites. Mite prevalence values and parasite load scores were determined. The procedure was replicated 11 times using the same colonies used in experiment 1 (one colony did not rear the second group of queens).

Analysis

We compared mite prevalence values and parasite load scores for the two groups of queen cells (experiments 1 and 2) using a randomized block design. Because the phoretic mite levels could have changed between experiments 1 and 2, we report these results with the caveat that there could be a bias in this comparison.

2.3. Experiment 3. Response of mites to cuticular extracts of queen and worker larvae in non-choice and binary-choice bioassays

Same-age worker and queen larvae were reared together in their parent colony and were collected 10–12 h before cell capping was completed. Intact larvae were extracted three times with hexane using 14–20 larvae per ml solvent per extraction. Each extraction lasted 5 min and was performed at room temperature. The combined extracts were filtered through glass wool in a Pasteur pipette and then concentrated under a stream of nitrogen gas. Worker and queen extracts were concentrated to 200 and 100 larval equivalents (Leq), respectively, per ml dichloromethane.

Responses of mites to extracts and controls were evaluated using both binary-choice and non-choice bioassays (Calderone and Lin, 2001). Responses to extracts in non-choice tests were evaluated at 0.5, 0.8, and 1.0 Leq. We tested 6 groups of mites (10–12 mites per group) at each combination of extract type and dose (36 groups total). Responses to extracts in binary-choice tests were evaluated at 0.5 and 0.8 Leq using 8 groups of mites (10–12 mites per group) for each combination of dose and extract type (32 groups total). For both assays, we replicated each treatment combination using mites from two colonies.

2.4. Experiment 4: Response of mites to crude extracts of royal jelly

Fresh royal jelly was collected from queen cells 10–12 h before cell capping was completed and extracted four times with dichloromethane, using 2 ml solvent per gram royal jelly for each extraction; and five times with dichloromethane:methanol, 1:1, using 1 ml solvent per gram royal jelly for each extraction. All extracts were filtered through glass wool in Pasteur pipettes. The dichloromethane:methanol, 1:1, extract was concentrated with a rotary evaporator at 30 °C down to an aqueous phase, which was partitioned three times with dichloromethane, using 0.5 ml solvent per 1 gram royal jelly equivalent (Rjeq). The dichloromethane phase was combined with the dichloromethane extract and then concentrated under a stream of nitrogen to 1 g Rjeq per ml dichloromethane for bioassay.

Responses of mites to extracts were measured with a repellent bioassay (see below), with solvent controls run for comparison. We tested 12 groups of mites (10–12 mites per group) at 5 mg Rjeq, 7 groups at 10 mg Rjeq, 7 groups at 20 mg Rjeq, and 10 groups for controls. Mites from four colonies were tested at each dose. The activity of methyl oleate (Sigma), a putative repellent of larval origin, was measured at three doses: 25, 50 and 100 µg, using a single group of 10–12 mites at each dose.

2.5. Repellent bioassay

Extracts were presented on a 5.8 × 5.8 cm glass plate with one frosted surface. A 5 × 5 cm pattern (Fig. 1) was printed on paper and attached to the underside of the glass plate to serve as an area marker. An aliquot (2–10 µl) of extract was applied evenly to the frosted surface in each of the treatment lanes ('T' in Fig. 1), and 10 µl dichloromethane were applied

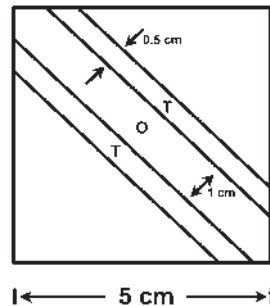


Figure 1. Pattern used in the bioassay used to measure repellent activity of royal jelly extract. Extracts were placed in the treatment lanes labeled 'T'. Mites were introduced to the central lane at the point designated 'O'.

for control runs. After air-drying, the plate and pattern were placed in a 9 cm Petri dish on a moistened filter paper.

Mites were obtained using established methods (Kuenen and Calderone, 1997; Calderone and Lin, 2001). Each mite was introduced to the central lane at the point designated 'O' and the dish cover was replaced. A mite was scored as not repelled if it moved into or through either of the treatment lanes during the 3 min observation period. A mite was scored as repelled if it moved off the plate while staying within the central lane, or if it remained in the central lane for the entire 3 min period, moving and making returns when it contacted either of the treatment lanes.

2.6. Data collection and analysis

Assays were conducted in an environmentally regulated room at 32–33 °C and 40% r. h. (Le Conte and Arnold, 1987; Kuenen and Calderone, 1997; LeDoux et al., 2000). For each group of mites, we calculated the proportion that were arrested or repelled. Non-choice bioassay data were analyzed with analysis of variance with caste (if appropriate), dose (where more than one dose was used), mite source and their interactions modeled as fixed effects. We compared extracts (types and doses) directly, without including data from control runs. We assessed extract activity separately by comparing the response to each extract with the $8.320 \pm 0.009\%$ (Ismean \pm SE) arrestment response in our standard control population (Calderone and Lin, 2001). Data are reported as percentages.

The proportions of mites arrested by the two treatments in binary-choice tests must sum to one and are not independent. Therefore, for the analysis of those data, we calculated the differences in the proportions of mites arrested by each type of extract at each dose and used the *t*-test to determine if those differences were greater than zero. Proportion data were transformed with the arcsine and square root functions to equalize variances. Data are reported as percentages.

3. RESULTS

3.1. Experiment 1. Mite prevalence values and parasite load scores in worker and queen cells

We examined an average of 98.33 ± 1.14 worker cells per colony (mean \pm SE; $n = 12$ colonies; and 1 180 total cells) and 27.42 ± 2.00 queen cells per colony ($n = 12$ colonies and 329

total cells). The average mite prevalence values (proportion of hosts with one or more mites) for the two host types differed significantly. We found $75.0 \pm 4.0\%$ (Ismean \pm SE) of worker cells infested with one or more mites; whereas only $5.1 \pm 4.0\%$ of queen cells were infested ($F_{1,11} = 198.64$; $P < 0.0001$; Fig. 2). The ratio of mite prevalence values for workers and queens was $MPV_w/MPV_Q = 15.0$. The parasite load scores (number of mites per host) for worker and queen brood were also significantly different in each colony ($|Z| > 4.0$ and $P < 0.0001$ each test; Fig. 3). The ratio of the average of the colony-average parasite load scores for workers to the corresponding value for queens was $PLS_w/PLS_Q = 48.0$.

Colony-average parasite load scores were correlated with mite prevalence values for both worker brood (using the non-linear model $y = ae^{bx}$ ($R^2 = 0.91$; $F_{1,10} = 112.62$; $P < 0.0001$) with $a = 0.11$ ($t = 2.2$; $P = 0.05$) and $b = 3.92$ ($t = 7.49$; $P = 0.0001$; $n = 12$) and queen brood ($r = 1.00$; $P < 0.0001$; $n = 12$). The correlation between worker and queen, mite prevalence values was not significant ($r = 0.34$; $P = 0.28$; $n = 12$). Similarly, the correlation between worker and queen, colony-average parasite load scores was not significant ($r = 0.19$; $P = 0.56$; $n = 12$).

3.2. Experiment 2. Effect of alternate hosts on mite prevalence values and parasite load scores in queen cells

Colonies reared an average of 21.64 ± 32.24 queen cells (mean \pm SE and 276 total queen cells). The average mite prevalence value was $4.9 \pm 1.6\%$, which was not significantly different from the $5.1 \pm 4.0\%$, the value obtained in the first experiment ($F_{1,10} = 0.01$; $P = 0.9181$; see Fig. 2). Similarly, the average parasite load score of 0.050 ± 0.016 mites per queen cell was not different from the 0.052 ± 0.015 mites per queen cell in experiment 1 ($F_{1,10} = 0.00$; $P = 0.9463$).

3.3. Experiment 3. Response of mites to extracts of queen and worker larvae in non-choice and binary-choice tests

In non-choice tests, $86.11 \pm 3.3\%$ (mean \pm SE) of mites were active. Averaged over all

Figure 2. (A) Mite prevalence values (% cells with one or more mites) for worker and queen hosts in experiment 1, and for queens in experiment 2. Values for workers and queens in experiment 1 were significantly different ($P < 0.0001$). Values for queens in experiments 1 and 2 were not significantly different ($P = 0.9181$). (B) Parasite load scores (average number of mites per cell) for queens in experiments 1 and 2 were not significantly different ($P = 0.9463$). Numbers atop each bar are the total number of hosts of that type examined in all colonies.

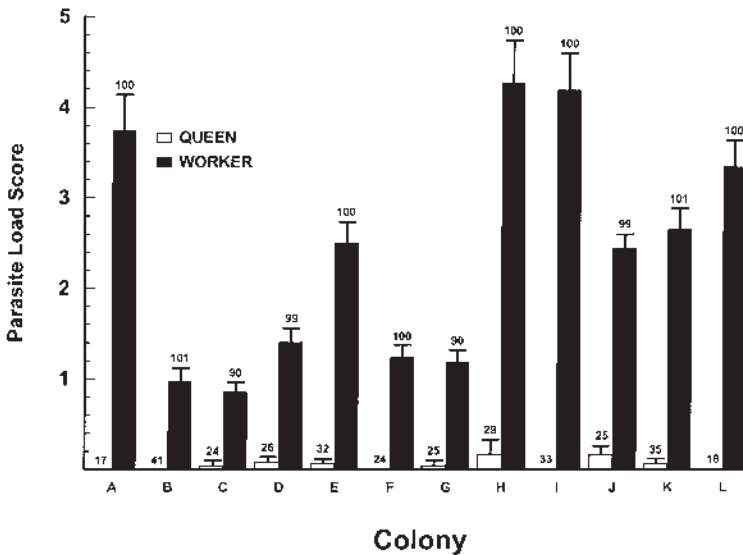
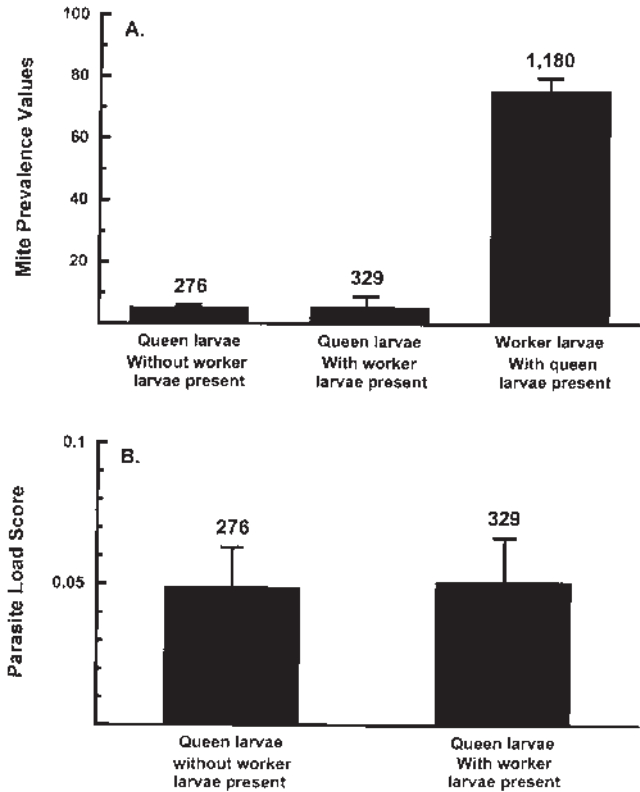


Figure 3. Parasite load scores for worker and queen hosts in each of the 12 test colonies in experiment 1. Numbers atop each bar are the total number of that type of host assayed in that colony. The differences between worker and queen hosts were significant in each colony ($P < 0.001$ each test). Where the error bar is absent, the value for that bar is zero.

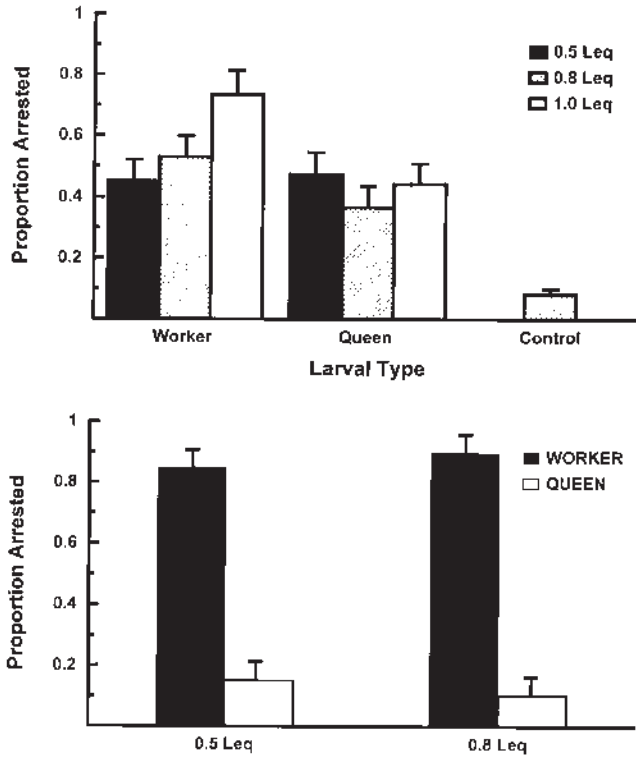


Figure 4. Percentage of mites arrested by cuticular extracts of 5th instar worker and queen larvae in (A) non-choice and (B) binary-choice test bioassays. For the non-choice tests, a greater percentage of mites was arrested by worker extracts ($P < 0.0528$). For the binary-choice bioassay, treatment effects were significant at both doses ($P < 0.0001$). Control data not included in either primary analysis.

doses, $57.13 \pm 4.94\%$ (lsmean \pm SE) of mites were arrested by worker extract, and $42.88 \pm 4.94\%$ by queen extract ($F_{1,24} = 4.15$; $P = 0.0528$) (Fig. 4A). Responses to both extracts were greater than the responses in control runs (except queen extract at 0.8 Leq). Dose ($F_{2,24} = 1.61$; $P = 0.2201$), mite source ($F_{1,24} = 2.71$; $P = 0.1130$), and the interactions between extract type and dose ($F_{2,24} = 1.72$; $P = 0.1999$); extract type and mite source ($F_{1,24} = 2.76$; $P = 0.1096$); dose and mite source ($F_{2,24} = 0.67$; $P = 0.5205$), and extract type, dose and mite source ($F_{2,24} = 0.77$; $P = 0.4761$), were not significant. In binary-choice tests, $53.13 \pm 2.70\%$ of mites were active. At 0.5 Leq, worker extract arrested $84.79 \pm 4.98\%$ of mites, while queen extract arrested $15.21 \pm 4.98\%$ (Fig. 4B) ($t_{14} = 6.98$, $P < 0.0001$). At 0.8 Leq, worker extract arrested $89.75 \pm 4.98\%$ of mites, while queen extract arrested $10.25 \pm 4.98\%$ (Fig. 4B) ($t_{14} = 7.98$; $P < 0.0001$).

3.4. Experiment 4. Response of mites to crude extracts of royal jelly

All mites were active in the bioassays. Royal jelly extract repelled $78.5 \pm 2.6\%$ of mites at 5 mg Rjeq; $85.6 \pm 3.7\%$ at 10 mg Rjeq;

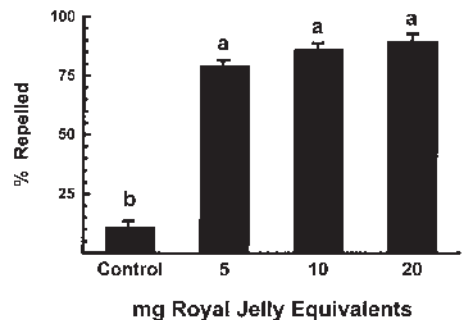


Figure 5. Percentage of mites repelled at each dose of royal jelly extract. Control data included in analysis. Means with different letters are significantly different ($P < 0.05$).

and $89.2 \pm 3.8\%$ at 20 mg Rjeq. The response at each dose was greater than the $10.5 \pm 2.9\%$ mites repelled by controls ($F_{3,20} = 67.85$; $P < 0.0001$) (Fig. 5). Differences among mite sources were marginally significant ($F_{3,20} = 2.87$; $P = 0.0622$); but, the interaction between dose and mite source was not significant ($F_{9,20} = 1.79$; $P = 0.1333$). Methyl oleate repelled $7.0 \pm 0.0\%$ of mites, which was not different from the response to solvent controls ($7.0 \pm 2.1\%$).

4. DISCUSSION

Mite prevalence values were 15 times greater for worker brood than for queen brood. The average parasite load score for worker brood was 48 times greater than the corresponding value for queen brood. These differences were found even though queen cells were reared in direct contact with worker comb, as normally occurs during supercedure, emergency queen replacement, and swarming. Our findings are similar to those of Harizanis (1991) who documented a low incidence of mites in queen brood in a population of *A. m. macedonica* in Greece using standard queen rearing techniques in which queens are reared on a separate frame.

Unlike Harizanis (1991), the incidence of mites in queen brood in our study did not appear to be affected by the presence of worker brood that could serve as an alternate host. The significance of this difference in our studies is not clear, but it is not likely to be a result of a reduction in phoretic mite levels during the second experiment. The time between the capping periods for the queen cells in our two experiments was only eight days. In addition, mite-infested workers were continuing to emerge in the queen-rearing colonies; and, since there were no immature hosts for them to enter, it is more likely that the phoretic mite levels on adult workers were greater during the second experiment. Since we did not measure phoretic mite levels and adult-bee to brood ratios, we cannot say which of these variables contributed to the variation in mite levels in the experimental worker brood in our 12 experimental colonies. However, regardless of which variables

are responsible, correlation analyses revealed that those factors had little if any effect on mite levels in queen cells. This finding suggests that mites discriminate between worker and queen larvae.

Our data provide the first evidence that mites use chemotactic cues to discriminate between worker and queen larvae. In non-choice and binary choice tests, the percentage of mites arrested by extracts of 5th instar worker larvae was greater than the percentage arrested by extracts of 5th instar queen larvae. These findings, combined with data from Trouiller et al. (1994) showing that levels of methyl palmitate, methyl linolenate and ethyl palmitate from queen larvae are lower than levels for worker larvae, suggest that those compounds could offer mites cues about caste.

Other data raise questions about the role of these compounds. Applications of methyl palmitate to worker larvae did not increase the number of mites in worker cells (Boot, 1994); and methyl palmitate was detectable in only 2 of 17 samples of volatiles from attractive brood. Rickli et al. (1992) were unable to detect the putative attractants in headspace analysis of larval volatiles, but did find palmitic acid and reported that mites on a servosphere tracked airstreams with palmitic acid. Calderone and Lin (2001) found that mites were arrested in similar proportions by extracts of worker and drone larvae at all ages during their susceptible periods. They also demonstrated arrestment behavior by mites in response to a blend of synthetic esters, but mites were not attracted to these compounds when presented as volatiles in a wind tunnel (Kuenen and Calderone, 1998). Similarly, Donzé et al. (1998) reported only a weak response to fatty acid esters when presented as volatiles.

An alternate explanation for the biased distribution of mites between worker and queen hosts is that mites are repelled by stimuli specific to queen cells, such as the royal jelly used to feed queen larvae. A crude extract of royal jelly repelled mites at each of the three doses tested, with a maximum of $89.2 \pm 3.8\%$ of mites repelled at 0.8 mg Rjeq. These data suggest that the low incidence of mites in queen cells is a result of their being repelled by chemicals in royal jelly. They also raise the

possibility that mites were arrested disproportionately by extracts of worker larvae because small amounts of royal jelly were present in the queen larvae extract. In contrast, worker and drone, brood food and brood food extracts have been shown to arrest mites in various bioassays (Calderone and Lin, 2001; Nazzi et al., 2001).

Non-chemical factors also distinguish worker and queen cells from each other and may play a role in the host acquisition process. Most notable is the difference in the orientation of the two cell types. The long axis of a queen cell runs perpendicular to the long axis of a worker cell. This difference could provide mites with geotactic cues that allow them to distinguish between cell types. Other characteristics, such as the distance between a larva and the rim of its cell, may also play a role, as has been suggested for worker and drone brood (Goetz and Koeniger, 1993; Boot et al., 1995).

Our findings suggest that the low incidence of mites in queen brood is due in part to the repellent activity of royal jelly, and possibly to intrinsic differences in larval chemistries.

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Résumé – Différence d'infestation du couvain d'ouvrières et de reine d'abeilles (*Apis mellifera*) par l'acarien *Varroa destructor*. Nous avons comparé les niveaux d'acariens dans le couvain d'ouvrières et le couvain de reines dans des colonies d'*Apis mellifera* L. de différentes régions des États-Unis dépourvues d'abeilles africanisées. Dans une expérimentation nous avons trouvé $75,0 \pm 4,0$ % (moyenne LS \pm SE) des cellules d'ouvrières infestées par au moins un acarien, alors que seulement $5,1 \pm 4,0$ % des cellules royales étaient infestées ($P < 0,0001$; Fig. 2). En outre les degrés d'infestation du couvain d'ouvrières et du couvain de reines étaient significativement différents pour chaque colonie ($P < 0,0001$ pour chaque test ; Fig. 3). La corrélation entre le taux d'infestation du couvain d'ouvrières et celui du couvain de reine n'était pas

significatif ($r = 0,34$, $P = 0,28$, $n = 12$). De même, la corrélation entre le degré moyen d'infestation par colonie du couvain d'ouvrières et celui du couvain de reines n'était pas significatif ($r = 0,19$, $P = 0,56$, $n = 12$). Ces résultats suggèrent que les acariens font activement la différence entre larves d'ouvrières et larves de reine.

Nous avons aussi examiné la réaction des acariens aux extraits de larves d'ouvrières et de larves de reines du 5^e stade. Dans les tests sans choix, toutes doses confondues, $57,13 \pm 4,94$ % en moyenne des acariens s'arrêtaient sur l'extrait brut de larves d'ouvrières et $42,88 \pm 4,94$ % sur celui de reines ($P = 0,0528$) (Fig. 4A). La réaction aux deux types d'extraits était significativement plus élevée que la réaction aux solvants (témoins). Dans les tests à deux choix à la dose de 0,5 équivalent larve (Leq), $84,79 \pm 4,98$ % des acariens s'arrêtaient sur l'extrait de larve et $15,21 \pm 4,98$ % sur l'extrait de reine (Fig. 4B) ($t_{14} = 6,98$; $P < 0,0001$). A la dose de 0,8 Leq, $89,75 \pm 4,98$ % des acariens s'arrêtaient sur l'extrait de larve et $10,25 \pm 4,98$ % sur l'extrait de reine (Fig. 4B) ($t_{14} = 7,98$; $P < 0,0001$).

Enfin nous avons étudié la réaction des acariens à des extraits de gelée royale. Nous avons trouvé que $78,5 \pm 2,6$ % des acariens étaient repoussés à la dose de 5 mg Rjeq (équivalent à 5 mg de gelée royale), $85,6 \pm 3,7$ % à la dose de 10 mg Rejq et $89,2 \pm 3,8$ % à la dose de 20 mg Rejq. La réaction à chaque dose était supérieure à celle suscitée par le solvant (témoin) : $10,5 \pm 2,9$ % ($P < 0,0001$) (Fig. 5). Nos résultats suggèrent que la faible présence des acariens dans le couvain de reine est partiellement dû à l'activité répulsive de la gelée royale et vraisemblablement à des différences spécifiques dans la chimie des larves.

Apis mellifera / *Varroa destructor* / acarien parasite / gelée royale

Zusammenfassung – Unterschiedlicher Infektionsgrad in Arbeiterinnen- und Königinnenbrut der Honigbienen (*Apis mellifera*) durch die parasitische Milbe *Varroa destructor*. Wir verglichen den Befallsgrad durch Milben (*Varroa destructor*) in Arbeiterinnen- und Königinnenbrut in Völkern von *Apis mellifera* aus verschiedenen Regionen der USA, in denen keine afrikanisierten Bienen vorkommen. In einer Untersuchung fanden wir $75,0 \pm 4,0$ % (LS Mittelwert \pm SE) der Arbeiterinnenzellen mit einer oder mehr Milben befallen, während nur $5,1 \pm 4,0$ % der Weiselzellen befallen waren ($P < 0,0001$; Abb. 2). Die Befallsrate für Arbeiterinnen- und Königinnenbrut war außerdem in jedem Volk signifikant verschieden ($P < 0,0001$ bei jedem Test; Abb. 3). Die Korrelation zwischen der Parasitenbelastung bei Arbeiterinnen und Königinnen war nicht

signifikant ($r = 0,34$, $P = 0,28$, $n = 12$). Entsprechend ähnlich war die Korrelation zwischen dem mittleren Verbreitungsdruck der Parasiten bei Arbeiterinnen- und Königinnenbrut nicht signifikant ($r = 0,19$, $P = 0,56$, $n = 12$). Dieser Befund legt nahe, dass die Milben aktiv zwischen Arbeiterinnen- und Königinnenlarven unterscheiden.

Wir untersuchten auch die Reaktion der Milben auf Extrakte des 5. Larvenstadium von Arbeiterinnen und Königinnen. In Tests ohne Wahlmöglichkeit (non-choice tests), durchschnittlich ermittelt über alle Dosierungen, befanden sich $57,13 \pm 4,94$ % der Milben auf dem Rohextrakt der Arbeiterinnenlarven und $42,88 \pm 4,94$ % auf dem der Königinnenlarven ($P = 0,0528$) (Abb. 4A). Die Reaktion auf beide Extrakttypen war signifikant höher als auf die Kontrolllösungen. In einem Simultan – Wahlversuch mit 0,5 Leq befanden sich $84,79 \pm 4,98$ % der Milben auf dem Larvenextrakt von Arbeiterinnen, während $15,21 \pm 4,98$ % der Milben den Extrakt der Königinnen gewählt hatten (Abb. 4B) ($t_{14} = 6,98$, $P < 0,0001$). Bei 0,8 Leq zog der Arbeiterinnenextrakt $89,75 \pm 4,98$ % der Milben an, während es bei Königinnenextrakt $10,25 \pm 4,98$ % waren (Abb. 4B) ($t_{14} = 7,98$; $P < 0,0001$).

Schließlich untersuchten wir noch die Reaktion von Milben auf Extrakte des Futtersaftes der Königinnen. Wir fanden eine Repellentwirkung bei 5 mg Rjeq von $78,5 \pm 2,6$ % der Milben; bei 10 mg Rjeq waren es $85,6 \pm 3,7$ % und bei 20 mg Rjeq waren es $89,2 \pm 3,8$ %. Die Repellentwirkung war bei jeder Dosis mit $10,5 \pm 2,9$ % Prozent der Milben größer, als die bei der Lösungsmittelkontrolle ($P < 0,0001$) (Abb. 5). Nach unseren Ergebnissen ist das geringe Vorkommen von Milben in Königinnenbrut zum Teil auf die abstoßende Wirkung des Futtersaftes der Königinnen und wahrscheinlich auch auf spezifische Unterschiede in der Larvenchemie zurückzuführen.

Apis mellifera / *Varroa destructor* / parasitische Milbe / Königinnenfuttersaft

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