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Observations on the removal of brood inoculated with *Tropilaelaps mercedesae* (Acari: Laelapidae) and the mite's reproductive success in *Apis mellifera* colonies

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Abstract This study assessed the response of *Apis mellifera* to brood deliberately infested with *Tropilaelaps mercedesae*. The reproductive success of *T. mercedesae* in mite-inoculated and naturally infested brood was also compared. The presence of *T. mercedesae* inside brood cells significantly affected brood removal. Thai *A. mellifera* removed 52.6 ± 8.2 % of the brood inoculated with *T. mercedesae* as compared to 17.2 ± 1.8 and 5.7 ± 1.1 % removal rates for the groups of brood with their cell cappings opened and closed without mite inoculation and the control brood (undisturbed, no mite inoculation), respectively. Brood removal peaked during the second and third days post inoculation when test brood was at the prepupal stage. Overall, non-reproduction (NR) of foundress *T. mercedesae* was high. However, when NR was measured based on the criteria used for *Varroa*, the naturally infested pupae (NIP) supported the highest NR (92.8 %). Newly sealed larvae inoculated with *Tropilaelaps* collected from newly sealed larvae (NSL) had 78.2 % NR and those inoculated with *Tropilaelaps* collected from tan-bodied pupae (TBP) had 76.8 % NR. Since *Tropilaelaps* is known to have a short development period and nearly all progeny reach adulthood by the time of host emergence, we also used two *Tropilaelaps*-specific criteria to determine NR. Foundresses that did not produce progeny and those that produced only one progeny were considered NR. Using these two criteria, NR decreased tremendously but showed similar trends with means of 65, 40 and 33 % for NIP, NSL and TBP, respectively. High NR in the NIP group may indicate increased hygienic behavior in Thai *A. mellifera* colonies. The removal of infested prepupae or

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tan-bodied pupae will likely decrease the reproductive potential of *Tropilaelaps*. Our study suggests that brood removal may be one of the resistance mechanisms towards *T. mercedesae* by naturally adapted Thai *A. mellifera*.

Keywords *Tropilaelaps mercedesae* · Non reproduction · Brood removal · Hygienic behavior · Resistance · Mesostigmata

Introduction

Tropilaelaps spp. are the most serious parasitic mites in *Apis mellifera* colonies in Asia (Burgett et al. 1983). Like *Varroa* spp., the reproductive cycle of *Tropilaelaps* spp. is synchronized with the developmental stages of *A. mellifera* (Sammataro et al. 2000; Oldroyd and Wongsiri 2006). Nonetheless, the developmental period (from egg to adult emergence) inside the brood cell is shorter for *Tropilaelaps* than for *Varroa* mites (Woyke 1987b). The first adult *Tropilaelaps* offspring are observed in purple-eyed pupae (referred to as Pr-Pd by Rembold et al. 1980) while the first adult *Varroa* mites are usually observed in tan-bodied pupae (Pdl) (Woyke 1987b; Ritter and Schneider-Ritter 1988). Also, *Tropilaelaps* females are reported to lay equal numbers of females and males, and that nearly all *Tropilaelaps* offspring become adult mites before their host bee emerges (Ritter and Schneider-Ritter 1988). This short life cycle and a short phoretic stage (Woyke 1987a; Koeniger and Muzaffar 1988; Rinderer et al. 1994) are probably the key factors that contribute to the rapid population development of *Tropilaelaps* in *A. mellifera* colonies.

Across *Apis* species, defense mechanisms are important factors that limit the reproduction and population growth of parasitic mites. *Apis cerana* is more efficient in removing *Varroa* or *Tropilaelaps* from their bodies than *A. mellifera* (Peng et al. 1987; Khongphinitbunjong et al. 2012). Similarly, mite drop varies among stocks of *A. mellifera*. For example, high mite drop was associated with decreased *Varroa* mite populations in Russian honey bee (RHB) colonies (Rinderer et al. 2003, 2013; Guzman-Novoa et al. 2012).

The removal of mite-infested brood has also been associated with resistance to *Varroa destructor*. One mechanism of resistance linked to hygiene is non-reproduction (NR) of *Varroa* mites. Initially, *Varroa*-resistant Varroa Sensitive Hygienic (VSH) bees were reported to be hygienic only to brood infested with reproductive mites (Harbo and Harris 2005). Hence, the bees were thought to be selected for high proportions of NR mites. However, further studies showed that these bees remove pupae infested with either reproductive or NR mites (Harris et al. 2010). The disruption of the reproduction of *Varroa* when the brood cells are uncapped or removed may cause NR in hygienic colonies (Harris 2007; Rinderer et al. 2010). Likewise, the production of males in the natal cells and females in the new host cells results in mating failure, and the lack of suitable hosts will force oosorption to occur in gravid mites exposed by brood removal (Kirrane et al. 2011).

Non-reproduction of foundress *Tropilaelaps* spp. has been examined by a few researchers but contradictory results have been reported. In their original host, *Apis dorsata*, a high NR of 65 % was reported by Kavinseksan (2004). A lower NR has been recorded in *A. mellifera* colonies: 18.3 % in Vietnam and 7.3 % in Afghanistan (Woyke 1990) and 27 % in Thailand (Ritter and Schneider-Ritter 1988). The highest NR of about 50 % was reported by Kavinseksan (2004) for Thai *A. mellifera* and RHB imported into Thailand in November 2001. This high NR reported by Kavinseksan may be due to an

increased hygienic behavior as demonstrated by responses to frozen brood by both Thai *A. mellifera* and RHB colonies (Kavinseksan et al. 2004), an observation also reported by de Guzman et al. (2002) for RHB in the United States.

So far, no studies have been conducted on the hygienic behavior of *A. mellifera* against brood infested with *Tropilaelaps*. Therefore, this study was conducted to determine the removal response of *A. mellifera* towards brood deliberately inoculated with *T. mercedesae*. The mites' ability to produce viable offspring in disturbed (mite-inoculated) and undisturbed (naturally infested) capped brood was also compared.

Materials and methods

This experiment was conducted in San Pa Tong district, Chiang Mai, Thailand between November 2011 and July 2012. The genotypic identity of *Tropilaelaps* used in this study was confirmed by DNA analysis as *T. mercedesae* (Khongphinitbunjong et al. 2012).

Experiment 1: Removal of brood deliberately infested with *Tropilaelaps mercedesae*

Five colonies of *A. mellifera ligustica* were used in this study. Each colony consisted of six or seven frames of adult bees and two or three frames of brood established in a standard 8-frame Langstroth hive. In each colony, brood of the same age was obtained by caging the queen on an empty frame using a screen (8 mesh) push-in cage. The test section of brood consisted of 20 rows with 20 brood cells per row. On the 8th day when the brood was sealed, 40 female *Tropilaelaps* were individually introduced into brood cells using a transfer technique (Garrido and Rosenkranz 2003; Kirrane et al. 2011). The inoculum mites (mites used for artificial infestation) were obtained from newly sealed brood of highly infested *A. mellifera* colonies. Brood cells randomly received one of the following groups: (1) brood inoculated with one female *Tropilaelaps*, (2) brood with capping opened and closed without mite inoculation (O/C), and (3) undisturbed brood cells as control. After mite inoculation, test frames were returned to their respective colonies. Prior to returning the frames, each test section was digitally photographed and then every 24 h for 7 days to assess the rate of brood removal.

Experiment 2: Reproduction of *Tropilaelaps mercedesae* in mite-inoculated and naturally infested brood

Fourteen *A. mellifera* colonies, different from the colonies used in Experiment 1, were used in this study. Reproduction of *T. mercedesae* was assessed using foundress mites collected from different sources: (a) *Tropilaelaps* collected from newly sealed brood and introduced into newly sealed brood (NSL, $n = 5$ colonies), (b) *Tropilaelaps* collected from tan-bodied pupae and then inoculated into newly sealed brood (TBP, $n = 3$ colonies), and (c) *Tropilaelaps* naturally infesting pupae (NIP, $n = 6$ colonies). For treatments a and b, the transfer technique used in Experiment 1 was employed. The mites for the NSL mite group were all foundresses (presumably all gravid) since they were collected from newly sealed larvae. However, the mites used to inoculate the TBP group came from tan-bodied pupae. Therefore, it is possible that both the foundress and the daughter mites were used for inoculation in this group. To prevent worker bees from removing inoculated brood, each test section was covered by a cage made of a plastic petri dish (diameter = 9 cm) modified with a single hole (2.54 × 2.54 cm) on the top covered with a nylon screen (8 mesh). The

screened hole provided ventilation for the caged brood. The cage also was reinforced with extra beeswax and rubber bands. The frames were then returned to their respective colonies. Eight days after inoculation when the pupae were tan-bodied, each pupa was examined under a dissecting microscope to determine mite reproduction. The same stage of brood was also examined to determine the mite reproduction in naturally infested brood.

The reproductive status of *T. mercedesae* was first assessed using categories used for *Varroa.* We considered a foundress *Tropilaelaps* to be non-reproductive (NR) when the infested cell consisted of: (a) a foundress but no progeny, (b) a foundress with a young daughter but no adult son, (c) a foundress with an adult son but no young daughter, and (d) a foundress with progeny too young to reach adulthood at the host bees' emergence (Kirrane et al. 2011; Dietemann et al. 2013). Second, we reformulated the criteria for determining NR *Tropilaelaps* foundresses. NR *Tropilaelaps* were those that did not produce any progeny or only produced one progeny. These two categories were based on the reports that *Tropilaelaps* lay female and male eggs in equal numbers (Ritter and Schneider-Ritter 1986) and that 99 % of nymphs reached adulthood by the time host bees emerged (Ritter and Schneider-Ritter 1988). For both methods, reproductive foundress mites were those that produced viable progeny (with an adult son and an adult daughter).

Data analyses

Rates of brood removal were calculated as the number of brood removed out of the total number of brood examined multiplied by 100. Arcsine square-root and square-root transformations were used to transform data on percentage of brood removed and the duration of brood removal, respectively to approximate normality. Means were then compared in a one-way ANOVA. Percentages of pupae removed through time were calculated as the number of pupae removed every day out of the total number of pupae removed. These data were transformed with an arcsine square-root transformation and means were compared with a two-factor ANOVA with day of observation and brood type as the factors.

Proportion of NR was calculated as the number of foundresses that followed the two categories as described above. For each treatment, comparisons within the different *Varroa* criteria were made using the Marascuillo procedure (http://www.itl.nist.gov/div898/handbook/prc/section4/prc474.htm) for multiple proportions. *T* test was used to compare proportions using the criteria for *Tropilaelaps.*

Results

Experiment 1

For the percentage of brood removed, no significant interaction between days of observation and brood type was detected ($F_{10,72} = 1.28$, $P = 0.26$). However, the inoculation of *Tropilaelaps* into brood cells significantly affected brood removal response ($F_{2,12} = 30.06$, $P < 0.001$) (Fig. 1). Of the 189 brood cells inoculated with mites, a total of 105 (56 %) was removed by worker bees by the end of experiment. The bees removed brood for 6 days with the highest rate of brood removal observed during days 2 and 3 post mite inoculation ($F_{5,72} = 7.27$, $P < 0.001$) (Fig. 2). The lowest removal rate was recorded on days 5 and 6. On average, duration of brood removal was similar ($F_{2,144} = 0.47$, $P = 0.63$) among the

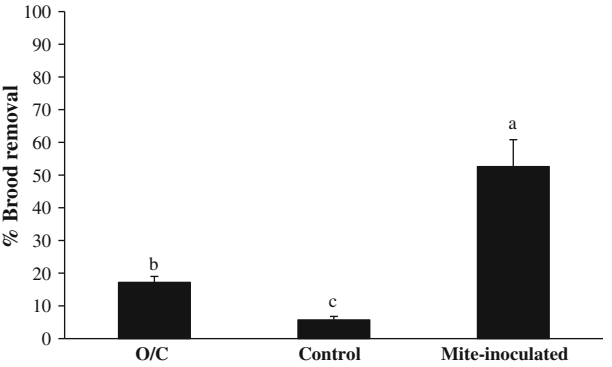

Fig. 1 Proportion of brood removed in brood with their cell cappings opened and closed without mite inoculation (O/C), control (undisturbed brood, brood without mite inoculation) and brood inoculated with *Tropilaelaps mercedesae*. *Bars with different letters* are significantly different (ANOVA, $P < 0.05$)

treatment groups: mite-inoculated brood = 2.9 ± 0.1 days; O/C = 2.9 ± 0.2 days and control (undisturbed cells) = 2.5 ± 0.2 days.

In addition, a low percentage of the opened brood was resealed within 24 h after opening. Of the 111 *Tropilaelaps*-inoculated and 18 undisturbed brood cells that were opened by the bees only 6.3 % (seven cells) and 5.5 % (one cell) were resealed by them, respectively. None of the O/C brood cells were resealed. In the control group, one pupa was found infested with a reproductive *Tropilaelaps* and one with a NR *Varroa* mite. None of the O/C group was infested by either mite genera.

Experiment 2

For the brood deliberately infested with *Tropilaelaps* collected from two different stages of brood, a total of 820 brood cells were examined (control = 326, O/C = 150 and mite-inoculated = 344 cells). Of the 344 inoculated mites, 91.73 % (n = 320) were successfully recovered and evaluated for mite reproduction. For the naturally infested brood, ≥1,000 tan-bodied pupae were examined. However, only 167 pupae were infested and used in the analyses.

Reproductive success of *T. mercedesae* was assessed two ways. Based on the infestation parameters standard for *Varroa*, proportions of NR *Tropilaelaps* were high. The NIP group (92.8 %) supported the highest NR with the lowest NR recorded in the NSL (78.2 %) and TBP (76.5 %) groups ($P < 0.01$) (Fig. 3). NR in the NSL group was largely composed of those foundresses that produced nymphs only, followed by those with an adult son but no adult daughter, and those that produced adult daughters but no adult son. For the TBP group, large proportions of the NR foundresses produced only nymphs, and had sons but no daughters. Only a few foundress *Tropilaelaps* did not produce progeny. The majority of the NR foundresses in the NIP group produced nymphs only. Females producing adult daughters with no son had the least contribution to NR in this group.

When we used the two *Tropilaelaps*-specific criteria to characterize NR, the proportion of NR decreased tremendously (Fig. 3). However, the same trend was observed. NR was higher in the NIP group (65.2 %) than in the NSL (39.9 %) or TBP (33.3 %) groups ($P < 0.05$). Most of the NR foundresses produced one progeny in both the TBP and NIP groups. However, similar proportions of foundresses that had one progeny and no progeny were recorded in the NSL group.

Moreover, more progeny per foundress were recorded in the NSL (1.86 progeny per foundress) and TBP (1.95 progeny per foundress) groups than those in the NIP group (0.79

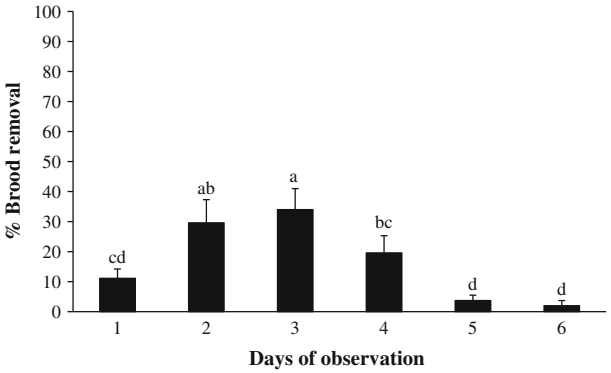

Fig. 2 Proportion of pupae removed during 6 days after mites' inoculation. *Bars with different letters* are significantly different (ANOVA, $P < 0.05$)

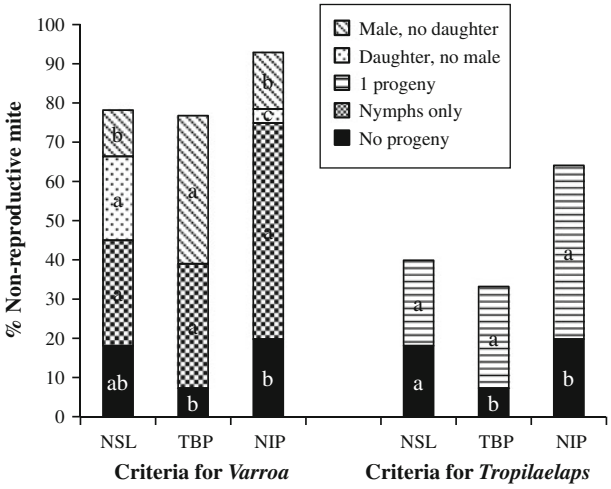

Fig. 3 Contribution of infestation parameters toward total non-reproduction of *Tropilaelaps mercedesae* in mite-inoculated and naturally infested brood. For each treatment, categories with *different letters* are significantly different ($P < 0.05$) within treatment. NSL = *Tropilaelaps* collected from newly sealed brood and introduced into newly sealed brood, TBP = *Tropilaelaps* collected from tan-bodied pupae and then inoculated into newly sealed brood, NIP = *Tropilaelaps* naturally infesting pupae

progeny per foundress) ($P < 0.05$). All pupae in the control and O/C groups were free from *Tropilaelaps* infestation. However, we found two cells infested with *Varroa*. No dead mite inside capped brood was observed.

Discussion

Our results suggest that on average, Thai *A. mellifera* colonies are also hygienic to brood infested with *T. mercedesae*. This observation is consistent with an earlier study which reported that high proportions of Thai *A. mellifera* and RHB colonies are hygienic towards freeze-killed brood (Kavinseksan et al. 2004). The removal of *Tropilaelaps*-infested brood occurred during 1–6 days post inoculation, which is similar to the observations reported by Harris (2007) using *Varroa* Sensitive Hygienic (VSH) bees against *Varroa*-infested brood. The peak brood removal was observed on the 2nd and 3rd day after mite inoculation. However, when brood removal for mite-infested brood was analyzed separately, significant brood removal (13–32 %) occurred during the first 4 days after mite inoculation, which coincided with the prepupal to white-eyed pupal stages of the bee hosts. During this period,

all mite progeny were still nymphs and thus were likely to die from bees' aggression or starvation upon brood removal.

According to Ritter and Schneider-Ritter (1988), *Tropilaelaps* mites lay their first eggs shortly after the brood is sealed. About 1 day from egg-laying to the development of protonymphs is required, and another 2 days are required for the mites to become deutonymphs (Woyke 1987b). Assuming that the inoculated foundresses laid eggs immediately after inoculation, the mite-inoculated brood should have had actively feeding mites (protonymphs and some deutonymphs) during the 2nd and 3rd day after mite-inoculation. Thus, the detection of mites and subsequent removal of infested brood may have been enhanced by the release of honey bee volatiles in response to nymphal feeding. Likewise, the presence of mites' feces inside the brood cells may have assisted worker bees in locating the infested cells. Insect frass and its volatile components are known to provide cues in habitat location (Weiss 2006). It is also possible that the movement of nymphs inside the brood cells may have assisted detection of infested brood.

About 25 % of *Varroa* mites successfully reproduced (produced an adult male and young daughter) when transferred from newly sealed larvae into newly sealed larvae (Kirrane et al. 2011). In this study, we followed Kirrane et al.'s procedure. Based on our results, *Tropilaelaps* could successfully reproduced with higher number of progeny per foundress (NSL and TBP) compared to NIP. This transfer technique including the caging method did not affect the reproduction of inoculum mites. We observed reproductive success similar to that of *Varroa* when *Tropilaelaps* were transferred from newly sealed (NSL, 22 %) or tan-bodied brood (TBP, 23 %) into newly sealed larvae. However, some aspects of *Varroa*'s life history are different from those of *Tropilaelaps*. *Varroa* lays one male egg only (Ifantidis 1983) while *Tropilaelaps* lays female and male eggs in equal numbers (Ritter and Schneider-Ritter 1986). Further, Ritter and Schneider-Ritter (1988) reported that adult sons and adult daughter *Tropilaelaps* are already present when bee hosts are at the red brown-eyed stage (Pr) and that the majority of the nymphs reached adulthood by the time the host bees emerged. In contrast, only 1 son and 1 or 2 adult daughters *Varroa* emerge with the host bee.

We used two *Tropilaelaps*-specific criteria to characterize NR: foundresses that did not produce any progeny and those that produced only one progeny were classified as NR. All other foundresses were classified as reproductive. Based on these criteria, the proportion of reproductive *Tropilaelaps* increased to 60 and 67 % for the NSL and TBP groups, respectively. Since the inoculum mites were collected from newly sealed larvae and tan-bodied pupae, it is likely that most of the mites (if not all) were already mated and ready to reproduce by the time they were used as inoculum mites for this study. In general, mated mites enter cells before they are sealed and son and adult daughters (especially for *Tropilaelaps*) are already present at the tan-bodied pupal stage (Ritter and Schneider-Ritter 1988).

NR of 30 % or more negatively affects *Varroa* mite population growth (Harbo and Hoopingarner 1997). Recently, Kirrane et al. (2011) reported that the removal of *Varroa* mite-infested brood may be the major cause of NR in hygienic colonies. Whether or not these observations also are true for *T. mercedesae* have yet to be established. Nonetheless, we observed high proportions of NR (more than 30 %) especially in the NIP group even when the more conservative criteria for *Tropilaelaps* were used. Although proportions of NR in the NSL and TBP groups decreased from 78 to 40 % and 77 to 33 %, respectively using these two criteria, these values were still higher than the NR reported from Vietnam (18.3 %) and Afghanistan (7.3 %) for *T. clareae* (Woyke 1990). These values were also higher than the NR (27 %) reported from Thailand by Ritter and Schneider-Ritter (1988).

The criteria for NR used by Woyke (1990) and Ritter and Schneider-Ritter (1988) were unclear. However, a higher NR of 50 % was recorded from Thai *A. mellifera* and RHB colonies when the *Varroa* criteria used by Kavinseksan (2004) were also used in this study. Further, volatiles from cocoons may also affect mite reproduction. Rates of NR were higher in combs built by the *Varroa*-resistant RHB than in combs built by susceptible Italian colonies (de Guzman et al. 2008). Some chemicals such as acaricides may also be present inside the colony that negatively affected *Tropilaelaps* reproduction. Thai beekeepers regularly treat colonies with acaricides to keep *A. mellifera* colonies alive.

Generally, *Varroa* mites will produce viable offspring in naturally (undisturbed) infested brood (LIG personal obs.), which contrasts with our observations with *Tropilaelaps*. NIP supported the highest proportion of NR *Tropilaelaps* and thus, had the lowest progeny per foundress. It is possible that these naturally infested colonies were hygienic towards *Tropilaelaps*-infested brood, a trend also suggested by our results in the brood removal experiment (Experiment 1). It is also possible that *Tropilaelaps* mites invading brood cells may not be ready for reproduction. Although *Tropilaelaps* are known to not survive long on adult bees (Woyke 1984; Rinderer et al. 1994), the actual phoretic phase for *Tropilaelaps* reproduction has not been studied. However, we can deduce from our results that a phoretic phase may unnecessary since high reproduction as observed on TBP. In *Varroa*, Kirrane et al. (2011) reported that using phoretic mites inoculated into NSL resulted in a high proportion of NR. Our observations may also suggest that after 40 years of exposure to *Tropilaelaps* (Akratanakul 1979; Wongsiri and Chen 1995), Thai *A. mellifera* may have slowly developed behavioral resistance to this parasite. Thai beekeepers usually use the presence of "bald brood" (a condition wherein the capping of a sealed brood is removed by worker bees) as an indicator of *Tropilaelaps* infestation in Northern Thailand where the majority of the country's *A. mellifera* colonies are kept. In addition to brood removal, Thai *A. mellifera* may also possess other mechanisms of resistance to *Tropilaelaps*. Although inferior to *A. cerana*, Thai *A. mellifera* removed 91 % of *Tropilaelaps* within 48 h of inoculation (Khongphinitbunjong et al. 2012). Likewise, the immune responses of Thai *A. mellifera* are also triggered by *Tropilaelaps* infestation (Khongphinitbunjong et al. submitted). Thus, a selection program for hygienic or other resistant traits may result in a stock of Thai *A. mellifera* that is resistant to *T. mercedesae.*

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References

Akratanakul P (1979) Occurrence of a parasitic bee mite *Tropilaelaps clareae* (Acari: Gamasida) in Thailand. BRL's Technical Report No.1/1979

Burgett DM, Akratanakul P, Morse R (1983) *Tropilaelaps clareae*: a parasite of honeybees in South-East Asia. Bee World 64:25–28

de Guzman LI, Rinderer TE, Stelzer JA, Beaman LD, Delatte GT, Harper C (2002) Hygienic behavior by honey bees from Far-Eastern Russia. Am Bee J 142:58–60

de Guzman LI, Rinderer TE, Frake AM (2008) Comparative reproduction of *Varroa destructor* in different types of Russian and Italian honey bee combs. Exp Appl Acarol 44:227–238

Dietemann V, Nazzi F, Martin SJ, Anderson DL, Locke B, Delaplane KS, Wauquiez Q, Tannahill C, Frey E, Ziegelmann B, Rosenkranz P, Ellis JD (2013) Standard methods for *Varroa* research. J Apic Res doi:10.3896/IBRA.1.52.1.09

Garrido C, Rosenkranz P (2003) The reproductive program of female *Varroa destructor* mites is triggered by its host *Apis mellifera*. Exp Appl Acarol 31:269–273

Guzman-Novoa E, Emsen B, Unger P, Espinosa-Montaño LG, Petukhova T (2012) Genotypic variability and relationships between mite infestation levels, mite damage, grooming intensity, and removal of *Varroa destructor* mites in selected strains of worker honey bees (*Apis mellifera* L.). J Invert Pathol 110:314–320

Harbo JR, Harris JW (2005) Suppressed mite reproduction explained by the behaviour of adult bees. J Apic Res 44:21–23

Harbo J, Hoopingarner R (1997) Honey bees (Hymenoptera: Apidae) in the United States that express resistance to *Varroa jacobsoni* (Mesostigmata: Varroidae). J Econ Entomol 90:893–898

Harris JW (2007) Bees with varroa sensitive hygiene preferentially remove mite infested pupae aged ≤five days post capping. J Apic Res 46:134–139

Harris JW, Danka RG, Villa JD (2010) Honey bees (Hymenoptera: Apidae) with the trait of Varroa Sensitive Hygiene remove brood with all reproductive stages of varroa mites (Mesostigmata: Varroidae). Ann Entomol Soc Am 103:146–152

Ifantidis MD (1983) Ontogenesis of the mite *Varroa jacobsoni* Oud. in worker and drone honeybee brood cells. J Apic Res 22:200–206

Kavinseksan B (2004) Defense mechanism of *Apis dorsata* Fabricius and ARS Primorsky honey bee *Apis mellifera* Linnaeus to the bee mite *Tropilaelaps clareae* Delfinado and Baker. Dissertation, Chulalongkorn University, Thailand

Kavinseksan B, Wongsiri S, Rinderer TE, de Guzman LI (2004) Comparison of the hygeinic behavior of ARS Russian and commercial honey bee in Thailand. Am Bee J 144:870–872

Khongphinitbunjong K, de Guzman LI, Burgett DM, Rinderer TE, Chantawannakul P (2012) Behavioural responses underpinning resistance and susceptibility of honeybees to *Tropilaelaps mercedesae*. Apidologie. doi:10.1007/s13592-012-0129-x

Kirrane MJ, de Guzman LI, Rinderer TE, Frake AM, Wagnitz J, Whelan PM (2011) Asynchronous development of honey bee host and *Varroa destructor* (Mesostigmata: Varroidae) influences reproductive potential of mites. J Econ Entomol 104:1146–1152

Koeniger N, Muzaffar N (1988) Lifespan of the parasitic honey bee mite *Tropilaelaps clareae* on *Apis cerana*, *dorsata* and *mellifera*. J Apic Res 27:207–212

Oldroyd BP, Wongsiri S (2006) Asian honey bees. Harvard University Press, Cambridge

Peng YS, Fang Y, Xu S, Ye L (1987) The resistance mechanism of the Asian honeybee, *Apis cerana* Fabr., to an ectoparasitic mite, *Varroa jacobsoni* Oudemans. J Invert Pathol 49:54–60

Rembold H, Kremer JP, Ulrich W (1980) Characterization of postembryonic developmental stages of the female castes of the honey bee, *Apis mellifera* L. Apidologie 11:29–38

Rinderer TE, Oldroyd BP, Lekprayoon C, Wongsiri S, Thapa R (1994) Extended survival of the parasitic mite *Tropilaelaps clareae* on adult workers of *Apis mellifera* and *Apis dorsata*. J Apic Res 33:171–174

Rinderer TE, de Guzman LI, Delatte GT, Harper C (2003) An evaluation of ARS Russian honey bees in combination with other methods for the control of *Varroa* mites. Am Bee J 143:410–413

Rinderer TE, Harris JW, Gregory J, Hunt GJ, de Guzman LI (2010) Breeding for resistance to *Varroa destructor* in North America. Apidologie 41:409–424

Rinderer TE, de Guzman LI, Frake AM (2013) Associations of parameters related to the fall of *Varroa destructor* (Mesostigmata: Varroidae) in Russian and Italian honey bee (Hymenoptera: Apidae) colonies. J Econ Entomol 106:566–575

Ritter W, Schneider-Ritter U (1986) *Varroa jacobsoni* and *Tropilaelaps clareae* in bee colonies of *Apis mellifera* in Thailand. Apidologie 17:384–386

Ritter W, Schneider-Ritter U (1988) Differences in biology and means of controlling *Varroa jacobsoni* and *Tropilaelaps clareae*, two novel parasitic mites of *Apis mellifera.* In: Needham GR, Page RE, Delfinado-Baker M, Bowman CE (eds) Africanized honey bees and be mites. New York, pp 387–395

Sammataro D, Gerson U, Needham G (2000) Parasitic mites of honey bees: life history, implications, and impact. Annu Rev Entomol 45:519–548

Weiss MR (2006) Defecation behaviour and ecology of insects. Annu Rev Entomol 51:635–661

Wongsiri S, Chen P (1995) Effect of agricultural development on honey bees in Thailand. Bee World 76:3–5

Woyke J (1984) Survival and prophylactic control of *Tropilaelaps clareae* infesting *Apis mellifera* colonies in Afghanistan. Apidologie 15:421–434

Woyke J (1987a) Length of stay of the parasitic mite *Tropilaelaps clareae* outside sealed honeybee brood cells as a basis for its effective control. J Apic Res 26:104–109

Woyke J (1987b) Length of successive stages in the development of the mite *Tropilaelaps clareae* in relation to honeybee brood age. J Apic Res 26:110–114

Woyke J (1990) Biology and control of the parasitic mite *Tropilaelaps clareae*. In: Proceedings of the Apimondia symposium recent research on bee pathology, Gent, Belgium, pp 90–99

Woyke J (1994) Mating behavior of the parasitic honeybee mite *Tropilaelaps clareae*. Exp Appl Acarol 18:723–733