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THE RESISTANCE BEHAVIOR OF *APIS CERANA* AGAINST *TROPILAELAPS CLAREAE.*

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ABSTRACT

The bee mite *Tropilaelaps clareae* Delfinado & Baker is a natural parasite of the giant honey bee, *Apis dorsata,* which is a native honey bee species of southeast Asia. *T. clareae* became a problem to beekeeping with *Apis mellifera* after it was introduced into Thailand. The mite does not appear to be a problem with the indigenous *Apis cerana.* We did not find *T. clareae* and symptoms of its damage on *A. cerana* bees, but we found mite damage on workers and drones of *A. dorsata.* A behavioral resistance mechanism of *A. cerana* against *T. clareae* has been observed. Our investigation indicates that the same sequence of behavior (function) exists as in *A. cerana* infested by *Varroa jacobsoni.* Mite parasitism induces *A. cerana* worker bees to perform a sequence of grooming-cleaning functions that effectively and quickly removes the mites from the bodies of the adult bees. The mites are subsequently killed and removed from the bee hive in a few seconds to minutes. Worker bees also remove mites from the brood effectively.

INTRODUCTION

The mite *Tropilaelaps clareae* Delfinado & Baker was originally described from a collection of dead honey bees *(Apis mellifera)* and from field rats nesting near a beehive in the Philippines (Delfinado & Baker 1961). Subsequently, the same mite was found infesting colonies of *Apis dorsata* in the Philippines (Laigo & Morse 1968) and in India (Bharadwaj 1968). Since then *T. clareae* has been reported in most southeast Asian countries, from Afghanistan, China, India, the Malay Archipelago to Papua New Guinea, as a pest of the imported European honey bee *Apis mellifera.*

T. clareae is undoubtedly as serious a pest of *Apis mellifera* as the better known *Varroa jacobsoni* in tropical Asia (Burgett *et al.* 1983). It is smaller than *V. jacobsoni* and less likely to be identified as the cause of a mite infestation in beehives. In some way, it suppresses the reproduction of *V. jacobsoni* in a simultaneous infestation (Burgett et al. 1983, Wongsiri et al. 1987). *T. clareae* is presently limited to tropical Asia and perhaps less likely than *Varroa* mites to be introduced to temperate areas as its natural host, *A. dorsata,* is restricted to tropical Asia. It was observed that *T. clareae* mites in colonies of *A. mellifera*

can only survive on adult bees for 1-2 days in the absence of bee brood. Since adult mites might be unable to feed on bee haemolymph, infestation of *A. mellifera* by *T. clareae* is not expected in temperate areas where winter interruption of brood rearing occurs (Woyke 1985). This is probably one reason that infestation by this mite has been restricted to tropical Asia.

Although *T. clareae* has been found in colonies of *A. cerana* (Delfinado-Baker 1982, Delfinado-Baker & Aggarwal 1987). It has not been reported parasitizing brood of *A. cerana* (Koeniger et al. 1983). The major question regarding *T. clareae* concerns its potential to feed successfully on haemolymph of *A. cerana*.

The resistance behavior of *A. cerana* against the mite *T. clareae* has not yet been recorded. This is the main question studied in the present investigation. The mite was also observed in the nests of *A. dorsata*, where the symptoms of dead brood and unemerged adult bees confirmed the report of Woyke (1984).

MATERIALS AND METHODS

This investigation was conducted at the following facilities: (1) Bee Biology Research Unit (BBRU), Chulalongkorn University in Bangkok, Thailand; (2) BBRU station, Samut Songkhram Province; and (3) BBRU station, Pitsanulok Province, in January-December 1987-1989.

The colonies of *A. cerana* and *A. mellifera* were established at BBRU facilities.

The presence of *T. clareae* in 100 colonies of *A. cerana* (60 colonies at Samut Songkhram, 20 colonies at Pitsanulok, and 20 colonies at C.U. campus), 10 colonies of *A. mellifera* (C.U. Campus) and 3 colonies of *A. dorsata* (Pitsanulok) was determined by removing the larvae from worker and drone cells, uncapping sealed brood of worker and drone cells, and searching for mites on unemerged adult workers and drones. For all 3 honey bee species, one hundred cells per colony were inspected and the number of cells invaded, the number of abnormal workers and abnormal drones (unemerged adults) and the number of normal and damaged mites recovered from bottom board inserts were recorded (Wongsiri *et al.* 1987).

Observation of the cleaning or grooming behavior and mite-biting behavior of the worker bees followed the method of Peng *et al.* (1987a). Small observation cages (4 cm × 12 cm) were designed with 4 replicates and a control in each species. Ten worker bees were released with ten mites in each cage and the amount of cleaning time in one minute was recorded (Table 2). *A. dorsata* colonies were observed also through a glass window. Similar observations were made of the cleaning behavior and mite removal from the body of *A. dorsata* and *A. mellifera*.

To study the percentage of normal and damaged dead *T. clareae*, 100 adult mites were released into the brood area of each of 10 *A. cerana* colonies previously fitted with

mite checking inserts. Mite checking inserts were also placed into 10 colonies of mite infested *A. mellifera*. The dead mites collected from the mite checking inserts were examined under a stereomicroscope to determine the number of damaged and normal dead mites after 24 hrs and after one week.

Experiments were also conducted to determine the survival of mites on young brood, capped brood and on newly emerged worker adults by releasing 50 female mites into each of 50 cells per colony on the first day of the experiment. Mites were counted 2, 4, and 6 days after the start of the experiment by opening the cells of capped brood and searching for mites present on the body surface of emerged adults. The cells from which these mites had emerged were also searched.

RESULTS AND DISCUSSION

1. *T. clareae* on *A. dorsata*, *A. mellifera*, and *A. cerana*

A. dorsata is the species-specific host of the mite *T. clareae*. We found *T. clareae* mites when we examined 100 cells but not in every cell each of larval workers, pupal workers, larval drones, pupal drones, unemerged adult workers, and unemerged adult drones from 3 colonies of *A. dorsata* (Table 1). Woyke (1984) also reported the presence of dead brood in cells infested by *T. clareae*. Similarly, we found *T. clareae* in those cells of *A. dorsata* and *A. mellifera* (Table 1). Invasion of *A. mellifera* colonies by *T. clareae* appears to be more destructive than that by *V. jacobsoni* and some believe that *T. clareae* is now a more serious pest of honey bees in South East Asia than *V. jacobsoni* (Burgett et al. 1983; Woyke 1984). The population ratio of *T. clareae* and *V. jacobsoni* in the same cells of *A. mellifera* was 2.6 : 1.4 (n = 2700 cells).

T. clareae was absent from all cells examined of 100 colonies of *A. cerana* at the BBRU, Chulalongkorn University, the BBRU station Samut Songkhram Province, and the BBRU station Pitsanulok Province. These results are similar to the previous reports by Koeniger *et al.* (1983) in Sri Lanka and in Malaysia (Koeniger, 1988).

2. Occurrence of abnormal honey bees

A. dorsata workers and drones with morphological damage such as deformed and vestigial wings were found in the cells and among discarded dead brood of three colonies. (Table 2). More than 100 abnormal dead adult bees were found per colony.

Ten colonies of *A. mellifera* infested with *T. clareae* were examined and more than 100 abnormal dead adult bees in each colony were found with the same symptoms of damage as in *A. dorsata*. No abnormal workers and drones were found in 10 colonies of *A. cerana* with *T. clareae*. Morphological damage to honey bees has been recorded before in *A. mellifera* in De Jong *et al.* (1982) and Burgett *et al.* (1983). However Laigo and Morse (1968) also reported the occurrence of morphological damage in *A. dorsata* as a result of *T. clareae* attack on these bees.

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TABLE 1 *Tropilaelaps* mites found in *Apis cerana, Apis mellifera* and *Apis dorsata* worker and drone cells.

Stage	*A. cerana*	*A. mellifera*	*A. dor*
Larval worker cells	−	+	+
Pupal worker cells	−	+	+
Larval drone cells	−	+	+
Pupal drone cells	−	+	+
Unemerged adult workers	−	+	+
Unemerged adult drones	−	+	+

\+ Mites were found in 100 cells examined per colony, 10 colonies of *A. mellifera* and 3 of *A. dorsata.*

− No mites were found in any of 100 cells in each of 100 colonies of *A. cerana*

TABLE 2 Abnormal adult workers and drones caused by *T. clareae* and cleaning behavior observed in small cages.

Observation	*A. cerana*[1]	*A. mellifera*[1]	*A. dor*
Abnormal workers	−	+	+
Abnormal drones	−	+	+
Abnormal dead mites	+ [3]	−	+ [2]
Cleaning behavior[4]	15.2	4.2	2.1

1 : 10 colonies were observed.

2 : 3 colonies were observed.

3 : *T. clareae* released in the experiment in Table 3.

4 : 20 replicates were observed and number of cleaning (times) in 1 min. (1 replicate = 10 bees and 10 mites).

TABLE 3 Percentage of normal and abnormal dead *T. clareae* mites collected in mite checking inserts in *A. cerana* and *A. mellifera* colonies.

Observation	Percentage of mites collected			
	A. cerana *		*A. mellifera* **	
	24 hrs ***	1 week	24 hrs	1 week
Abnormal dead	21	72.1	0	8.5
Normal dead	10	27.9	0	91.5
Total	31	100.0	0	100.0

* 100 mites were released in each of 10 colonies.

** From natural population of mites in 10 colonies, no mites were released.

*** Times are after insertion of mite checking inserts.

TABLE 4 Survival rate of *Tropilaelaps* mites in *Apis cerana* cells.

Days	Percentage of mites surviving		
	Young brood	Capped brood*	Emerged adults
2	0%	79.2 (n = 24)	0%
4	0%	53.3 (n = 15)	0%
6	0%	44.4 (n = 9)	0%

* n = no. of recaptured mites (dead and alive) 50 mites were released for each set (1 mite/cell)

3. Damaged dead mites due to behavioral resistance

Dead *T. clareae* mites were found on bottom board inserts in *A. cerana* colonies 24 hrs after the mites were released into the colonies. The mites had damaged bodies and legs, as shown in Figure 1.

After mite release, *A. cerana* worker bees were able to immediately detect and remove mites encountered in brood cells during nursing activities. They subsequently removed the mites from the hives or chewed on them with their mandibles as reported by Peng et al. (1987a) in their resistance mechanism study of *A. cerana* to *Varroa* mites. *T. clareae* mite parasitism induces *A. cerana* to perform a cleaning and biting behavior sequence that effectively removes the mites from the bodies of the adult host bees, which is reported here for the first time (Fig. 2., Table 2). The mites were subsequently killed and removed from the hives in very short time periods such as a few seconds to several minutes. The grooming behavior of nurse bees consists of four different phases (1) self-cleaning, (2) grooming dance, (3) nestmate-cleaning and (4) group-cleaning behavior.

Worker bees of *A. dorsata* were also observed and showed the same effective mite removal behavior. Once again the mites were eliminated from the body surface of the bees in a few seconds to several minutes, but the cleaning behavior was not as frequent as that observed in *A. cerana* colonies (Table 2). *T. clareae* is not a serious pest of *A. dorsata,* as compared to *A. mellifera,* because the absconding or seasonal migration of *A. dorsata* takes place during a dearth period every year and the mites are left to die in the old nest. This is why *A. dorsata* is not seriously impaired by *T. clareae* mites.

4. Comparison of mites detected in *A. cerana* and *A. mellifera* colonies.

When *T. clareae* mites were placed on adult *A. cerana* worker bees, an average 15.2 times per minute the worker bees attempted to perform the previously described self-and nestmate-cleaning (Table 2). A total of 31% of the released mites were recovered on the inserts after 24 hrs (Table 3). Of these 31%, 21% were damaged mites (Fig. 1) and 10% were normal dead mites. Some mites were lost, probably as a result of hiding in the brood cells or by being removed to the outside by house bees of the colonies. At the end of 1 week of observation, of all the dead mites recovered, 72.1% were damaged and 27.9% were normal in appearance (Table 3). In the case of *A. mellifera,* no mites were found dead after 24 hrs and after 1 week only 8.5% of the dead mites were found to be damaged. This is similar to the report of Peng et al. (1987a) who also found that only 0.3% of the bees were successful in removing *Varroa* mites from their bodies.

5. *T. clareae* in young brood and capped brood of *A. cerana*

A. cerana colonies were found to leave no mites in the cells of young open brood examined 2, 4 and 6 days after the release of *T. clareae* mites (Table 4). This might be due to the cleaning behavior previously described. However we found 79.2%, 53.3%, and 44.4% of the mites to survive in capped brood cells, 2, 4 and 6 days after introduction. According to Woyke (1984), *T. clareae* might survive no longer than 2 days, without food. In our work

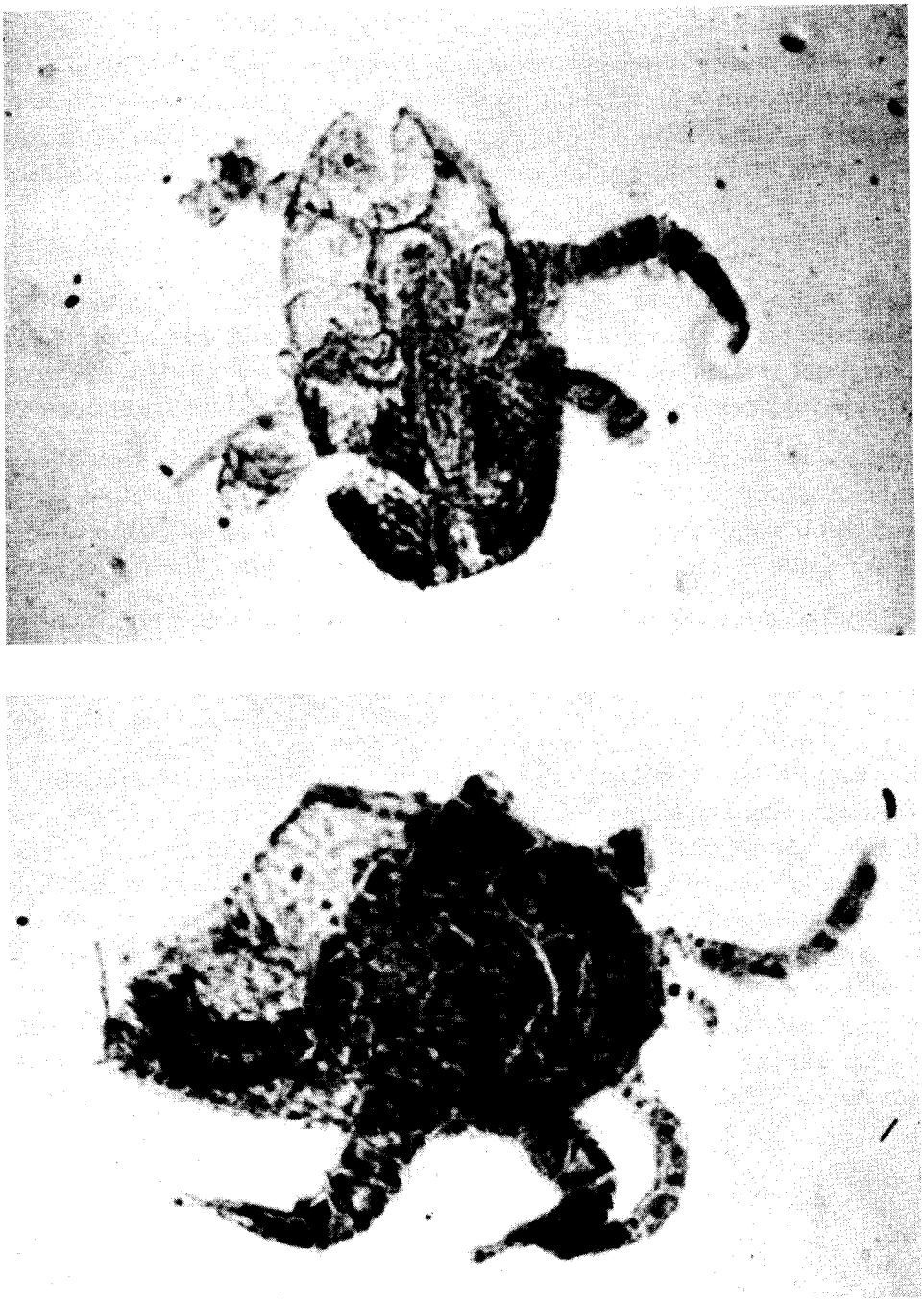

Fig. 1. Damaged *T. clareae* mites killed by *A. cerana*.

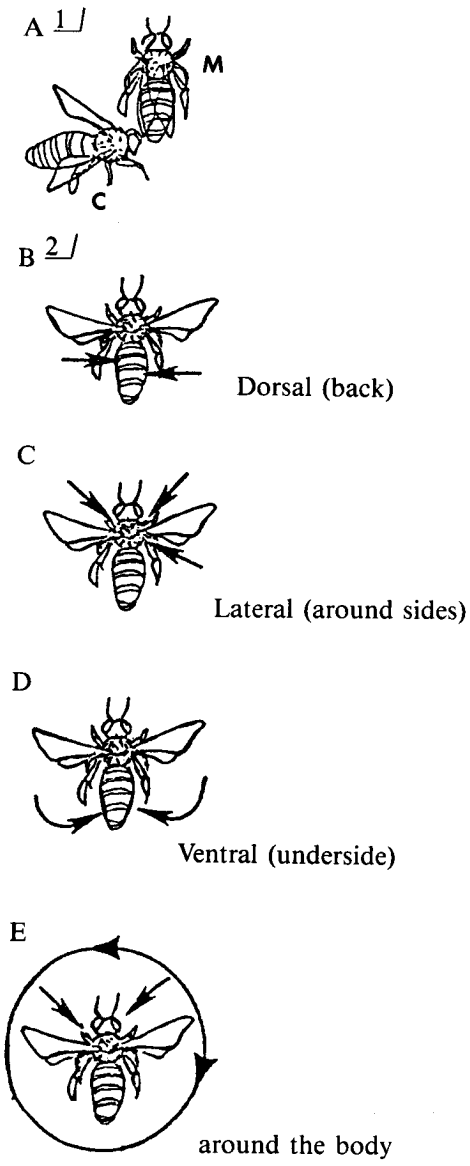

Fig. 2. Pattern of cleaning and biting behavior to remove mites.

1/ M = bee with mite, C = cleaning bee (s)
2/ Arrows show approach of cleaning bees.

with *A. cerana* we found a similar survival period without food, but if offered larvae in sealed brood cells, the mites lived for up to 6 days. However, the mites could not complete their life cycle. No eggs of *T. clareae* were found in capped brood cells nor young mites on the bodies of newly emerged adult bees. This might be the result of a some physiological mechanism, but the causes for this phenomenon are presently not known. More research is needed to find the answer to the problem of reproductive failure of the mites.

CONCLUSIONS

Results presented in this paper show that *T. clareae* cannot survive in *A. cerana* colonies due to the behavioral adaptations of *A. cerana*. Thus, this mite has been found occasionally on the body of workers in *A. cerana* colonies, but it has not been found inside capped brood cells (Delfinado-Baker 1982, Delfinado-Baker and Styer 1983, Nixon 1983).

A dorsata also shows a behavioral adaptation to remove *T. clareae* but at a lower frequency than observed in *A. cerana*. Also, it has a lower success rate in cleaning *T. clareae* from colonies than that of *A. cerana*. This explains why *T. clareae* appears to be more destructive in *A. dorsata* and *A. mellifera* colonies than in *A. cerana* colonies.

Based on the success of *A. cerana* in controlling mites and the inability of *A. mellifera* to do so, the concept of using inter-specifically mixed colonies of *A. cerana* and *A. mellifera* to control parasitic mites clearly deserves more study (Peng *et al.* 1987b, Wongsiri *et al.*, 1987).

As a long term solution to the control of external mites in *A. mellifera,* the most promising avenue is the transfer of the behavioral and physiological resistance adaptations of *A. cerana* to *A. mellifera*. This would be through the use of biotechnology, since all research efforts to date indicate that fertile hybrids between these two species cannot be made (Ruttner and Maul, 1983; Dietz and Wongsiri, 1990). There are two major obstacles to such a transfer at present. The first is that we do not yet have any knowledge of the genetic basis in *A. cerana* for this mite resistance. Obtaining such knowledge will take a large amount of work by many researchers over several years. The second obstacle is that the technology to make such transfers in honey bees is not yet available. Similarly, this will require much work and time. However, work in both areas has begun by us and other researchers.

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