

## CHANGE IN SHAPE OF *TROPILAELOPS CLAREAE* FEMALES AND THE ONSET OF EGG LAYING

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### Summary

Time was recorded in which brood cells were sealed in colonies infested with *Tropilaelaps clareae*. The cells were opened at successive 4-h intervals, and bee brood developmental stage was determined as well as presence of *T. clareae* females, eggs and developmental stages. The size of *T. clareae* females was also recorded as a measure of their readiness to oviposit.

The first *T. clareae* egg was found 48-52 h after sealing, in a cell with a prepupa; the first larva, 64-68 h; the first protonymph, 68-72 h; and the first deutonymph, 88-92 h after sealing, respectively. The thickness of *T. clareae* females doubled from 0.3 mm for those moving freely on the combs up to 0.6 mm for females during the egg laying period. It is concluded that *T. clareae* females do not lay eggs in brood cells before they are sealed, but 40 or 48 h after sealing. Both sexes of *T. clareae* run freely on comb surfaces.

### Introduction

*Tropilaelaps clareae* causes much more severe damage to beekeeping in southeast Asian countries, where it exists, than *Varroa jacobsoni*. This is mainly caused by the quicker population growth of *T. clareae* compared with *V. jacobsoni* (Woyke 1987a, c). A non-chemical method of controlling *T. clareae* has already been described (Woyke, 1984, 1985). It consists of depriving bee colonies of all their brood for a few days. However, many beekeepers do not like to remove brood from bee colonies. Therefore, investigations are being conducted to control *T. clareae* in the presence of bee brood. Whatever method is finally used, the biology of *T. clareae* must still be known, in order to succeed.

Only two papers deal with the reproduction and development of *T. clareae*. One is the unpublished thesis of Kitprasert (1984) and the other is that of Woyke (1987b). According to the first author, the total developmental period of *T. clareae* lasts 8.76 days. Different authors disagree about the beginning of oviposition. Burgett et al. (1983) report that fecund female mites enter an open brood cell containing a fourth-instar larva. Oviposition takes place shortly after the mite enters the cell. According to Grobov et al. (1983), Gliński and Chmielewski (1984), and Akrotanakul (1987, unpublished), a *T. clareae* female lays 3-4 eggs before the cell, with its larva, is sealed. Kitprasert (1984) found the first young imaginal mites on bee brood, 12-16 days after the honeybee eggs were laid. Thus with a figure of 8.76 days for mite development, the mite eggs have to be laid in bee brood cells 7 days after bee egg deposition or earlier, in cells with 4-day-old bee larvae, or younger. Ritter and Schneider-Ritter (1988), taking into account the 9-day development period of the mite, also suggested that mite eggs must be laid before the cell is sealed. But Woyke (1987b) showed that the development period of *T. clareae* lasts only 6 days. He found the first *T. clareae* eggs somewhat later, that is in sealed cells with spinning larvae. But the exact age of those larvae, at the time when the first mite eggs were found on them, was not determined.

This study was carried out to more accurately determine the commencement of oviposition by *T. clareae* females. The female shape changes were also recorded as a measure of their readiness to oviposit.

### Materials and Methods

This investigation was conducted in the Apicultural Institute of the Chinese Academy of Agricultural Sciences in Beijing in October 1986. The bee colonies in which investigation was carried out were of a hybrid Italian type.

To find out how many hours after cell sealing *T. clareae* females started to lay eggs, the time of brood cell sealing had to be determined. For that purpose, a plastic strip 1 cm wide was

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attached to one side of a comb containing older bee larvae (Ifantidis, 1983). Each cell row was given a number, recorded on the strip, and the presence of larvae at the developmental stage prior to sealing, was noted along each row. The comb was returned to a bee colony infested by *T. clareae*, and then removed from the hive at 4-h intervals to record brood cells sealed in the meantime. Most combs were returned to bee colonies, but a few were kept in an incubator. The comb was removed from the colony or incubator again at determined periods of 4 hours, and cells sealed at successive 4-hourly intervals (from 0–4 to 92–96 h) were opened. The stage of bee brood was determined, as well as the presence or absence of *T. clareae* adults and developmental stages. Generally, cells sealed in each interval, were opened until 10 containing *T. clareae* mites and developmental stages in each cell were recorded. Some adults as well as developmental stages were collected, preserved in 75% alcohol, and measured. Specimens for scanning electronmicroscopy were preserved in alcohol (80%, 90%, 100%), then transferred to equal parts of 100% alcohol and benzene, and rinsed in benzene. Dry specimens were coated with silver.

## Results

### Infestation

The percentage of cells infested with *T. clareae* in different bee colonies was not determined, but the number of *T. clareae* females which entered one cell was quite high (Table 1). On average, 3.3 females per cell were found, but often 7–8 females were encountered, and in one cell inspected 44–48 h after sealing, 15 adult females were found. Simultaneous infestation of the same cells with *T. clareae* and *Varroa jacobsoni* was very common. Between 60 to 100% of brood cells infested with *T. clareae* were also infested with *V. jacobsoni*, with an average of 81%.

### Contents of cells

Synchronization between bee brood development and the beginning of oviposition by *T. clareae* was investigated. Results presented in Table 1 show that spinning bee larvae were found in all cells up to 32 h after sealing, and based on the examination of 20 cells, the last few were found up to 40–44 h after sealing. Bee prepupae were found in cells up to 88–92 h after sealing.

Table 1 shows that no *T. clareae* eggs were found in cells containing spinning bee larvae, although 59 cells containing 184 *T. clareae* females had been examined. No eggs or any other

TABLE 1. Contents of brood cells at determined periods after cell sealing in bee colonies infested by *T. clareae*. I, comb in incubator; s, spinning larva; pr, prepupa; p, pupa; T., *Tropilaelaps clareae*; prn, protonymph; dn, deutonymph.

<i>h</i> after cell sealing	Bee brood stage	No. cells with <i>T.</i> examined	No. <i>T.</i> females found	<i>T.</i> eggs or offspring
0–4	s	10	20	No eggs
4–8	s	9	31	No eggs
16–20	s	10	24	No eggs
20–24	s	10	35	No eggs
24–28	s	10	28	No eggs
28–32	s	10	46	No eggs
40–44	Is, 6 pr	7	42	No eggs
40–44I	Is, 9 pr	10	42	No eggs
44–46	pr	10	21	No eggs
44–48I	pr	8	48	No eggs
48–52	pr	10	49	1 egg
52–56	pr	3	3	No eggs
64–68	pr	15	51	7 eggs, 15 larvae
68–72	pr	6	7	3 eggs, 3 larvae, 1 prn
88–92	4 pr, 6 p	10	14	1 egg, 1 larva, 3 prn, 1 dn
92–96	p	4	4	No eggs

developmental stages of *T. clareae* were found in cells with bee prepupae 40–44 and 44–48 h after sealing, although some 35 cells containing 153 females had been examined. Thus, till 48 h after cell sealing, no eggs nor any other developmental stages of the mite were found, although, altogether, 94 cells containing 337 female mites had been examined. Consequently, no mite egg could have been deposited in an unsealed brood cell.

The earliest single egg was found in a cell containing a prepupa 48–52 h after cell sealing. *T. clareae* eggs and larvae were then found in cells 64–68 h after sealing, and then continued to be found until observations ceased, 88–92 h after sealing. No eggs were found 92–96 h after sealing, but only 4 cells with *T. clareae* had been examined. The first *T. clareae* protonymph was found on a prepupa 68–72 h after cell sealing, and the first deutonymph, 88–92 h after sealing.

In this investigation, the first *T. clareae* eggs were found later in bee brood development than reported elsewhere in the literature. Therefore, additional examinations were conducted on combs left in the incubator, to exclude the possible influence of the low outside temperature (13–15°C). Mite eggs were again not found until 48 h after bee brood sealing, in combs which were left in the incubator (34°C) shortly after the cells were sealed (40–44I and 44–48I, Table 1).

### Body enlargement of *Tropilaelaps clareae* females

Efforts were undertaken to find correlations between the shape change of a *T. clareae* adult female, the duration of stay in a sealed brood cell, and the beginning of oviposition.

Results presented in Table 2 show that the average length of *T. clareae* varied between 0.93 and 1.1 mm, and the width, between 0.51 and 0.58 mm. No correlation was noticed between these sizes and the developmental stage of the bee brood, but the dorso-ventral thickness of the mite changed according to the time the mite stayed in the cell. The average thickness of both sexes of mites moving freely on the combs was 0.29 mm. The thickness of males was 0.25 mm and that of females 0.30 mm (Fig. 1a). Only females were found in recently sealed brood cells.

In cells with spinning bee larvae, the thickness of female mites increased after brood sealing as follows: 0–8 h, 0.31–0.35 mm (Fig. 1b); up to 24 h, 0.43 mm (Fig. 1c); and up to 44 h, 0.50 mm. In cells with prepupae, further increase of thickness was noticed, until at 48 h, the thickness increased to 0.56 mm. But afterwards, until the end of the prepupa stage at 92 h

TABLE 2. Size (mm) of *Tropilaelaps clareae* moving freely on combs or in brood cells at determined periods after sealing. I, combs in incubator.

<i>h</i> after cell sealing	No. of mites measured	Mean $\pm$ SD		
		Length	Width	Thickness
Free on combs	53	0.95 $\pm$ 0.0020	0.51 $\pm$ 0.0020	0.29 $\pm$ 0.0090
0–4	18	1.00 $\pm$ 0.0059	0.52 $\pm$ 0.0039	0.35 $\pm$ 0.0154
4–8	21	1.00 $\pm$ 0.0064	0.53 $\pm$ 0.0025	0.31 $\pm$ 0.0090
16–20	9	0.99 $\pm$ 0.0139	0.51 $\pm$ 0.0073	0.41 $\pm$ 0.0125
20–24	26	1.01 $\pm$ 0.0063	0.54 $\pm$ 0.0032	0.43 $\pm$ 0.0100
24–28	10	1.05 $\pm$ 0.0069	0.58 $\pm$ 0.0096	0.51 $\pm$ 0.0152
28–32	28	0.98 $\pm$ 0.0066	0.53 $\pm$ 0.0041	0.52 $\pm$ 0.0098
40–44	27	0.99 $\pm$ 0.0058	0.52 $\pm$ 0.0042	0.50 $\pm$ 0.0150
40–44I	34	0.99 $\pm$ 0.0052	0.51 $\pm$ 0.0026	0.56 $\pm$ 0.0159
44–48	14	0.98 $\pm$ 0.0059	0.52 $\pm$ 0.0038	0.56 $\pm$ 0.0136
44–48I	33	0.98 $\pm$ 0.0030	0.52 $\pm$ 0.0026	0.55 $\pm$ 0.0051
48–52	21	0.96 $\pm$ 0.0035	0.52 $\pm$ 0.0077	0.60 $\pm$ 0.0168
52–56	2	0.95 $\pm$ 0.0000	0.54 $\pm$ 0.0010	0.64 $\pm$ 0.0250
68–72	5	0.93 $\pm$ 0.0138	0.50 $\pm$ 0.0095	0.58 $\pm$ 0.0162
72–76	11	0.96 $\pm$ 0.0048	0.52 $\pm$ 0.0042	0.60 $\pm$ 0.0205
88–92	14	0.97 $\pm$ 0.0074	0.53 $\pm$ 0.0034	0.62 $\pm$ 0.0239
92–96	3	0.96 $\pm$ 0.0066	0.52 $\pm$ 0.0100	0.58 $\pm$ 0.0300
10–12 days	37	0.95 $\pm$ 0.0029	0.52 $\pm$ 0.0025	0.29 $\pm$ 0.0072

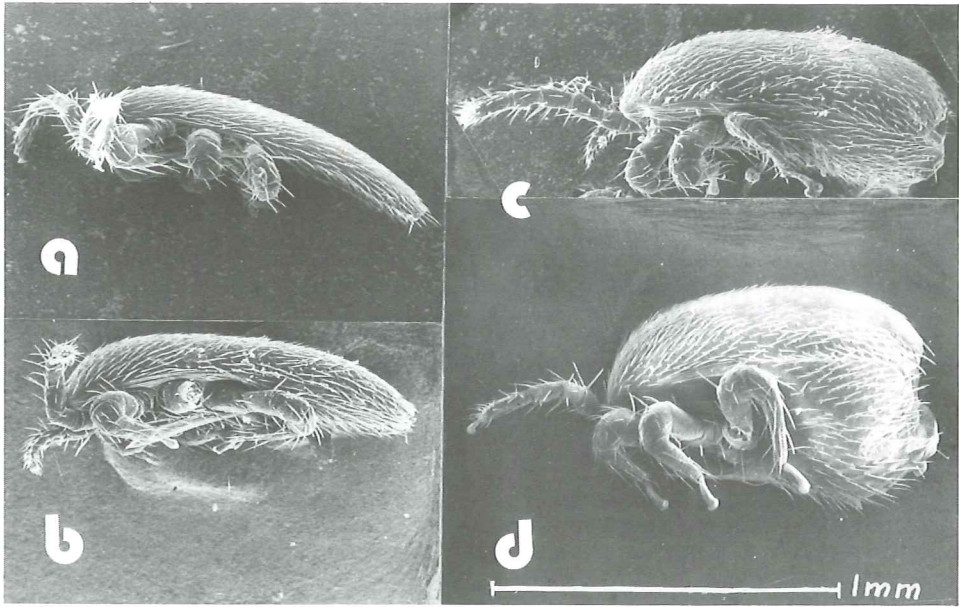


FIG. 1. Females of *Tropilaelaps clareae*.

(a) Young female moving freely on comb. (b) Mated female on brood, 0–4 h after cell sealing. (c) Gravid female, 20–24 h after cell sealing. (d) Gravid female, 48–52 h after cell sealing.

after sealing, and further on up to 96 h after cell sealing, the thickness of *T. clareae* females only varied between 0.58 and 0.64 mm (Fig. 1d). Thus the thickness of the females, 48 to 96 h after cell sealing, doubled in relation to that of mites moving freely on the combs. Again, there was no correlation between the thickness and the time of stay in the cells in that brood interval of 48–96 h after cell sealing. In this time of maximal thickness of *T. clareae* females, the first and further eggs, were found in the cell.

Swollen females with an egg half-way out of the genital orifice were found, but this was never seen in thin females. In cells with older bee pupae, *T. clareae* females became thin again and no more eggs were found. Shortly before worker bees emerged from comb cells (pupae 8–9 days old), *T. clareae* mites were thin, and their thickness averaged 0.29 mm. At this stage of worker bee development, old mite females as well as young imagines of both sexes were present in the cells. Unfortunately, discrimination between males and females was not conducted. The thickness of the adult mites ranged from 0.24 to 0.34 mm. Thus, thick females were not present in sealed comb cells shortly before worker bees emerged.

### Sex of mites moving freely on combs

Both sexes of *T. clareae* emerged from the cells, together with the worker bees. Both sexes were running on the surface and in the cells of the combs. The following numbers of males (M) and females (F) were discriminated among those collected alive from the combs: colony No. 1, 15 M, 11 F; No. 2, 4 M, 25 F; No. 15, 4 M and 2 F; other colonies, 6 M, 15 F. The number of mites collected from different bee colonies is too low to reach conclusions about mite sex ratio in different bee colonies, but mites of both sexes were running freely on combs of every colony. Altogether, 29 males and 53 females were collected from the comb surfaces. Thus the total sex ratio of males to females on comb surfaces was 1.0 : 1.8.

### Discussion

According to direct reports (Grobov et al., 1983; Gliński & Chmielewski, 1984) or indirect ones (Kitprasert, 1984; Ritter & Schneider-Ritter, 1988), *T. clareae* females lay eggs in cells which contain older bee larvae, before the brood is sealed. In this investigation, the first egg

was found in a sealed cell with prepupa, 48–52 h after cell sealing. All eggs were found in cells with swollen females. Swollen females were found on older spinning larvae and prepupae as reported by Woyke (1987a, 1989) and Ritter and Schneider-Ritter (1988). No eggs were found in cells in which the females were not swollen, both shortly after brood sealing as well as in those with older bee pupae. It is therefore concluded, that only swollen females lay eggs. No swollen females were found among *T. clareae* mites moving freely on broodcombs. Additionally, Woyke (1987a) showed that *T. clareae* females stay outside sealed bee brood cells for only 2 days. It is concluded that thin *T. clareae* females do not lay eggs in unsealed bee brood cells during the short period they are moving freely on the combs.

Woyke (1987b) found the first eggs in sealed cells with spinning larvae, but their exact age was not determined. In this investigation, eggs were not found even in cells containing the youngest prepupae (40–48 h after cell sealing), although 40 cells containing 148 females were examined. The eggs found by Woyke (1987b) in Vietnam may have been laid in cells with the oldest spinning bee larvae. But nevertheless, the first eggs found in China were laid about 10 h later. This difference may be due to one or more of the following factors: the high infestation rates of *T. clareae* found in China; simultaneous infestation of the same cells by *V. jacobsoni*; different external conditions; or the existence of different ecotypes of *T. clareae*. The times of appearance of the first *T. clareae* protonymph and deutonymph found in this investigation are similar to those reported by Woyke (1987b).

Contrary to *V. jacobsoni*, where only females are present outside sealed brood cells, both sexes of *T. clareae* run freely on comb surfaces. The sex ratio of 1 M : 1.8 F found in this investigation, is similar to the 1 M : 2.3 F ratio found among mite offspring in Afghanistan (Woyke, 1987c).

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