

LENGTH OF SUCCESSIVE STAGES IN THE DEVELOPMENT OF THE MITE *TROPILAE LAP S CLAREAE* IN RELATION TO HONEYBEE BROOD AGE

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Summary

Combs of sealed honeybee worker brood (*Apis mellifera ligustica* hybrid) were taken from colonies infested by *Tropilaelaps clareae* and the numbers of mites in different developmental stages recorded. The duration of successive stages was estimated from the times at which the first and last individuals in each stage appeared and also from the relative frequency of occurrence of the different stages in the mite population of infested cells. Altogether 22 eggs, 42 larvae, 187 protonymphs, 251 deutonymphs and 659 young or old imagos were detected. The first individuals of successive stages in the development of the mite were found on honeybee brood of the following stages: eggs and larvae on spinning larvae, protonymphs on prepupae, deutonymphs on day-old pupae and young imagos on pupae 4 days old. The most advanced age of a developing bee (calculated from the time of egg-laying) with which each stage of mite was associated was: egg, 14 days; larvae, 15 days; protonymphs, 16 days and deutonymphs, 19 days. The calculated lengths of successive *T. clareae* stages were: eggs, 0.3–0.4 days; larvae, 0.3–0.6 days; protonymphs, 1.7–2.0 days and deutonymphs, 3.0–3.8 days. Length of the total developmental period was 6 days. All mites completed their development before the honeybees emerged, a factor that probably contributes to the faster population build-up of *T. clareae* than of *Varroa jacobsoni*.

Introduction

The parasitic honeybee mite *T. clareae* causes severe damage to beekeeping with *A. mellifera* in South-east Asia. To combat it the biology of the mite must be known. The mite was first described by Delfinado and Baker (1961). At first only adults were found in honeybee colonies and a life cycle outside the hive, possibly with a wild bee as the native host, was suggested (Delfinado, 1963; Sevilla, 1963). Atwal and Goya (1971) photographed a deutonymph but could not find either eggs or larvae. Burgett et al. (1983) reported the presence of *T. clareae* eggs in honeybee brood cells. Detailed descriptions of the protonymph and deutonymph were presented by Delfinado et al. (1985). Kitprasert (1984) reported the duration of successive developmental stages. Woyke (1984) found that the mite can live on adult bees for only about two days, and that it can be combatted without medication by removing all brood from honeybee colonies (Woyke, 1985). Repeated fumigation of colonies with brood was unsuccessful because of the short time spent by the mite outside sealed brood cells (Woyke, 1987).

Materials and Methods

The investigations were conducted in Kabul, Afghanistan in 1983–1984 and in Hanoi, Vietnam in 1985.

Combs of sealed brood were taken from colonies of a hybrid of *A. m. ligustica* infested with *T. clareae*. The cells were opened under a stereomicroscope, the brood pulled out and the presence or absence of *T. clareae* mites in any stage of development determined. If mites were detected, the age of the honeybee brood was recorded together with the numbers of mites in each stage of development. Except for spinning larvae or prepupae, honeybee age was estimated within a day. In Afghanistan only the colour of the compound eyes was taken into account, permitting identification of pupae aged 1, 2, 3–4, 5–6, 7–8 and 9 days old. In Vietnam other characteristics described by Jay (1962) were also considered, which enabled the discrimination of all 9 days of pupal age (Table 1).

Next the percentages of the different developmental stages of *T. clareae* associated with honeybee brood of each successive age group was calculated. The results were arranged in a

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diagram (Fig. 1). Comparing the day in which the first mites in a particular stage of development were detected with the last one gave an indication of the length of each stage. More accurate estimates were obtained from the relative numbers of mites in each developmental stage found in infected brood cells.

Results

Female mites can be distinguished from males by the sex characteristics on the ventral side, and young imagos from older ones by their lighter colour.

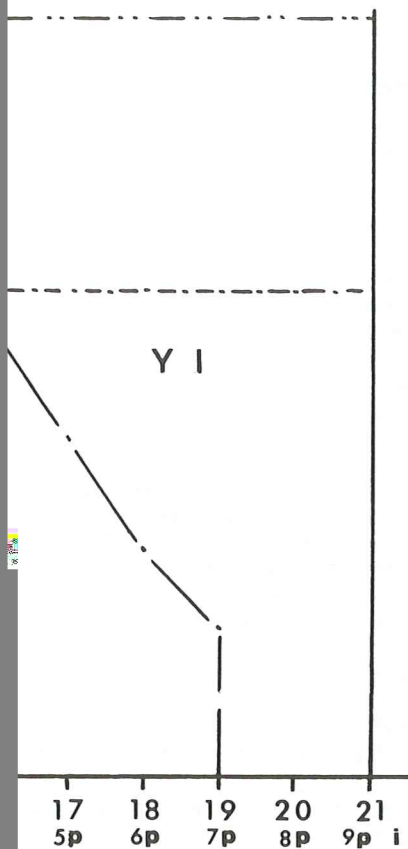
Males were on average, 0.92 mm long and 0.49 mm wide. Females collected outside the brood cells were 0.97 mm long, 0.49 mm wide and 0.30 mm thick. In sealed brood cells with spinning honeybee larvae about half the females were 30 mm thick but among the other half females 0.40–0.50 mm thick or 0.60 mm thick were found. Thus the thickness increased twice. In cells containing honeybee prepupae or pupae one day old female mites 0.60 mm thick were present. But in cells with 2-day pupae only a few thick and many thin females were present. Only thin *T. clareae* females were found in cells with older pupae. Several thick females, but no thin ones, were found with an egg protruding from the genital opening. Eggs were associated only with thick females, in cells with spinning honeybee larvae, prepupae, or pupae one-day old. It is evident that the female mite was thin when she entered the cell, becoming thick only after feeding on the brood, and laying eggs some time after entering the cell. After egg-laying the female became thin again.

Altogether 1161 individuals in the different developmental stages were detected, about half of them in Afghanistan and half in Vietnam. Of that number 22 were in the egg stage, 42 were larvae, 187 protonymphs, 251 deutonymphs and 659 old or young imagos. Numbers of eggs and larvae were much fewer than those of any other developmental stage. This indicated a much shorter duration for the first two mite stages than for the other stages. However, ratios of absolute numbers of mites in successive developmental stages do not reflect exact ratios for the duration of those stages because the numbers of honeybee cells were not equal for different ages of brood (Table 1). Accordingly, relative numbers of mites in different stages of development associated with successive ages of honeybee brood were calculated. Results for 595 mites found in Vietnam are presented in Fig. 1.

Unfortunately, ages of sealed honeybee larvae in their first or second day of spinning were not distinguished nor were the exact ages of prepupae one or two days old. The first *T. clareae* eggs and larvae were detected in sealed cells containing spinning larvae, i.e., on brood 9 or 10 days of age (from egg laying). However, it was not certain if the eggs were deposited during the first or the second day of larval spinning. As indicated above, eggs were not laid immediately after the cells were sealed. The highest percentage of honeybee cells with mite eggs (6.3%) occurred among cells containing prepupae (11 and 12 days after egg-laying). The first

TABLE 1. Numbers of *T. clareae* mites in different stages of development found in sealed cells with brood of different ages in honeybee colonies in Vietnam. Age of honeybee brood is calculated from day of egg-laying. DSM = developmental stage of mite; SL = spinning larva; PP = prepupa; P = pupa.

DSM	No. mites in sealed cells associated with indicated age of brood (upper) or pupae (lower):											TOTAL		
	9 SL	10	11 PP	12	13 P1	14 2	15 3	16 4	17 5	18 6	19 7		20 8	21 9
Egg	1		5		2	1								9
Larva	1		2		1	1	2							7
Protonymph			4		9	6	9	21						49
Deutonymph					5	6	13	36	17	20	15			112
Young imago								6	8	24	35	20	26	119
Adult	36		68		38	13	25	33	13	23	26	10	14	299
Total	38		79		55	27	49	96	38	67	76	30	40	595



DEVELOPMENT

the various stages of development found in
of different ages.

—8p = pupae 1-8 days of age; i = imago.

Deutonymphs were detected two days
(g) and the first young imagos three
Mite eggs were found from 4 or 5
of honeybee brood. The last
found only on honeybee pupae; the
the last larvae a day later on brood 15
The last deutonymphs were found
Only mite imagos were present on

successive developmental stages of
nymphs and 3 days for deutonymphs.
6-7 days and between last eggs and
the oldest 2-day honeybee pupa. It
development time for *T. clareae* was 6

TABLE 2. Duration (days) of developmental stages of *T. clareae* mites estimated from the average percentage occurrence of various stages and from sums of percentage incidence.
 APO = average percentage occurrence; SPO = sums of daily percentage occurrence.

Stage	APO Afghanistan (1984)	APO Vietnam (1985)	APO Average	SPO Vietnam	Data of Kitprasert* (1984)
Egg	0.35	0.47	0.4	0.3	1.05
Larva	0.78	0.35	0.6	0.3	1.85
Protonymph	2.34	1.98	2	1.7	2.11
Deutonymph	2.53	3.20	3	3.7	3.75
Total	6.00	6.00	6	6.0	8.76

*Thailand.

The relative percentage occurrence of mites in successive stages of development in association with honeybee brood of a given age was calculated and the 6-day span of *T. clareae* development divided accordingly among the successive developmental stages (Table 2). The relative times differed for mites in Afghanistan and Vietnam. These differences could be accidents of sample size, sampling bias because of a more accurate determination of age in Vietnam, differences in environmental conditions, different ecotypes of *T. clareae*, etc. The greatest difference was in the length of the larval period which in Afghanistan was longer than the egg period and in Vietnam slightly shorter. In contrast, the deutonymphal stage was shorter in Afghanistan. The average successive periods for the two countries taken together were: egg, 0.4 day; larvae, 0.6 day; protonymph, 2 days and deutonymph 3 days.

These estimates agree quite well with calculations based on times of detection of the first and last individuals in successive developmental stages (Fig. 1). They also support the supposition that the first mite eggs are not laid immediately after cell capping on honeybee brood 9 days of age but rather on the second day of larval spinning when brood is 10 days old.

Next the 6-day developmental period of *T. clareae* was apportioned among the successive stages according to the sums of their percentage occurrence in the mite populations of infested honeybee brood cells (rather than according to the average percentages as above). With this method, the lengths of the successive stages of mite development differed from the earlier estimate for Vietnam; they were shorter for the first three stages and longer for the deutonymph stage than before (Table 2).

The maximum number of offspring produced by a single *T. clareae* female (by direct counts for individual old females) was found to be four. Since the eggs were detected over 4 or 5 days, and the development time for an egg was less than one day, the female must have laid eggs at intervals of about a day.

Discussion

According to Kitprasert (1984/1985) the total developmental period for *T. clareae* is 8.76, or roughly 9 days. If this were so, and the first young mites were found on honeybee brood 16 days old, then the first eggs would be deposited in cells containing larvae 4 days old, 1.5 days before cell capping, which does not occur. The last deutonymphs in this study were found on brood 19 days of age (the last found by Kitprasert were on brood 17–19 days old). Thus, with a 9-day developmental period the last eggs would be deposited in cells containing brood 10 days old, i.e. spinning larvae. In the present investigation, however, most eggs were found one or two days later, on prepupae.

Also, the relative lengths of successive stages differ between Kitprasert's investigation and this one. The protonymph stage in this study lasted 3.5 times as long as the larval and 5 times as long as the egg stage. This explains why it was so difficult to find eggs. According to Kitprasert the developmental period for the protonymph (2.11 days) was only slightly longer than that of the larva (1.85 days) and only twice as long as the egg stage (1.05 days). If these times were accurate, larvae should be as easy to find as protonymphs and one egg should be found for every two protonymphs. However, Atwal and Goyal (1971) and Sevilla (1963) were

unable to find either eggs or larvae despite a thorough examination of numerous cells containing honeybee larvae of different ages. Older nymphs, however, were very abundant. The developmental period of the deutonymph, calculated from the sums of percentage occurrence of various stages, was in the present investigation almost the same as reported by Kitprasert (1984/85).

Differences in reported lengths of successive stages of *T. clareae* development are probably caused by differences in methods. Kitprasert investigated the mites on honeybee brood in the laboratory, where, out of 60 larvae, only four reached the adult stages, whereas the present investigation utilized honeybee colonies, where conditions are more favourable for mite development.

Conclusions

Developmental stages of *T. clareae*, estimated by two methods, were as follows: egg, 0.3–0.4 days; larva, 0.3–0.6 days; protonymph, 1.7–2.0 days and deutonymph, 3.0–3.7 days. The overall period was 6 days. The brief duration of egg development was the reason for the difficulty in detecting eggs. All individuals completed their development before the honeybees emerged from the comb cells. This is not true of *Varroa jacobsoni* and may be one of the factors responsible for the more rapid increase of *T. clareae* populations.

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