

## Short communication

# Repeated egg laying by females of the parasitic honeybee mite *Tropilaelaps clareae* Delfinado and Baker

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**Summary** — *T clareae* females were collected from pupae with dark eyes. At this time the old females had finished egg laying, and the young ones were in early development stages. A few days later, the old females were mated and introduced into sealed cells with spinning bee larvae. After 5–8 d, the cells were opened and examined. A total of 18.5% of *T clareae* females were found gravid or with offspring.

### *Tropilaelaps clareae* / egg laying / honeybee mite / parasite

## INTRODUCTION

*Tropilaelaps clareae* Delfinado and Baker is a much more dangerous parasite of *Apis mellifera* L than *Varroa jacobsoni* Oudemans, partly because its population increases at a geometrically higher rate than that of *V jacobsoni* (Woyke, 1987b). This is caused by the 2 d stay of *T clareae* females outside sealed brood cells (Woyke, 1987a), which is only a small fraction of the time spent outside by *V jacobsoni*. The population increase depends upon several factors including the percentage of fertile females and their number of offspring. One

additional factor is the percentage of females that enter brood cells to lay eggs for a second time. This question concerning *T clareae* is investigated in the present study. Preliminary results of the present investigation (Woyke, 1993) and that of Chen and Li (1993) have been presented previously.

## MATERIALS AND METHODS

*T clareae* females were collected from honeybee pupae with dark-brown eyes (Pd pupae according to Rembold *et al*, 1980). At this time old females had completed egg laying, and the young ones

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were light or in earlier development stages. Thus, females of both generations could be differentiated. The popular method of catching *T. clareae* mites with the aid of a wet brush was avoided. Instead, the mites were caught by the method described by Woyke (1994). The old females were put on pupae of the same age as those from which they were collected. They were placed into small glass test-tubes in an incubator at 34°C. The pupae were removed shortly before their last moult. *T. clareae* males were then collected from combs with emerging workers or from brood cells with workers ready to emerge. Mites were sexed and a *T. clareae* male was added to each female in the test-tube. Several matings occurred within a short time. Since the length of time of mating in a bee colony is unknown, the mites were left in the tube for 2 d together with a honeybee prepupa or white pupa (unpublished results). After 2 d, *T. clareae* females were put into brood cells originating from mite-free bee colonies, using the following method. First, a piece was cut off from brood comb with recently sealed spinning bee larvae. A small hole was then made in the capping. The mites inside the test-tube were sexed, and the female was released on white paper. A piece of wire 1 mm thick and about 6 cm long was placed near the mite, which caused the female to climb onto it. When the *T. clareae* female approached one end of the wire it was put inside a brood cell through the hole. As the wire was withdrawn the hole was capped with the opened piece of capping. The edges of the opening were melted together with a piece of hot metal. The comb pieces with mites were located in glass or plastic containers, and were placed in an incubator at 34°C. After 5–8 d, cappings of the cells were removed and the contents were examined under a stereo-microscope.

## RESULTS

Out of the 36 *T. clareae* females put in bee brood cells, 1 female was not found after the cells were opened and 8 females (23%) died. The remaining 27 females were alive. In one cell with 1 old female, 1 *T. clareae* larva and 1 protonymph were present. In another cell with female, 1 protonymph was found. In other cells, 3 gravid females 0.40–0.50 mm thick were detected. Those females would probably have laid eggs in

a short time. Thus, 5 of the females (18.5%) did or would have reproduced under the conditions described. The calculated binomial 95% confidence interval showed that 7.4% (2) to 37.0% (10) of the females could be expected to lay eggs for the second time, if more repetitions were conducted.

If the opening of the cells had been delayed further, following introduction of the female mites, perhaps more *T. clareae* offspring would have been found. However, since many *T. clareae* females did not become gravid within 5–8 d, I believe that the percentage of *T. clareae* females repeating egg laying during the second entrance into brood cells would not have increased by much.

## DISCUSSION

The results revealed that some mites died in the cells. Woyke (1985) showed that in a single bee colony more than 1 200 mites died every day. In the present investigation, *T. clareae* females were introduced into bee brood cells but would not enter the cells in a bee colony and thus died. In a bee colony, some *T. clareae* female mites die in brood cells. Thus, the deaths were not caused by unfavorable conditions in the incubator.

With the short development period of *T. clareae* (3 d), discussed by Chen and Li (1993), the gravid females found in this investigation had the chance to produce adult offspring. Chen and Li (1993) also found that 30% of *T. clareae* females repeated egg laying. This fits into the 7–37% binomial confidence interval calculated in the present paper.

The delayed resumption of egg laying by some females observed in this investigation was even higher in the Chen and Li (1993) experiments. The rest time between 2 layings was 4–5, 11–13 or 20–23 d. Thus, some females would not have resumed

repeated egg laying during the second, but during the third entrance into honeybee brood cells. If only the period of the second entrance into brood cells is considered, then the percentage of *T. clareae* females repeating egg laying in the Chen and Li investigation would be similar to the 18.5% presented in this investigation.

The finding that 7–37% *T. clareae* females can repeat egg laying should be taken into account in calculations of population growth rate of *T. clareae* mites (Woyke, 1987b; Ritter and Schneider-Ritter, 1988).

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**Résumé — Les femelles de *Tropilaelaps clareae* pondent 2 fois de suite.** Les populations de l'acarion *Tropilaelaps clareae* Delfinado & Baker croissent beaucoup plus vite que celles de *Varroa jacobsoni* Oudemans. On ignore le pourcentage de femelles de *T. clareae* qui pondent une 2<sup>e</sup> fois. On a prélevé des femelles de *T. clareae* sur des nymphes d'abeilles au stade yeux noirs. À ce moment-là les femelles âgées ont fini de pondre et les jeunes sont encore à un stade initial de développement. Des femelles âgées ont été mises sur des nymphes d'abeilles dans des tubes à essai placés dans une étuve à 34°C. Quelques jours plus tard, après introduction de mâles, les

femelles s'étaient accouplées. Puis elles ont été introduites dans des cellules de couvain operculé contenant des larves en train de filer. Cinq à 8 j plus tard les cellules ont été ouvertes et examinées. Sur 36 femelles introduites, 27 ont été retrouvées vivantes. Dans une cellule avec une femelle âgée on a trouvé une larve de *T. clareae* et une protonympe. Dans une autre cellule on a trouvé aussi une protonympe et dans d'autres cellules 3 femelles gravides de 0,40 à 0,50 mm d'épaisseur. Elles auraient probablement pondu dans peu de temps. On peut donc conclure que 5 femelles (18,5%) s'étaient reproduites ou allaient le faire dans les conditions décrites. D'après l'intervalle de confiance binomial à 95% on peut estimer que 7 à 37% des femelles pondent une seconde fois.

#### *Tropilaelaps clareae* / ponte / acarien / parasite

#### Zusammenfassung — Wiederholte Eiablage von *Tropilaelaps clareae*-Weibchen.

Die Population von *T. clareae* wächst viel schneller als die von *Varroa jacobsoni*. Der Anteil an *T. clareae*-Weibchen, die ein zweites Mal Eier ablegen, ist nicht bekannt. *T. clareae*-Weibchen wurden von Puppen mit dunklen Augen abgesammelt. Zu dieser Zeit hatten die alten Weibchen ihre Eiablage beendet, und die jungen befanden sich noch in einem frühen Entwicklungsstadium. Die alten weiblichen Milben wurden auf Bienenpuppen in einem Glasröhrchen im Brutschrank gehalten. Wenige Tage später wurden die Weibchen begattet und auf spinnende Bienenlarven in verdeckelten Zellen gesetzt. 5-8 Tage später wurden die Zellen geöffnet und untersucht. Von 36 so behandelten *T. clareae*-Weibchen waren zu dieser Zeit 27 noch am Leben. In einer Zelle mit einem alten Milbenweibchen wurde eine *T. clareae*-Larve und eine Protonympe gefunden. In einer anderen Zelle wurde ebenfalls eine Protonympe gefunden, und

in weiteren Zellen drei gravide Weibchen, 0,40–0,50 mm dick. Diese Weibchen würden wahrscheinlich in Kürze Eier ablegen. Es kann also geschlossen werden, daß unter den beschriebenen Verhältnissen 5 Weibchen (18,5%) Eier ablegen würden. Nach dem binomialen Konfidenzintervall bei 95% kann bei 7–37% der Weibchen eine nochmalige Eiablage erwartet werden.

### ***Tropilaelaps clareae* / Eiablage/ Bienenmilbe**

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