Mating behavior of the parasitic honeybee mite *Tropilaelaps clareae*

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ABSTRACT

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The mating behavior of adult *Tropilaelaps clareae* males and females was observed in glass test tubes. The male jumped on the dorsum of the female, stretched legs I forward, and hooked their distal ends on the frontal margin of the female dorsum. He then slipped sideways to the female venter. In the venter-to-venter position, the male clasped the female between her legs I and II, with his legs I. The male then moved backwards until his gnathosoma reached about 1/2 way along the epigynial plate of the female. The male vibrated and probably pushed the spermatophore out of his body. Next he moved laterally and placed the gnathosoma between coxae III and IV where the gonopore is located. Probably the sperm was introduced into the gonopore by means of the spermatodactyl. Subsequently the male moved to one side and stroked the anterior part of the epigynial plate and the gonopore area with his leg II. He repeated these contacts on average 282 times (variation 100 up to 522). The male then moved on the other side of the female venter and repeated the whole procedure. Finally, the male withdrew himself backwards from the female. The total mating process lasted between 3 and 42 min, with an average of 23 min. Multiple matings of both, males and females were observed.

INTRODUCTION

The mite *Tropilaelaps clareae* Delfinado and Baker, is a serious parasite of the honeybee, *Apis mellifera*. To control it efficiently its biology must be known. There are few publications on the natural history of *T. clareae*. Recently some new facts about the biology of this mite were described (Woyke, 1993a, b), including different aspects of reproduction (Woyke, 1994b, c). Rath *et al.* (1991) described the three main positions used during mating, based on relatively few observations (about 10).

Michael (1892) was the first to describe the mating behavior of various Gamasida (Mesostigmata), to which *T. clareae* belongs. He also described the sacculus foemineus (Michael's organ), which is connected through paired tubuli annulati

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with the extragenital openings (solenostomes, gonopores) on the base of coxae III. Evidently, the reproductive system of *T. clareae* is the laelapid-type, similar to that of *V. jacobsoni* (Akimov and Yastrebtsov, 1984, 1985; Alberti and Hänel, 1986; Akimov *et al.*, 1988). Insemination occurs by means of podospermy. The sperm is introduced into the gonopore by means of the spermatodactyl (Evans, 1992). In the present paper, some actions occurring during mating of *T. clareae* are described for the first time and some erroneous descriptions are corrected.

MATERIALS AND METHODS

The investigations were conducted at the Institute of Apicultural Research of the Chinese Academy of Agricultural Sciences, Beijing, China in 1992 and 1993. Brood combs with emerging worker bees were removed from *Apis mellifera ligustica* colonies infested by *T. clareae*. The mites were mainly collected from cells with emerging worker bees. Forceps with fine points were used to catch the mites (Woyke, 1994a). The sex of the mites was determined and one male and one female were placed in an empty glass test tube (4 × 0.8 cm). Mating behavior was observed under a stereoscopic microscope.

A total of 135 matings of 55 different pairs of *T. clareae* were observed. Of these 83 matings were successful and 52 were unsuccessful, the latter lasting about 1 min. Detailed timing and counts of different stages in the mating process were recorded during some matings as described below.

RESULTS

Mating behavior

The mites ran rapidly inside the glass tubes in a manner similar to that observed on the comb. No special searching or courtship behavior was observed by either sex prior to mating. Apparently males and females find each other by random movement. If the male and female met each other face-to-face, they touched their first pair of legs. Then the male moved behind the female and jumped on her dorsum. A frontal meeting was not necessary for mating to begin. After mounting the female, the male stretched legs I forward and hooked the tarsi on the frontal margin of the female dorsum. He then tapped that margin with tarsi I. At the same time, he moved from one tarsus to another. This behavior may have bee a kind of sexual encouragement for the female to accept the male. The male remained on the female dorsum for about 1 min. Next, he moved one leg of pair I on its side and probed with tarsus I between female coxae II and III. Then he moved laterally to the female side, and slipped sideways beneath female legs III and IV to her venter (Fig. 1). In many observations, the male moved to the venter from either side of the female idiosoma. Of 21 detailed observations, the male was observed to move ventrally 9 times from the right side and 12 from the left. One male moved ventrally from the left side on each of the four occasions when he encountered

females. Mostly males which mated several times alternated from one side to the other.

In the venter-to-venter position, the male faced the female in the same direction more or less centrally (Fig. 2). He stretched his legs I forward and clasped the female frontal margin of the idiosoma between her legs I and II. Legs II of the male were placed between female legs II and III, and male legs III and IV were wrapped around the female opisthosoma behind legs IV. The male then moved backwards until his gnathosoma reached about 1/2 way along the epigynial plate of the female. At this point the male opisthosoma protruded by about one third of its length behind the female opisthosoma (Figs. 1 and 2). The male vibrated, possibly as a prelude to secreting a spermatophore. The male genital orifice has an oval shape and measures about 45μm × 20μm. It is located in front of the holoventral plate, behind the gnathosoma, with its anterior edge 14μm behind the base of the tritosternum (Fig. 3 g). It is therefore on the level of the anterior margin of coxae II. Observation from the side revealed an elongated white body coming out of the male genital orifice (Fig. 4 h), which is probably the spermatophore. Unfortunately the further fate of the spermatophore was not seen clearly because the act was screened from sight by the mites' bodies. According to Krantz and Wernz (1979) and Rath *et al.* (1991), the male carries the spermatophore between pedipalps and chelicerae. The male remained in the central position of the female venter for about 3 min. The male then moved backwards a little and sideways to the left or right side of the female. As a result, he moved his leg II from a position anterior to the female's leg III to anterior of leg IV on the same side (Fig. 5). Thus legs II of the male were now placed asymmetrically on the body of the female. On the side that the male had

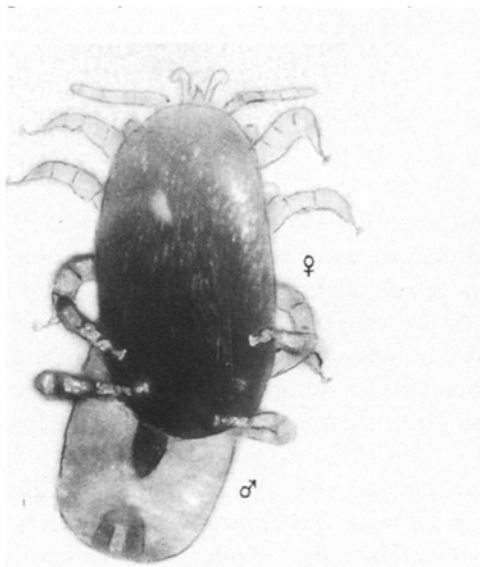

Fig. 1.Mating of *T. clareae,* male beneath the female.

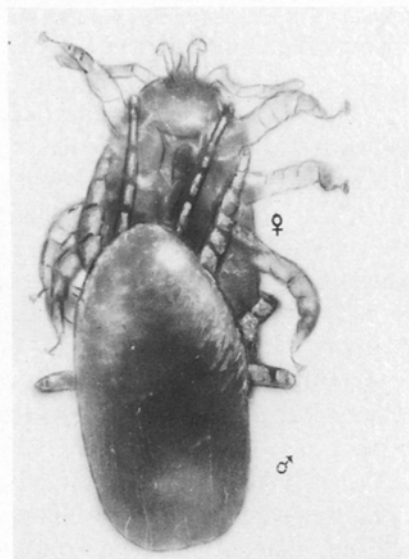

Fig. 2. Male legs II placed between female legs II and III.

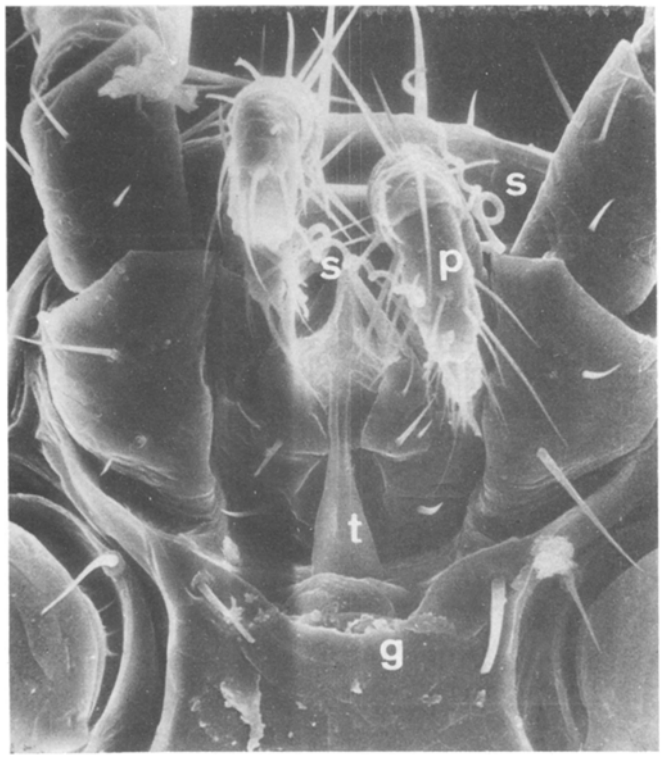

Fig. 3. Ventral view of male gnathosoma and anterior part of podosoma. (g) genital orifice, (p) pedipalp, (s) spermatodactyl, (t) tritosternum.

moved to, his leg II was between the female's leg III and IV. On the opposite side, the male leg II was between the female's legs II and III. The male was now a little skewed along the long axis of the female (Fig. 5).

The male placed his mouth parts (gnathosoma) between female coxae III and IV (Fig. 6). A small opening, the gonopore or solenostome, is located on either side behind coxa III. The solenostomes are connected with the female sperm sacculus. The sperm material probably was introduced into the solenostome by means of the spermatodactyl (Fig. 3 s), an elongate structure measuring about 170μm long and 3μm wide. However, the introduction was not visible through the stereoscopic microscope under the conditions described. The male remained in this position for about 3 min.

Next, the male moved further laterally so that the whole epigynial plate of the female was uncovered (Fig. 6). Sometimes the male was almost completely outside the lateral margin of the female idiosoma. Then the male detached his leg II from the opposite side of the female body between her leg II and III, and rubbed with that leg the anterior part of the female epigynial plate and the region of the

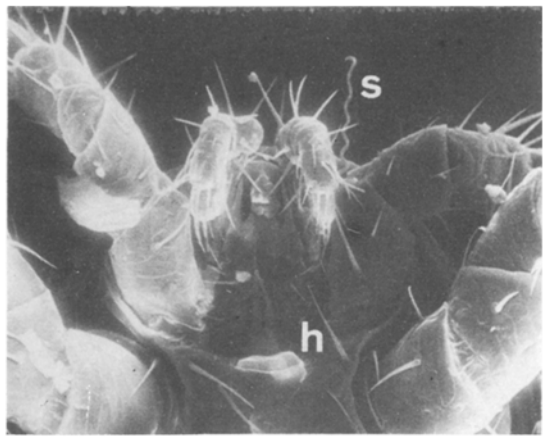

Fig. 4. Ventral view of male gnathosoma and anterior part of podosoma with spermatophore (h) appearing in genital orifice, (s) spermatodactyl.

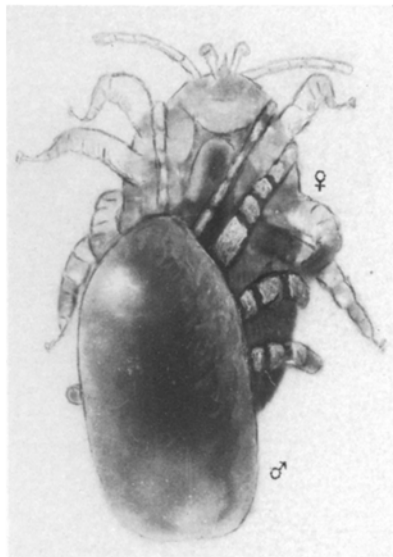

Fig. 5. Male shifted on right side of the female, and moved his left leg II between female legs III and IV.

solenostomes (Fig. 7). Thus when the male was on the left side of the female he stroked her with his left leg. Similarly, when he was on the right side, he stroked the female with his right leg. The male moved the leg rapidly, making about 5 oral-anal motions in about 3 seconds. He repeated this operation a varying number of times. In 14 observations the number ranged from 100 to 522 times, with an average of 282 ± 141 (x ± s.d. n = 14). The middle 50% of males repeated these 3-second strokings between 140 and 385 times. When the numbers of strokings were grouped into classes of 50, then the most numerous was that of class 100 to 150. The whole procedure lasted from 5 to 20 minutes. It is suggested that this was a means of stimulating, either directly or indirectly the passage of sperm into the female body.

Next, the male returned to the central position on the ventral side of the female, but the asymmetric position of the male legs was retained (Fig. 5). Therefore it was possible to know whether the male was in the starting position (legs positioned symmetrically, Fig. 2) or in a later stage of the mating process (Fig. 5). The male again began the vibration phase and then moved to the other side of the female venter where the whole procedure was repeated. At the conclusion of the procedure, the male came back to the central position on the venter of the female and withdrew himself backwards from her idiosoma.

The total mating process lasted between 3 and 42 min., with an average of 23 ± 9 min. (x ± st.dev., n = 55). The middle 50% of the observed males copulated within 16 and 29 min.

Temperature was found to influence mating behavior. In July, 1992 the laboratory

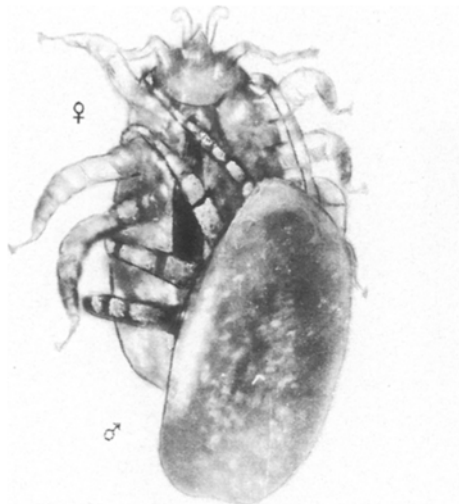

Fig. 6. Male moved so far on the side, that the whole female epigynial plate is visible.

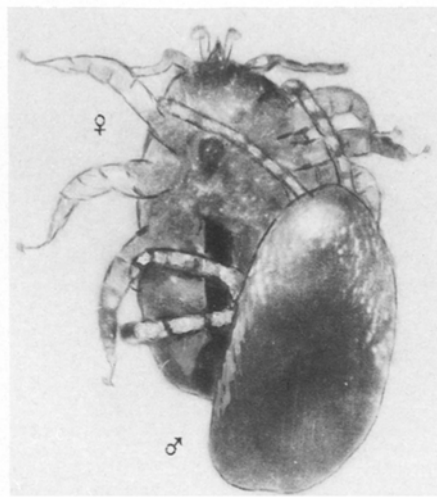

Fig. 7. Male stroking with his left leg II (dimmed on photo), the anterior part of epigynial plate and the solenostome area of the female.

temperature was around 30°C–33°C and in September 1993 it was 26°C. In 1993, the male did not stroke the epigynial plate and the area of the solenostomes. However after the glass tube with a pair of mites was warmed by a lamp, the male started to rub the female epigynial plate.

Multiple mating

Continuous observations over some hours revealed that the male mated several times within an interval between 3 and 39 min with the same or other females. A few examples of the mating times of particular females are presented in Table 1. Matings of 1 min were unsuccessful. Here the male mounted on the female dorsum but did not succeed in moving on to her venter. Two to five successful matings were observed among the five couples presented above. Altogether multiple, successful matings by 16 females were recorded. When a female in a test tube was replaced by a fresh specimen, the male mated with the new female again several times.

Mating with old females

Among mites collected from brood cells with emerging worker bees, both light and dark *T. clareae* females were present. Apparently the former were young and the latter were old females. Mating with both light and dark females was observed.

To prove the phenomenon of mating of previously inseminated females, the

following observations were made. Old females which had already laid eggs were collected from sealed brood cells containing honeybee pupae with dark brown eyes (experiment of Woyke, 1994b). At this time, it was easy to discriminate between old *T. clareae* females and their offspring. These females mated normally with males in glass tubes. Examples of two females are presented in Table 1. Altogether 12 single and multiple matings of old females were observed.

In one observation, a gravid female with an enlarged opisthosoma (Woyke, 1989) was taken from a comb cell with a honeybee prepupa and was placed in a test tube with a male. At first normal copulation was observed. After the male slipped to the venter of the female, he moved to her left side. However, the female opisthosoma was so large, that the male was unable to wrap his legs around her right body margin. With his gnathosoma at the level of coxae III and IV, the male went through the stroking procedure 100 times. He then moved to the right side of the female where he repeated the procedure another 100 times. The whole mating process lasted 19 minutes. The male made over 10 more attempts to copulate with that female again more. However, he did not succeed in moving to the female venter.

Unsuccessful matings

After a female was mated several times, she ran away when the male approached her. Sometimes the males mounted such females, and would ride her dorsum as she ran. The male shifted from one tarsus to the other and hooked and tapped the frontal margin of the female with tarsi I. However, the male did not succeed in moving to the venter of the female, regardless of whether the female was running or

TABLE 1

Duration of mating (M) and interim (I) time, (in minutes) of some *T. clareae* couples.

Female No	M I	Successive matings 1	2	3	4	5	6	7	8
Females of undetermined age									
11	M	27	22	4	13	1	17	1	1
	I	12	3	15	16	4	9	17	
12	M	29	7	1	14	13	29		
	I	8	9	6	8	3			
26	M	32	20	18	24				
	I	3	11	39					
Old females									
7	M	22	14						
	I	13							
8	M	18	23	1	1				
	I	5	3	1					

stationary. When the male was mounted on the dorsum of an unreceptive female and put his leg I between her leg II and III, the female stacked her legs so fast to the substrate that the male could not slip to her venter. In some cases the male slipped behind female leg IV, or even from the rear of the female. In this case, the male legs II were placed between female legs III and IV or wrapped the female opisthosoma behind her legs IV. The male stretched his legs I forward, but he did not reach the frontal margin of the female idiosoma between legs I-II. In this circumstance, the male did not move forward to locate himself in the initial position for copulation, but withdrew posteriorly from the female rear. After 3 matings of couple No 1, I observed 15 unsuccessful attempts by the male to mate with the same female.

DISCUSSION

Usually fewer details of the mating behavior of Gamasida are presented in the literature than are described here. Usually the male mite climbs on the female dorsum from behind, turns around to face the opposite direction and then crawls over her posterior end to take a ventral position like in the Phytoseiidae: *Typolodromus* species (Dosse, 1959), *Amblyseius andersoni* (Amano and Chant, 1978), *Amblyseius cucumeris* (Castagnoli and Liguori, 1991), in Laelapidae: *Echinolaelaps echidninus* (Jakeman 1961), in Rhodacaridae (Lee, 1974) in Macrochelidae: *Glyptholapsis americana* (Krantz and Wernz, 1979), and the Varroidae: *Varroa jacobsoni* (Hänel, 1983; Donzé, 1993). By contrast, *T. clareae* males always slip sideways to the female venter via the lateral route.

During mating, the male may stay in a skewed direction along the long axis of the female like *Typhlodromus* sp.(Dosse, 1959), or it may assume an angle of 45°–90° as in Macrochelidae (Oliver and Krantz, 1963; Costa, 1966, 1967; Krantz and Wernz, 1979). In *Athiasella dentata* (Rhodacaridae), the male lies in a lateral position outside the female sterno-genital area with his axis at right angle to the female axis (Lee 1974). *T. clareae* males copulate in an almost parallel (little skewed) position, like *Echinolaelaps echidninus* (Jakeman 1963).

According to Costa (1966), copulation of *Macrocheles robustulus* has been observed only between males and newly emerged females. However, the *Amblyseius gossipi* (Phytoseiidae) female accepted the male again after she had finished oviposition (Elbadry and Elbenhawy, 1968). Rath *et al.* (1991) did not observe mating with old *T. clareae* females previously inseminated. In this investigation, mating of old *T. clareae* females was observed, similar as occurs in *V. jacobsoni* (Ifantidis and Rosenkranz 1988).

Rath *et al.* (1991) claim that one mating may serve to fertilize the entire egg production of a *T. clareae* female. However, there was no evidence presented that all the observed females were not previously inseminated. Multiple matings of females were described in *Phytoseiulus macropilis* (Prasad, 1967), *Amblyseius*

gossipi (Elbadry and Elbenhawy, 1968), *Glyptholaspis americana* (Krantz and Wernz, 1979), and *Amblyseius cucumeris* (Castagnoli and Liguori, 1991). Multiple matings of *T. clareae* females were observed in this investigation.

Woyke (1994a) suggested, that unmated *T. clareae* females do not oviposit, similar to females of *Typhlodromus* sp (Dosse 1959) and *Amblyseius gossipi* (Elbadry and Elbenhawy 1968).

An unreceptive female of *Glyptholapsis americana* fends off the male with legs IV, or catapults him from her dorsum (Krantz and Wernz, 1979). Such a phenomenon was not observed in *T. clareae*.

The average mating time varies in different Mesostigmata: 4 min in *Glyptholaspis americana* (Krantz and Wernz, 1979), 90 min in *Phytoseiulus macropilis* (Prasad, 1967), 185 min in *Amblyseius andersoni* (Amano and Chant, 1978), 243 - 380 min in 3 species of Phytoseiidae (Castagnoli and Liguori, 1991) and 6-12 hrs in different *Typhlodromus* species (Dosse, 1959). In *T. clareae* mating lasted about 25 min.

Populations of *T. clareae* in honeybee colonies are over-represented by females (Woyke, 1987, 1989; Rath *et al.*, 1991). Thus it is obvious that multiple mating of males must also occur in bee colonies. The present investigation demonstrated multiple matings by both young and old females.

The question arises as to where the mating of *T. clareae* occurs in the honey bee colony. Wei (1992) and myself have seen mating couples of mites inside brood cells after the emerging worker bee was removed. I have also seen several copulating mites outside sealed brood cells. Thus mating of *T. clareae* in the honeybee colony takes place inside as well as outside sealed brood cells.

CONCLUSIONS

The following events concerning the mating behavior of *T. clareae* were described in addition to information presented earlier by Rath *et al.* (1991): the behavior of the male on the female dorsum, the manner in which the male slips to the female venter, the position and movements of legs II, the male vibration while in the centre of the female venter, stroking of the female epigynial plate and the area of the solenostomes (gonopores), which is the most lasting event during the mating process, and the manner of disengagement of the couple. Mating positions were redescribed. The position described and presented in Fig. 1A. by Rath *et al.* (1991) is not the initial but a subsequent phase (asymmetrical position of legs II). In the two other sequential positions, the rear margins of male and female opisthosomas are not situated on the same level (Fig. 1 B and C), but the male opisthosoma protrudes behind the female opisthosoma. The size ratio of female and male on these figures is unproper. Unsuccessful matings were described and explained. In addition to multiple matings of males, multiple matings of young and old females were recorded.

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