Mating behavior of the astigmatid mite, *Caloglyphus rodriguezi* Samšínák (Acarina: Acaridae)¹

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Abstract

The mating behavior of the astigmatid mite, *Caloglyphus rodriguezi* Samšínák (Acarina: Acaridae), was observed in the laboratory. The typical sequence of the behavior can be classified into the following four phases: (I) encounter, (II) recognition and mounting by males, (III) positioning to achieve the correct posture between both sexes and (IV) sperm transfer. In phase (I), males searched for females more vigorously than females did for males. Further, approaches to males by females tended to end with unsuccessful mating. It was concluded that males play a more active role in the search for sexual partners at an early stage in mating behavior.

Key words: Mite, Acaridae, *Caloglyphus rodriguezi*, mating behavior, sex pheromone

INTRODUCTION

Among the astigmatid mites, the mating behavior of the flour mite, *Acarus siro* (Acarina: Acaridae), has been the most intensively analyzed (Boczek and Griffiths, 1979; Levinson et al., 1989). The analyses were similar to that used for the two-spotted spider mite (Tetranychidae) (Lehr and Smith, 1957; Cone, 1979; Potter, 1981), the phytoseiid mites (Phytoseiidae) (Amano and Chant, 1978; Hoy and Cave, 1985; Tsunoda, 1994) and the ixodid ticks (Ixodidae) (Sonenshine et al., 1991). The behavior of *A. siro* consists of a four-phased stereotype sequence as follows: The female approaches the male and circles him several times (phase 1). When the opisthosoma of the female comes close to the male's gnathosoma, the male is aroused and mounts the female laterally (phase 2). The male settles on the female's dorsum in a parallel position, while the female commences walking (phase 3). The male turns his body by $\approx 180^\circ$ and transfers gnathosoms and part of his idiosoma beyond the margin of the female's opisthosoma (phase 4) (Levinson et al., 1989).

In addition to these behavioral observations, acetone extracts from *A. siro* males and females have been demonstrated to function as an attractant for the females and as a sexual excitant for the males, respectively. This indicates that the mating of this species is mediated by two different male and female sex pheromones (Levinson et al., 1989). However, the chemical structures of these pheromones are presently uncharacterized.

The female sex pheromone which elicits sexual activity in the acarid male mite, *Caloglyphus polyphyllae* (Acarina: Acaridae) has been identified as β -acaridial, a monoterpenoid, but a comprehensive behavioral examination has not been carried out (Leal et al., 1989). The following 5 female sex pheromones have been subsequently identified from each of the following acarid mites using similar criteria: 2-hydroxy-6-methylbenzaldehyde from *Aleuroglyphus ovatus* (Acaridae) (Kuwahara et al., 1992) and *Acarus immobilis* (Acaridae) (Sato et al., 1993), undecane from *Caloglyphus rodriguezi* (Acaridae) (Mori et al., 1995), (2R,3R)-epoxyneral from an unidentified *Caloglyphus* sp. MJ

¹Chemical ecology of astigmatid mites LIV.

(tentatively named in a study, Mori and Kuwahara, 1995; Mori et al., 1996), and rosefuran also from another unidentified *Caloglyphus* sp. HP (also tentative name, Mori et al., in preparation as chemical ecology of astigmatid mites LII).

Among these species, only one species, namely *A. immobilis*, has been clearly shown to possess a male sex pheromone which functions as an attractant for females, as reported for *A. siro* (Levinson et al., 1989). The active principle(s) of this attractant have been identified as a mixture of hydrocarbons (Sato et al., 1993). No chemical evidence is presently known to definitely demonstrate the existence of a male sex pheromone among the other four astigmatid mites. Based on these facts, it can be concluded that pheromonal communication systems relative to the mating behavior in astigmatid mites can be classified into at least two groups; one in which both male and female sex pheromones are involved in the mating sequence, and the other, in which only a female sex pheromone is responsible for mating. In order to better understand this, it is necessary to demonstrate that the mating behavior of males in certain astigmatid mites is triggered only by the female sex pheromone, not only from chemical, but also from an ethological point of view, including the possibility of the existence of a male sex pheromone.

The present report describes the mating behavior of *Caloglyphus rodriguezi*, and discusses the function of the female sex pheromone from an ethological point of view.

MATERIALS AND METHODS

Mites. *Caloglyphus rodriguezi* Samšínák (Acarina: Acaridae) was originally collected from farm soil in Tsukuba (Mori et al., 1995) and maintained in our laboratory for more than 5 years at 20–25°C and at conditions of high humidity by feeding moistened dry yeast in a plastic Petri dish (85 mm i.d., 20 mm ht.), which also contained two sheets of moistened filter paper. To maintain the humidity in the Petri dishes, the dishes were enclosed in a zippered plastic bag (240 × 170 × 0.04 mm). The plastic bags were then placed in an incubator at 25°C. For behavioral experiments, both sexes

were randomly selected from the stock culture and were used with no prior knowledge of previous mating experiences, because it is well known that in Astigmata, multiple mating commonly takes place even in females (Pillai and Winston, 1969; Boczek and Griffiths, 1979; Radwin, 1991).

Observation. Mating behavior of the mite was observed using a mini Petri dish (10 mm i.d., 5 mm ht.) as a chamber. For this experiment, the bottom of the dish was covered with moistened filter paper and a small portion of moistened dry yeast was then introduced. Test mites were introduced in two different ways, in order to offset the effects of handling the mites; a male was first transferred by a needle into the Petri dish and conditioned for more than 1 h, and a female was then exposed to the conditioned male (when a female was exposed to a conditioned male, this method was abbreviated as the F-method). The second procedure was in the opposite order and involved the conditioning of a female in the chamber, followed by the introduction of a male (when a male was exposed to a conditioned female, this method was abbreviated as the M-method). Throughout the conditioning and observation period, the dish was covered with a glass plate (cover glass 18 mm × 18 mm) to prevent the mites from escaping and also to minimize air currents in the chamber. The behavior was observed for 15 min under a binocular stereoscopic microscope using fluorescent illumination (6 V–12 W) at room temperature, until more than 85% of tested pairs succeeded in mating.

The basic sequences of mating behavior and its variation were preliminarily confirmed from observations of 25 pairs each for both the F- and the M-methods. Detailed recordings on each component of behavioral patterns were made by a total of 120 examples of mating behavior, with particular emphasis on determining which sex was more active at each phase and in which direction one sex approached the other.

RESULTS

Classification of mating behavior

The preliminary observations of mating behavior, using a total of 50 pairs of *C. rodriguezi*, revealed that a typical sequence

Fig. 1. Classification of mating behaviors in *Caloglyphus rodriguezi*.

could be classified into the following four phases; (I) encounter, (II) recognition and mounting by males, (III) positioning to achieve the correct posture between both sexes and (IV) sperm transfer (Fig. 1). The frequencies of each behavior for each sequence were calculated for the two introduction methods and are summarized in Figs. 2 and 3.

The behavior of both sexes during each phase was detailed as follows.

Encounter

After conditioning in the chamber, the mites largely either settled in a motionless state or fed on the moistened dry yeast. When an opposite sex was transferred into the chamber, mating behavior proceeded. In most cases, the males actively moved around the Petri dish, while the females either moved or rested.

Phase (I) involves an encounter between a male and a female. There were five possible encounter modes, as follows (Fig. 1): male approaches female from her lateral side (I-a); male proceeds to female from her posterior side (I-b); male and female meet front to front (I-c); female encounters male from his lateral side (I-d);

and female chases male (I-e).

In the F-method (Fig. 2), the frequencies of encounter for 60 trials were 26, 29, 5, 0 and 0 for (I-a), (I-b), (I-c), (I-d) and (I-e), respectively, while for the M-method they were 19, 17, 8,

Fig. 2. Diagram of mating behavior of *Caloglyphus rodriguezi* by the F-method. The numbers in brackets indicate the total number of pairs observed in each category out of 60 replicates.

Fig. 3. Diagram of mating behavior of *Caloglyphus rodriguezi* by the M-method. The numbers in brackets indicate the total number of pairs observed in each category out of 60 replicates.

6 and 2 (Fig. 3). To confirm which sex was the more active participant in the encounter, the frequencies of male-to-female encounters (I-a+I-b), female-to-male encounters (I-d+I-e) and front-to-front encounters (I-c) were rated separately (Table 1). The numbers of male-to-female encounters were 55 (26+29) and 36 (19+17), and those of female-to-male encounters were 0 and 8 (6+2) for the F- and the M-methods, respectively. The numbers for the male-to-female encounter were each significantly higher than those of the female-to-male for both methods ($p < 0.01$ by test for the proportion). Frequencies of mating success among 60 trials were 49 (46+3) and 30 (24+4+2) for the F- and the M-methods, respectively (Table 1), in which male to female encounter was the major process for successful mating for both methods ($p < 0.01$ by test for the proportion; 46 and 24

for the male-to-female encounter, and 0 and 2 for the female-to-male encounter). Although the difference between the two methods was not significant in terms of the numbers of successful and unsuccessful matings ($p = 0.13$ by Fisher's exact probability test; 46 and 0 for F-method, 24 and 2 for M-method), a significant difference was detected between the two methods in the numbers of male-to-female and female-to-male encounters ($p < 0.01$ by Fisher's exact probability test; 55 and 0 for F-method, 36 and 8 for M-method) depending upon the observation methods.

These results imply that males, playing a leading role at an earlier stage of the mating behavior, were more affected than females by handling, however, the effect appeared to be restricted to the phase of encounter, and no further effect was detectable in the subsequent processes after encounter. Because males walk faster than females in the field, the results stated above might be obtained, even if males don't play the leading role in mating behavior. Therefore, we paid close attention to the behavior that males showed in the female-to-male or male-to-female encounter. Males tended to run away from females and, thus, failed in mating in the former encounter (Fig. 3). Even when males met females, most of the females also attempted to escape. Males, however, were more eager to pursue females and mounted them successfully. The behavioral sequence proceeded successfully from phase (I) to phase (II), when males approached females. We concluded that males played a more active role in mate-search than females at the early stages of mating behavior.

Recognition and mounting

At phase (II), the male touched the female with the forelegs and palps. This type of contact is defined as a first contact. The males continued to touch the female's body with their forelegs and palps during mating behavior, after males mounted the female's back.

The mode by which a male approached, touched and mounted the female body was also examined (Figs. 2 and 3). The direction of the approach was divided into four routes; lateral (II-a), back (II-b), front-to-front (II-c)

Table 1. Encounter mode and mating success of *C. rodriguezi*

Encounter mode	Number of encounters (success)	
	F-method	M-method
Male to female	55 (46)	36 (24)
Front to front	5 (3)	8 (4)
Female to male	0 (0)	8 (2)
Failure	0	8

and failure (II-d). As a result, males which faced females in one direction, mounted the females in the same direction in both methods (Figs. 2 and 3); for example, 92% of the males which approached females in lateral modes, subsequently mounted the females laterally (Fig. 2).

Orientation towards the mate

After mounting a female, the male folded his fourth pair of legs characteristically to hold tightly to the female's dorsum at phase (III), as was observed for *Caloglyphus anomulus* (Pillai and Winston, 1969). The male drummed the female's dorsum with his forelegs and contacted the female surface with his palps as was shown for the initial touching during this stage.

Phase (III) could be classified into two phases: that when a male walked around on the female's dorsal region (III-1), and then when he crawled over the female's opisthosoma and proceeded to the ventral surface (III-2); (I) → (II) → (III-1) → (III-2) → (IV) and (I) → (II) → (III-1) → (IV). In the F-method, the frequency for proceeding from (III-1) → (III-2) was 34 (61%) and for (III-1) → (IV), it was 18 (32%) among the 56 pairs in (III-1), as shown in Fig. 2. Furthermore, in the M-method, the former was 27 (71%) and the latter was 7 (18%) among 38 pairs (Fig. 3). These observations indicate that the major mating sequence proceeded as (I) → (II) → (III-1) → (III-2) → (IV) for both methods.

Sperm transfer

When the male finally attached himself to the female's opisthosoma, both sexes then oriented themselves tail to tail in opposite directions. Although phases (I) → (II) → (III) proceeded rapidly, this positioning lasted, on average, $1,071 \pm 511$ s ($n=32$, mean $\pm$ standard deviation). Females were often observed moving about in this fashion, dragging males on their backs.

DISCUSSION

The present studies reveal that the mating behavior of *Caloglyphus rodriguezi* is similar to that of species belonging to other suborders, such as ixodid ticks (Sonenshine et al., 1991) and the phytoseiid mites (Phytoseiidae) (Amano

and Chant, 1978; Hoy and Cave, 1985; Tsunoda, 1994), where males search for females more actively than females do for males.

An observable difference, however, was found in the mating behavior between the present species and *A. siro* (Levinson et al., 1989) or *A. immobilis* (Sato et al., 1993), which belongs to the same family (Acaridae) as the present species. *A. siro* females are first attracted to males by a male sex pheromone (Levinson et al., 1989). *C. rodriguezi* males, on the contrary, tended to run away from females in the case of the female-to-male encounter, resulting in failed mating. Successful mating, furthermore, resulted more frequently from male-to-female encounters than female-to-male encounters. It is not the male sex pheromone, but the female sex pheromone, that plays an important role in mating behavior. In addition, it is known that hexane extract of males of the present species does not show any activity toward *C. rodriguezi* females, while it does elicit sexual behavior from males (Mori et al., 1995). From these results, we conclude that there might be no chemicals functioning as a male sex pheromone in the mating behavior of *C. rodriguezi*. Leal et al. (1989) also observed that *C. polyphyllae* females do not respond to the male extract. This is different from the cases of *A. siro* (Levinson et al., 1989) and *A. immobilis* (Sato et al., 1993), in spite of the fact that *A. siro*, *A. immobilis*, *C. rodriguezi* and *C. polyphyllae* belong to the same family of acarid mites.

It is also known that ixodid tick males search for females more actively than females do for males, and that the following three types of female pheromones are responsible for this mating behavior: a volatile attractant sex pheromone; a mounting sex pheromone; and a genital sex pheromone (Sonenshine et al., 1991). Each pheromone functions during different phases of the mating behavior of the male ticks. In the case of an unidentified *Caloglyphus* sp. MJ whose female pheromone is known to be (2*R*, 3*R*)-epoxyneral (Mori et al., 1996), the males were attracted to a small piece of paper impregnated with the female sex pheromone, and gathered around the paper, tapping it with their forelegs and touching it with their palps. This observation suggests that (2*R*, 3*R*)-epoxyneral

functions not only as an attractant but also as a stimulant for mounting and an adherent. *C. rodriguezi* males also touched females with the palps, and actively tapped the female's body with their forelegs, after an encounter was made. Similar behavior toward a paper impregnated with the pheromone, however, was not observed for the case of *C. rodriguezi*, as shown in *Caloglyphus* sp. MJ. We hypothesized that such dissimilarities might be due to differences in the volatility of the sex pheromone component.

To our knowledge, the present study is the first report dealing with the mating behavior of the species belonging to the genus *Caloglyphus*. The possible presence of a male sex pheromone was not confirmed based on behavioral observations made herein. Inter-specific comparison of mating behavior, not only from a chemical, but also from an ethological point of view, will be required to conclude that the astigmatid mites can be classified into two groups; namely species whose successful mating depends only on the female sex pheromone, and species who require both male and female sex pheromones.

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