Oviposition behavior of *Eumicrosoma blissae* (Maki) (Hymenoptera: Scelionidae), an egg parasitoid of the oriental chinch bug, *Cavelerius saccharivorus* Okajima (Heteroptera; Lygaeidae)

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Abstract

Oviposition behavior of the parasitoid, *Eumicrosoma blissae* (Maki) toward unparasitized eggs of the oriental chinch bug, *Cavelerius saccharivorus* Okajima was observed in the laboratory. After the female encountered a host egg mass, her oviposition behavior could be divided into nine discrete steps: drumming, extension of ovipositor sheath, probing, drilling, insertion of ovipositor, withdrawal of ovipositor, withdrawal of ovipositor sheath, marking, and retraction of ovipositor sheath. In some cases, insertion of ovipositor did not occur. The parasitoid egg was found only in hosts where a female exhibited ovipositor insertion and marking. When a female attack a parasitized host, she often marked it without ovipositor insertion after drilling. The duration of marking was long when the ovipositor was inserted. The frequency distribution of the number of attacks per host egg was not random. There was a tendency for *E. blissae* to attempt to insert its ovipositor in the same host egg only once. The characteristics of the oviposition behavior of *E. blissae* are discussed in comparison with those of other egg parasitoids.

Key words: Egg parasitoid, *Eumicrosoma blissae*, oriental chinch bug, oviposition behavior

INTRODUCTION

In biological control by parasitic wasps, it is necessary to clarify ecological characteristics of the potential parasitic wasp, especially its normal oviposition behavior to a common host to analyze not only the host discrimination/acceptance but also the meaning of behavioral changes under various conditions (Noda, 1993).

Eumicrosoma blissae (Maki) is a solitary egg parasitoid of the oriental chinch bug, *Cavelerius saccharivorus* Okajima, which is one of the most important pests of the sugarcane *Saccharum officinarum* L. in the Southwestern Islands of Japan. *E. blissae* has been considered as a potential natural enemy of *C. saccharivorus* because parasitism by *E. blissae* exceeds 50% in Spring and Fall (Azuma, 1977). However, there has been only one brief report on the development time, longevity, and fecundity of adult *E. blissae* (Azuma, 1977). Females of *C. saccharivorus* oviposit an egg mass in a line containing about 20 eggs. They are laid along an edge of

the inner part of a contacted leaf sheath of the sugarcane. The parasitoid approaches the eggs by creeping (Azuma, 1977). However, there have been no reports on the oviposition behavior of *E. blissae* after they make contact with the host egg mass.

In this paper, I describe the results of laboratory observations of oviposition behavior in *E. blissae* females without previous oviposition experience when exposed to unparasitized host eggs.

MATERIALS AND METHODS

Insects. Adults of *C. saccharivorus* were collected from sugarcane fields in the southern and central parts of Okinawa Island, Japan, in September 1993, and December 1994, respectively. One or two pairs of adults were placed in a glass bottle (70 × 40 mm ϕ) with a leaf sheath stalk (long length about 30 mm). They were kept at 25 ± 1°C and LD 14:10 (light phase 6:00 to 20:00). Eggs of *C. saccharivorus* which were laid in the leaf sheath were collected daily. The

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egg period of the chinch bug is 21.4 d at 25°C (Azuma, 1977).

The strains of parasitoid used for observation were kept under laboratory conditions at $25 \pm 1^\circ\text{C}$ or $30 \pm 1^\circ\text{C}$ and LD 14:10 (light phase 6:00 to 20:00) in generations after being collected from sugarcane fields in the Okinawa Prefectural Agricultural Experiment Station and the central part of Okinawa Island in October 1993, and June 1994, respectively. In Okinawa Island, *E. blissae* exists as a thelytokous species. Successive rearing was conducted in a manner where one or two adult females were placed in a glass tube ($105 \times 18 \text{ mm}\phi$) and given a piece of filter paper ($8 \times 20 \text{ mm}$) on which 15 to 25 unparasitized host eggs were glued. These females were not fed.

Observation of oviposition behavior. The inside of a glass tube ($105 \times 18 \text{ mm}\phi$) was moistened by water to attach 10 host eggs (2–5 d old) to the inner wall, and a female without oviposition experience (2 d after emergence) was placed in the tube. After the female's contact with a host egg, the sequence of oviposition behaviors were observed through a binocular and the number and duration of each behavioral component were recorded for each of 16 females in total. The insertion of the ovipositor into the host egg was observed under an adequate intensity of light to enable the egg shell of light yellow color to be semitransparent. Observation was discontinued when the female left a host egg mass and failed to return within 10 min.

Dissection of parasitized host eggs. To examine the relationship between the occurrence of ovipositor insertion and egg laying, eggs exposed to oviposition behavior were dissected. An unexperienced female (2 d after emergence) was placed in a glass tube ($105 \times 18 \text{ mm}\phi$) in which 5 host eggs (3 d old) were attached to the inner wall with water. Oviposition behavior was observed for ten females in total. Thirty host eggs were dissected on one-hole-slide-glasses to count the number of parasitoid's egg after being stained with methylene blue.

RESULTS

Sequence of oviposition behavior

The sequence of oviposition behavior of *E.*

blissae is shown in Fig. 1. After the female encountered a host egg mass, she antennated the surface of a host egg while walking on and around them (Drumming). After that, the female extended her ovipositor sheath (Extension of ovipositor sheath) and intensively drummed a host egg. The female dismounted the egg, directed the tip of the ovipositor sheath to the host egg by turning her body 180° , then selected a boring site by rubbing the surface with the tip of the ovipositor sheath (Probing). After determining the boring site, the female grasped the substrate with all of her tarsi and started to drill while reciprocally moving two of the terebra (Drilling). In this position, the female's wings were horizontally held in contact with the abdomen. No upward flagellation of the antennae or wings was observed. After the drilling, two different behaviors were observed: one was insertion of the ovipositor into the host egg (In-

Fig. 1. Sequence of ovipositional behaviors of a female, *E. blissae*, observed on host eggs. Arrows indicate the direction that behavioral sequences proceeded. After drilling, solid lines indicate the sequence when the parasitoid inserted the ovipositor into the host egg, and dashed lines indicate the sequence when parasitoid did not insert ovipositor. The figures in parentheses indicate the frequency of the behavior observed.

section of ovipositor), and the other was withdrawal of the ovipositor sheath without insertion of the ovipositor. Whether or not females laid eggs in hosts was not confirmed in this observation. When the ovipositor was inserted, the ovipositor sheath was withdrawn from the host egg (Withdrawal of ovipositor sheath) after the ovipositor was taken into the sheath (Withdrawal of ovipositor). Subsequently, after withdrawal of the ovipositor sheath, the female rubbed the surface of the host egg with the tip of her ovipositor sheath (Marking). Marking was done on the eggs at both ends of an egg mass, covering the entire surface of the host eggs. On the contrary, it was concentrated only around the boring spot on the other eggs. After marking, the female moved to another host egg while holding her ovipositor sheath pointing at the egg and resumed oviposition behavior. Subsequent trials mainly started with probing, but in some cases, oviposition behavior was restarted at drumming after the ovipositor sheath was once retracted to the abdomen (Retraction of ovipositor sheath). In some cases when the ovipositor was not inserted, immediately after the female withdraw her ovipositor sheath, she started drumming or probing without marking. Having oviposited in the last egg in the egg mass and retracted her ovipositor sheath, the female repeatedly came back to the egg mass after walking around it. Gradually the distance of her walking got further and finally she left the egg mass.

There were various sequences in the order of egg attack by female *E. blissae*. Ten host eggs were given to 16 test individuals. Some females attacked the left (or right) end first, and then continued in order until reaching the other end (Fig. 2A), whereas others started with the egg in the middle, and attacked subsequent eggs in an irregular manner (Fig. 2B). However, it was impossible to clearly categorize the 16 females into systematic and non-systematic groups.

A total of 290 attacks by females were observed on 160 host eggs. The occurrence of the insertion of the ovipositor and marking was recognized for 235 observations. Marking behavior was always observed when the ovipositor was inserted into a host egg (144/144, 100%). Among 91 attacks without ovipositor insertion,

Fig. 2. Two examples of the order of attack to the host egg in *E. blissae*. Figures indicate order of attack. In A, host eggs were attacked systematically. In B, they were occasionally attacked at irregular intervals. The circles indicate that the parasitoid inserted the ovipositor into the host egg at the oviposition behavior.

65 attacks (71.4%) were followed by marking, whereas 26 attacks (28.6%) were not ($\chi^2 = 46.261$, $p < 0.0001$, Table 1).

The duration of some behavioral events

The durations from the start of drilling to the end of withdrawal of the ovipositor sheath and that for marking were measured in 227 cases and 94 cases of a total 290 ovipositions, respectively. The duration between drilling and withdrawal of the ovipositor sheath was 166.89 ± 45.84 s (mean $\pm$ S.D. $n = 139$) when the ovipositor was inserted, and 97.52 ± 44.28 s ($n = 88$) when it was not (Mann-Whitney's *U*-test, $p < 0.0001$, Table 2).

The duration between ovipositor insertion and withdrawal of the ovipositor was 42.06 ± 9.76 s ($n = 139$) when the ovipositor was inserted.

The durations required for marking were 34.07 ± 12.46 s ($n = 59$) when the ovipositor was inserted and 18.06 ± 6.28 s ($n = 35$) when it was not. The parasitoid spent a significantly longer time marking when the ovipositor was inserted (Mann-Whitney's *U*-test, $p < 0.0001$, Table 2).

Table 1. Frequency of marking in relation to the insertion of ovipositor

Insertion of ovipositor	No. of observed behaviors	Marking		χ^2
		Yes	No	
Yes	144	144	0	46.261
No	91	65	26	($p < 0.0001$)

Table 2. Duration between drilling and withdrawal of ovipositor sheath and duration of marking in relation to occurrence of the ovipositor insertion

Insertion of ovipositor	No. of observed behaviors	Time (s) required for behavior (mean±S.D.)	<i>U</i>
Drilling-withdrawal ovipositor sheath			
Yes	139	166.89±45.84	−10.28***
No	88	97.52±44.28	
Marking			
Yes	59	34.07±12.46	−6.68***
No	35	18.06±6.28	

*** $p < 0.0001$, Mann-Whitney's *U* test.

The number of attacks per host egg

The frequency distribution of the number of attacks per host egg is shown in Fig. 3. A total of 16 females performed oviposition behavior from drilling to marking at least once on almost all of the host eggs presented to them. Of the 160 host eggs, only one was not attacked. There were 66 host eggs which experienced oviposition behavior once, 59 eggs twice, 30 eggs three times, and four eggs four times. The mean number of attacks per host egg by a single female was 1.81 ± 0.28 (mean $\pm$ S.D.).

The frequency distribution of attacks per host egg was greater around the mean value than the value expected by a Poisson distribution indicating that the attacks took place randomly regardless of whether or not the hosts were parasitized, but when the frequency of attack was zero or four, the value was smaller than the expected value ($\chi^2 = 48.847$, $p < 0.001$). The I_δ -index value, which indicates the degree of concentration, was smaller than 1 ($I_\delta = 0.6605$), but the difference was not significant ($F = 0.382$,

$p > 0.05$). This value suggested that the frequency distribution of attacks per host egg was a uniform distribution rather than a random distribution.

No significant difference was found among individual females in terms of the number of attacks per host egg ($\chi^2 = 12.99$, $p > 0.05$).

Pattern of oviposition behavior on the same host egg

Of the 160 host eggs, occurrence/non-occurrence of ovipositor insertion and marking on the same host egg were confirmed for 110 host eggs. There were three different types of oviposition trials: 1) a trial with an unparasitized host, 2) a trial with a singly attacked host, and 3) a trial with a doubly attacked host. The frequency of oviposition behavior in relation to the combination of the occurrence of ovipositor insertion and marking toward these hosts is shown in Table 3. Of the 110 cases of oviposition trial to the unparasitized host, 107 (92.27%) showed ovipositor insertion followed by marking (type I+M). Whereas, of the 50 cases of oviposition trial to the singly attacked host, only five (10.00%) cases showed the oviposition behavior of type I+M. A large proportion (39 cases, 78.00%) showed no ovipositor insertion, but only marking (type NI+M). There was a significant difference in the frequency of ovipositor insertion between unparasitized and singly attacked hosts ($\chi^2 = 124.675$, $p < 0.0001$). Only one (7.1%) of the 14 oviposition trials to the doubly attacked host showed the behavior of type I+M. The 11 cases (78.57%) showed the behavior of type NI+M. A significant difference was also recognized in

Fig. 3. Frequency distribution of the number of attacks per host egg. The line indicates the expected distribution when a parasitoid randomly attacks host eggs.

Table 3. Frequency of the 4 types of oviposition behavior which shows different combinations of ovipositor insertion and marking in relation to the type of host egg having different oviposition experiences

Type of host eggs	Combination of the occurrence of ovipositor insertion and marking			
	I + M ^a	I + NM ^b	NI + M ^c	NI + NM ^d
Unparasitized	107	0	0	3
Singly attacked	5	0	39	6
Doubly attacked	1	0	11	2

^aOvipositor insertion was followed by marking.^bOvipositor insertion was not followed by marking.^cOvipositor non-insertion was followed by marking.^dOvipositor non-insertion was not followed by marking.Table 4. Relationship between occurrence of ovipositor insertion and egg laying in *E. blissae*

Insertion of ovipositor	No. of hosts examined	No. of hosts containing parasitoid eggs		<i>p</i> ^a
		Yes	No	
Yes	25	22	3	0.0004
No	5	0	5	

^aFisher's exact probability test.

the frequency of ovipositor insertion between unparasitized and doubly attacked hosts (Fisher's exact probability test, $p < 0.0001$). There was no difference in the frequency of ovipositor insertion between singly attacked hosts and doubly attacked hosts (Fisher's exact probability test, $p > 0.9999$). Of the 110 host eggs, self-superparasitism occurred only in three host eggs (2.7%). These include two cases on the singly parasitized hosts (by the same female), and one case on a doubly parasitized host.

The relationship between insertion of the ovipositor and egg laying

To clarify the relationship between the occurrence of ovipositor insertion and egg laying, a total of 30 host eggs were examined for the presence of parasitoid eggs in the host eggs. The parasitoid's egg was found in 22 of 25 host eggs into which females inserted their ovipositor, whereas no eggs were found in 5 host eggs into which females did not insert the ovipositor (Fisher's exact probability test, $p = 0.0004$, Table 4).

DISCUSSION

The sequence of ovipositional behavior of *E. blissae* on a host egg was as follows: drumming, extension of the ovipositor sheath, probing, drilling, insertion of the ovipositor, withdrawal of the ovipositor, withdrawal of the ovipositor sheath, marking, and retraction of the ovipositor sheath. The sequence is similar to other scelionid egg parasitoids such as *Trissolcus mitsukurii*, *Telenomus nakagawai* (Hokyo and Kiritani, 1966), *T. fariai* (Bosque and Ravinovich, 1979), *T. heliothidis* (Strand and Vinson, 1983), *Trissolcus plautiae* (Ohno, 1987), *Gryon japonicum* (Noda, 1993), and *T. triptus* (Higuchi and Suzuki, 1996).

E. blissae performed a reciprocal back-and-forth movement of two pieces (tereбра) composing the ovipositor sheath at the surface of a host egg. A similar drilling performance was reported for *E. benefica* (McColloch and Yuasa, 1915).

Ovipositor insertion of *E. blissae* was easily observed through the semitransparent chorion of chinch bug eggs. In the case of *T. heliothidis*, the first and the second valvulae (tereбра) were

inserted into the host, whereas the third valvulae was fixed on the surface after drilling (Strand and Vinson, 1983). This is similar to the ovipositor insertion of *E. blissae*.

Females of *T. mitsukurii* and *T. plautiae* hold both fore and hind wings vertical to the body during ovipositor insertion (Hokyo and Kiritani, 1966; Ohno, 1987). However, such action was not observed for *E. blissae*. Actually, it is physically impossible for *E. blissae* to do this because the inside of the sugarcane leaf sheath where the host eggs are located is too cramped for the parasitoid to hold its wings vertically.

The majority of parasitic Hymenoptera can discriminate between parasitized and unparasitized hosts (Salt, 1937; van Lenteren, 1976; Vinson, 1976) by 1) detecting a kind of cue inside of the host (internal cue) when it inserts its ovipositor into the host, and 2) detecting a cue inside and on the surface of the host (external cue) at the time of ovipositor insertion and drumming (Takasu, 1989). External cues include a scratch by the ovipositor and a secretion like a pheromone emitted from various accessory glands of wasps (Rabb and Bradley, 1970; Vinson, 1976; Bosque and Ravinovich, 1979; Okuda and Yeargan, 1988). Noda (1993) found a thread-like marking substance attached on the surface of the host parasitized by *G. japonicum*, and thought that it functioned physically or chemically as a nonvolatile or low volatile contact stimulus. Although it appears that marking by *E. blissae* is for the purpose of leaving a cue, and drumming is to discriminate between parasitized and unparasitized hosts, the females often attacked the same hosts twice or more. However the frequency distribution of the number of attacks per host egg was proved to be significantly different from random distribution (see Fig. 3). Also, I_{δ} -index value indicated that the oviposition behavior of *E. blissae* occurred uniformly in the host eggs given to each female. These results suggest that *E. blissae* recognizes the previous parasitism of a host egg and avoids it. Higuchi and Suzuki (1996) gave 20 host eggs to each of 12 females (inexperienced with oviposition) of *T. triptus*, and reported that self-superparasitism occurred in 24 out of 240 host eggs (10.0%). This study gave similar results where self-superparasitism of *E.*

blissae occurred in only 3 out of 110 host eggs (2.7%), suggesting that *E. blissae* discriminates between parasitized and unparasitized host eggs quite accurately. In this observation, the parasitoids were given a limited number of host eggs in small test tubes. They may leave more effective marks for host discrimination under field conditions.

E. blissae attacked the same host eggs frequently but rarely laid eggs (Table 3). A parasitoid undoubtedly lays eggs only when she inserts her ovipositor into the host egg (Table 4). All of these experimental results indicate that *E. blissae* can perceive internal cues during the period from drilling to ovipositor insertion to discriminate the status of the host egg. Strand (1986) reported that females of *T. heliothidis* recognized the status of the host from an internal cue, the necrosis of the host cell. Noda (1993) considered the pupation of the parasitoid and the disappearance of the host egg yolk as the internal cues in *G. japonicum*. At present, the internal cue for *E. blissae* has not yet been identified, and further investigations are needed.

Scelionid wasps including *T. mitsukurii*, *T. nakagawai*, *G. japonicum*, and *T. triptus* exhibit no marking behavior when they do not lay eggs. Therefore, the presence of a parasitoid's eggs can be recognized by the occurrence of marking behavior (Hokyo and Kiritani, 1966; Noda, 1993; Higuchi and Suzuki, 1996). However, marking behavior occurred even when the ovipositor was not inserted in *E. blissae* (Tables 1 and 3).

The external cues of *T. sphingis*, *T. fariai*, and *T. euschisti* are water soluble (Rabb and Bradley, 1970; Bosque and Ravinovich, 1979; Okuda and Yeargan, 1988). If the marking substance of *E. blissae* is water soluble, the effective duration of marking may be short because the inside of the leaf sheath of the sugarcane, where eggs of *C. saccharivorus* can be found, is very moist. The marking behavior after oviposition trial even without ovipositor insertion may occur in order to publicize more clearly that the host egg has already been parasitized by herself. In fact, marking behavior unrelated to the ovipositor insertion occurred when females attacked hosts parasitized

by themselves (Table 3).

To understand the host acceptance and host discrimination ability of *E. blissae*, further investigations concerning the behavioral changes associated with the status of the host eggs and the female's oviposition experience are needed.

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