

Oviposition Behavior of *Ooencyrtus kuvanae* (Howard) (Hymenoptera: Encyrtidae), Egg Parasitoid of *Lymantria dispar* L. (Lepidoptera: Lymantriidae)

매미나방알좀벌, *Ooencyrtus kuvanae* (Hymenoptera: Encyrtidae)의
매미나방 난에 대한 산란행동

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ABSTRACT The oviposition behavior of *Ooencyrtus kuvanae* (Howard) (Hymenoptera: Encyrtidae) was examined in a small petri dish containing eggs of gypsy moth, *Lymantria dispar* L. The behavioral sequence from initial searching movement toward the host egg through oviposition, departure, and auxiliary phase activities such as resting, glooming, and host feeding were described. Oviposition experience shortened the approach time to subsequent eggs. The time spent for contacting the host egg also decreased with oviposition experience. Oviposition experience may play an important role in host recognition and increasing oviposition efficiency.

KEY WORDS *Ooencyrtus kuvanae*, oviposition behavior, oviposition experience, parasitoid

抄 錄 매미나방알좀벌, *Ooencyrtus kuvanae* (Hymenoptera: Encyrtidae), 이 기주를 발견한 후 산란을 끝내고 기주를 떠날 때까지 11단계의 연속행동과정, 그리고 자기몸청소, 휴식 등과 같은 부수적인 행동이나 산란관에 의한 상처로부터 나오는 기주체액을 먹는 습성 등이 조사되었다. 기생벌에 기주난이 연속적으로 주어졌을 때는 처음 산란을 마친 매미나방알좀벌은 활발히 주변을 움직이며 다음 산란대상이 되는 기주에 보다 빨리 접근하는 경향을 나타내고, 또한 기주접촉단계에 소요하는 시간이 첫번째 난에서 보다 두번째 세번째난에서 단축되는 경향을 나타내는 것으로 관찰되어 산란경험이 기주발견과 인식에 중요한 영향을 미치는 것으로 조사되었다.

檢 索 語 매미나방알좀벌, 산란행동, 산란경험, 기생천적

It has been pointed out that the host specificity of a parasitoid is attained by four consecutive processes of host elimination, namely (1) host habitat finding, (2) host finding, (3) Host recognition and acceptance, and (4) Host suitability (Flanders 1953). Vinson (1976) divided the events into five steps; (1) host habitat location (2) host loca-

tion (3) host acceptance, (4) host suitability, and (5) host regulation. Through the successive processes, the host list of a parasitoid becomes restricted to few species than are potentially available to it in nature (Doutt 1964). These phases are not isolated from each other but are studied independently to understand better the behavioral patterns.

The acceptance of the host by the parasitoid, through a specific behavioral sequence, is part of the innate behavior of parasitoid

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species (Doutt 1964) and such sequences have been previously described by Lee et al. 1989, Loke et al. 1983, Tucker and Leonard 1977, van Lenteren 1976, Schmidt 1974, and Edwards 1954.

As the augmentation (regular rearing and release) approach to biological control became more important, the factors involved in successful parasitoidism have been more frequently studied. Strand and Vinson (1982) reported that females of *Cardiochiles nigriceps* are more likely to search for and attack host if they have previously encountered a host than an inexperienced female. *Apanteles marginiventris* that have experienced a period of contact with host larvae and/or frass of larvae, spend less time resting and attack a greater number of their host, *Spodoptera frugiperda* larvae, than inexperienced females (Lewis et al. 1982).

Ooencyrtus kuvanae was first reported to attack gypsy moth eggs in Korea by Park (1966) and this species was found to have a high potential in reducing gypsy moth population in this country because of its high reproductive capability and high rate of parasitoidism (Lee 1978). *O. kuvanae* was imported into the northeastern United States (Crossman 1925) and is quite abundant there presently (Brown & Cameron 1982, Bellinger et al. 1988).

The objectives of this study were to describe the host acceptance behavior of *O. kuvanae* on *L. dispar* eggs and to analyze the influence of the prior oviposition experience on its activation and host recognition.

MATERIALS AND METHODS

Gypsy moth was reared on the artificial diet described by ODell et al. (1984). A pair of adult moths was kept in a cardboard box

(28 × 17 × 10 cm) with 10% honey water solution. The container was lined with a paper towel and kept in a C.T. room with 25 ± 1°C and 16L : 8D photoperiod. Eggs were deposited on the paper towels and held in the laboratory for 2 weeks embryonation period to allow the 1st instar larvae to be fully formed. The eggs were then transferred into an incubator at 4°C. Eggs, dehaired by the method described by Schaefer et al. (1988) were used for this experiment for easier observation.

Parasitoid colony maintenance

O. kuvanae, obtained from field collected gypsy moth eggs, was reared on eggs of gypsy moth at 25 ± 1°C, 60 ± 10% RH. and 16L : 8D photoperiod. Adult *O. kuvanae* were fed with clover honey.

Behavioral bioassay

Active, 4~6 days old, females were selected for this study because wasps of this age showed the greatest ovipositional activity (Schieferdecker 1969). Females were placed individually in a petri dish for the conditioning for 30 minutes prior to bioassay. The laboratory bioassay technique was as follows. Three eggs were attached, in a triangular pattern, 1.5 cm apart from each other, on a 5.5 cm diameter filter paper (Whatman No. 1) using Elmer's glue. The filter paper was then fitted to the upper part of a 5.5 cm diameter plastic petri dish and placed upside down. *O. kuvanae* female was, then, introduced into the egg containing-petri dish by switching the inverted bottom of the petri dish, where she was conditioned, with the inverted bottom of the petri dish containing the eggs. This procedure was done to avoid possible aspirator associated trauma that could alter the parasitoid's behavior.

Two sets of observations were made. The

first was to describe the behavioral sequence of *O. kuvanae* host acceptance on gypsy moth eggs. The second set of observation was made to examine the influence of oviposition experience on the efficiency of following ovipositions. For the second set of observations, the time required for the phase of acceptance behavior at each of the three eggs was checked with stopwatch. The female showing no response to the eggs up to 20 minutes since transferred into the petri dish containing eggs, were replaced with a new female. The behavioral bioassays were carried on 1~3 p.m. everyday in a laboratory under 16L : 8D photoperiod, $25 \pm 1^\circ\text{C}$ and $60 \pm 10\%$ RH.

RESULTS AND DISCUSSION

Behavioral sequence for oviposition of *O. kuvanae* on a gypsy moth eggs consisted of 11 or more steps.

The typical patterns are as follow:

Searching. Random movement of female *O. kuvanae* with its antennae moving upward and downward successively.

Approach to the egg. When she approaches the approximate site of egg (usually about one body length away), she usually makes a sharp turn toward the egg.

Host contact. She moves on and off the host egg drumming with her antennae (the antennae is held obliquely) and rapidly taps, contacting the egg with the tips of the antennae.

Mounting with antennal drumming. She stays on top of the egg, and then turns clockwise or counterclockwise with her antennal drumming (the antennae are brought to a vertical position and very rapidly tap the egg).

Grasping and ovipositor tapping. The female grasps the egg with her legs and taps

it with the tip of her abdomen. The body is arched and the ovipositor is still covered by its sheath during tapping. The antennal drumming ceases when the female grasps the egg. This step is immediately followed by drilling.

Drilling. Ovipositor is unsheathed and the surface is drilled with turning its ovipositor 180° clockwise and counterclockwise alternately. A parasitoid may stop drilling after a few seconds and return to drumming on a different part of the same egg.

Ovipositor probing. The ovipositor is inserted into the egg and moved around within.

Oviposition. A single egg is inserted into the host through the slender ovipositor and a stalk of the egg is protruded out of the host egg shell after the successful oviposition.

Withdrawal. Ovipositor is withdrawn from the host and ensheathed.

Post antennal drumming. This step occurs for 1~3 seconds immediately after the removal of the ovipositor. It is similar to the antennal response which occurs prior to grasping the host.

Departure. The parasitoid steps off the egg.

Grooming. The parasitoid cleans various body surfaces (head, antennae, thorax, legs, abdomen, genitalia, etc.) by using its legs and mouth parts.

Resting. This is a sedentary phase in which the parasitoid stays in motionless.

Feeding. The feeding on the host egg juice occasionally occurs without oviposition after the egg is pierced.

The sequence, from "approach to egg" through "departure", described above always occur for successful oviposition. However, the sequence may be interrupted at any point, with the parasitoid reverting to "searching" or digressing to "grooming" or "resting" (Fig.

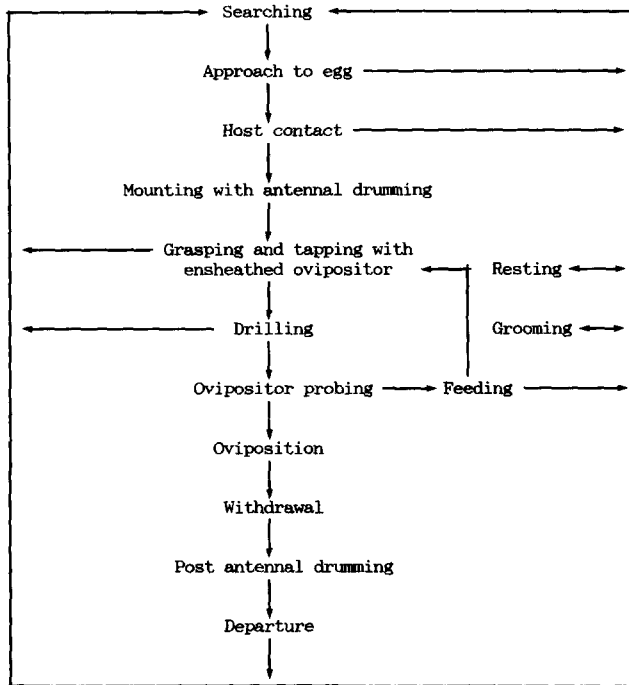


Fig. 1. Schematic presentation of the host-acceptance behavior of *O. kuvanae* toward egg of gypsy moth *L. dispar*.

1). Once the sequence is interrupted, the process must begin again with "searching".

Schmidt(1974) mentioned that "grooming" and "resting" probably were required to maintain the integrity in the parasitoid *Camponotus sonorensis* (Cameron). He also defined these phases as auxiliary phases because they do not contribute directly to the host acceptance process. Feeding behavior of *O. kuvanae* was observed in this study although it was rare. Once the parasitoid assumed feeding activity, it did not stop until several repetitions of drilling or probing and feeding were performed. The parasitoid entered directly into "graping and ovipositor tapping" phase soon after feeding was finished as shown in fig. 1. It was reported that many species of Chal-

cidoids show host feeding behavior. It may play a role as nutritional source for egg maturation (Bartlett 1964). Egg stalk was found on parasitized host egg. Superparasitoidism was not observed although *O. kuvanae* frequently contacted already parasitized egg during the petri dish bioassay. It visited already parasitized eggs after oviposition but it always moved away from the egg soon after antennal drumming on the parasitoid egg stalk. The stalk or some other chemicals left during oviposition may function to help the recognition of already parasitized egg. However, when the parasitoid was confined with eggs for more than one day, several egg stalks were found on one egg, but always only one wasp was emerged from the host indicating

superparasitism. This corresponds to Vinson's report (1977) that while host marking pheromones are important in reducing superparasitism, many parasitoids may still oviposit on a host several times, depending on host availability and other condition.

Table 1 shows the time spent in oviposition of *O. kuvanae* as influenced by oviposition experience. The oviposition activity could be divided into four phases: (1) the time before host contact (approach to the egg), (2) contact with antennal palpitation (host contact), (3) mounting, grasping, and tapping with the ensheathed ovipositor (mounting-tapping with ensheathed ovipositor). (4) drilling through ovipositor withdrawal (drilling-withdrawal). The approach to the first host egg is somewhat different from that on the 2nd and third egg; the former represents the steps from initial walking following exposure to egg through approach at egg, and the latter shows the steps, from departure from the first egg following oviposition to an approach at next eggs. The time required for each phase of acceptance behavior in response to three successive eggs was compared. The time required for the approach phase on the second and the third eggs was significantly less than on the first egg. Part of the difference in the approach times to eggs could be explained by the differential distances that the wasp needed to move to the eggs. The approach to the first egg was from a random starting point within the petri dish, whereas the distance to the second and third eggs was usually 1.5 cm away from an egg. Since the petri dish arena was only 5.5 cm in diameter, the difference in distance is not thought to explain fully an approach time to first egg (99.7 sec.) being greater than twice that of subsequent eggs (42.8 and 37.7 sec.). This time difference suggests that *O. kuvanae*

females exhibit success motivated searching defined by Vinson (1977), as the behavior of continuing to search a patch after a host encounter. Beever et al. (1981) demonstrated in tests with host eggs present, that a kairomone reduced the amount of time before contact with the first egg. The time between subsequent eggs, however, was not reduced by the kairomone. This is probably attributable to the intensified search pattern created by the egg itself (Laing 1937). Beevers et al. (1981) demonstrated the same trend for *Trichogramma pretiosum* Riley. Thus, contact with a host egg can contribute significantly to the probability that another egg, located near the first, will be discovered and parasitized.

Our observation revealed that in most cases the approach phase to second egg didn't include an auxiliary phase (resting or grooming). Sometimes *O. kuvanae* did pass through the auxiliary phase making the time required for the approach phase longer. The reduced time for "approach to egg" could be explained partly by appetitive behavior which is motivated by drive for a oviposition. *O. kuvanae*'s approach to egg was comparable to the non-searching movements of *Apanteles marginiventris* (Cresson) which is governed by the appetitive behavior (Loke et al. 1983).

Host contact by *O. kuvanae* with first egg was not always resulted in the mounting and drumming phase. The parasitoid could give up the egg, depending on its condition. The situation, however, was quite different in a parasitoid with prior ovipositional experience. After a short period of antennal contact, the egg is immediately mounted. The contact by the parasitoid on third egg is much shorter than on the second egg (table 1). These results demonstrate that the prior experience of oviposition increases the efficiency of sub-

Table 1. Time spent in various host acceptance activity by female *Ooencyrtus kuvanae* in three successive *Lymantria dispar* eggs

Activity	Mean time (Sec.) \pm SE		
	1st	2st	3rd
Approach to host	99.7 \pm 28a	42.8 \pm 6.5b	37.6 \pm 13b
Host contact	37 \pm 10.3a	1.3 \pm 0.4b	0.2c
Mounting-tapping with ensheathed ovipositor	49.5 \pm 5a	57.3 \pm 7.4a	48.4 \pm 6.1a
Drilling-withdrawal	477 \pm 35.7a	468.4 \pm 70a	341.3 \pm 51.4b
Replication	18	12	10

Means in the same row followed by the same letter are not significantly different (5% level) by Kramer's modification of Duncan's new multiple range test.

sequent oviposition by the parasitoid, which is similar to that demonstrated for *Brachymeria intermedia* Nee by cardé and Lee (1989) and *Cotesia marginiventris* (Cresson) by Democh et al. (1985). The authors reported that experienced females are more active and that the number of contacts with hosts required prior to attack decreases with number of oviposition experiences the parasitoid has had.

O. kuvanae did not spend significantly different time on the three eggs during the third phase (mounting, antennal drumming, and ensheathed ovipositor tapping). It then appears that this phase is not influenced by the previous experience.

The time spent in the ovipositing phase, including drilling through egg departure, decreased gradually from the first, to second and third eggs. The time spent in this phase may be mainly related to physical factor such as thickness and hardness of egg shell. The reduced time spent on three successive eggs is due to the shorter period of ovipositor probing which is believed to play a role on determining whether host is suitable for development of its progeny (Vinson & Iwantsch 1980). Female which already have laid in an egg, begin to oviposit sooner and are more likely to complete oviposition.

Searching or investigative behavior appears

to be influenced by sign stimuli (Lewis et al. 1982, Loke et al. 1983). Although the experiment described in this paper did not examine the chemical nature of the stimuli involved, a kairomone may play an important role in host recognition. Preliminary test showed that glass beads treated with a hexan extract of the gypsy moth eggs released the similar behavior in *O. kuvanae* but untreated control glass beads did not. The hair on the egg mass, sloughed from the abdomen of the female gypsy moth as she oviposited, also functioned to release host searching behavior. The host acceptance behavior by *O. kuvanae* on egg masses was a little different from that on the dehaired eggs. There was no post antennal drumming and more frequent grooming, which is probably due to the hair.

Weseloh(1972) examined the intertree variation in gypsy moth egg parasitization by *O. kuvanae* and suggested that females do not disperse readily from one tree to another, or perhaps that they exploit fully each egg mass before moving to others. The effectiveness of *O. kuvanae* was severely limited by egg mass size directly related to the availability of eggs to it (Crossman 1925). But gypsy moth egg parasitization rates as high as 81.6% were reported by Brown and Cameron (1979).

They observed *O. kuvanae* actively parasitizing eggs in an egg mass not yet completed by the female moth as we did. The success motivated searching characterized by arrestment response to stimuli and the ovipositional may experience related to oviposition efficiency help maximize gypsy moth egg parasitization by *O. kuvanae* especially if she locate the eggs left naked during egg deposition of gypsy moth.

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