

# 空間的構造からみたキツネノボタンハモグリバエとその寄生 蜂Kratochviliana sp.関係に関する研究1

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Ecological Studies on the Relationship between the Ranunculus Leaf Mining Fly, *Phytomyza ranunculi* SCHRANK (Diptera : Agromyzidae) and Its Parasite, *Kratochviliana* sp. (Hymenoptera : Eulophidae) from the Viewpoint of Spatial Structure I. Analysis of Searching and Attacking Behaviors of the Parasite<sup>1</sup>

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The present paper studies how a parasite, *Kratochviliana* sp., of the Ranunculus leaf mining fly searches and attacks its host larvae. When the parasite alights on a Ranunculus leaf, it walks about rather directly until it encounters a mine formed by the host larva. It then searches ingeniously for its host along the mine using its antennae. Simulation models revealed that this characteristic host searching procedure is adaptive for locating leaf mining insects. The parasite seemed to recognize the host and to receive stimuli for inserting its ovipositor into the host from the somewhat swollen surface which the host larva pushes up within the mine. The parasite, after finding the host, often inserted its ovipositor into the host body through the epidermis of the leaf. Sometimes, however, it left the host without ovipositor insertion after roaming over it, or passed it without any reaction. When insertion occurred, the parasite laid an egg in the host or fed on its body fluid after killing the host by injecting venom into its body. In some cases, however, it did not kill the host even when inserting its ovipositor. No eggs were laid in a dead host though the parasite sometimes fed on the host. The parasite attacks mainly the second and the third-instar larvae. On the average a parasite killed 182.3 hosts and laid an egg in each of 103.4 of them during its life span of 22.8 days.

#### INTRODUCTION

As one of the steps to clarify the spatial pattern of attack by a parasite, *Kratochviliana* sp., on a larval population of the Ranunculus leaf mining fly, *Phytomyza ranunculi* SCHRANK, the mechanisms of host searching and host attacking are analyzed with some other problems in the present paper.

Host-searching and host-attacking, of course, from significant parts of the mechanism by which the relationship between the host and the parasite is established in the host

<sup>1</sup> This paper constitutes a part of Doctoral Thesis presented to the Department of Agriculture, Kyoto University by the present author.

habitat. Generally, the parasite seems to find and attack hosts efficiently due to a species-specific searching and attacking pattern (LAING, 1938; DOUTT, 1959). Such a specific searching and attacking pattern must have developed in correspondence with characters of the host. First, the host searching in the parasite concerned has a two-fold structure, i.e., searching for a leaf infested with host larvae and searching for host larvae on the leaf; only the latter is discussed in the present paper. Particular attention is paid to how the parasite characteristically searches for the host on a leaf and how adaptive the searching pattern is to search for the host larva which forms a mine in the leaf. Next, an analysis is made of parasite recognition and attack of the host once the host is encountered on the leaf. These two points, discussed in the present paper, play essential roles in host selection, from which the relationship between the host and the parasite arises. Host selection consists of four steps: (1) host-habitat-finding, (2) host-finding, (3) host acceptance and (4) host suitability (SALT, 1935; DOUTT, 1959). Under this classification, the present paper is mainly concerned with (2) and (3) of the four steps.

*Kratochvilian* sp. is the most predominant among the many larval parasites of the *Ranunculus* leaf mining fly and seems a common parasite species of Agromyzid larvae in Japan.

#### MATERIALS AND METHODS

To obtain adults of the parasite, *Kratochvilian* sp., used in the present experiment, host plants, *Ranunculus glaber* MAKINO, which contained the parasitized host larvae, were collected in the field and kept at 25°C in the laboratory until adult parasites emerged. In the present study female parasites obtained by such a procedure were used during 2 weeks after their emergence, with the exception of their preovipositional period (of about 3 days) because they are relatively active during that period. Also laboratory-reared host larvae and their host plants, *R. glaber*, were used in the course of this experiment. In many of the observations concerned in the present paper, a female parasite was liberated in a transparent plastic cylinder (30 cm high, 17 cm wide) covered with a nylon film over the top, in which one or more leaves infested with host larvae were placed in a bottle with water.

#### RESULTS AND DISCUSSION

##### I. *Host-searching on a leaf by the parasite*

LAING (1938) pointed out that the parasite *Trichogramma evanescens* had a characteristic and efficient pattern of searching for the eggs of the moth *Sitotroga cerealella*. Because the parasite turns continually as it leaves an egg of its host, it covers a great deal more of the area in the vicinity of that host, thereby increasing its chance of encountering any other eggs which may be in the immediate vicinity of the first. The present parasite normally searches for the host larva along the mine formed by the latter, after finding the mine (Fig. 1), though it sometimes found the host regardless of the mine. Therefore, its host-searching on a leaf consists of two processes, i.e., searching for a mine on the leaf and searching for a host along the mine.

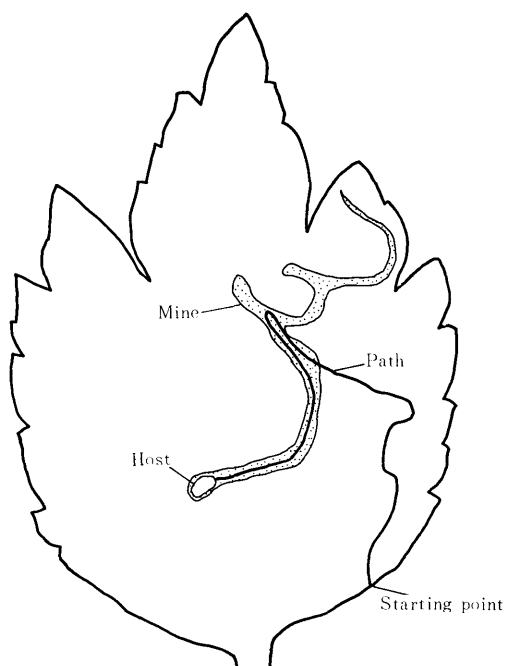


Fig. 1. Path taken by the parasite searching for the host on a leaf.

I-a. *Mine-searching by the parasite.*

To determine the pattern of host-searching by the parasite on a leaf, a parasite was liberated for a while on a leaf infested with a host larva, which was placed on the stage of a universal projector. The parasite was kept in a petri dish inverted over the leaf. Shortly after it settled on the leaf, the petri dish was removed and the position of the searching parasite on the leaf was recorded successively on tracing paper spread on the screen of the universal projector. At the same time, the time spent in each of two processes described above was recorded. Fig. 1 shows the path along which a parasite walked in finding a host. It is obvious in this figure that the parasite, after encountering a mine, searched for the host along the mine. According to Table 1, until it encountered the mine its walking speed on the leaf was about twice as fast as on the mine. Also it walked rather directly on the leaf until it encountered the mine, as in *T. evanescens* (LAING, 1937) or in *Coeloides brunneri* attacking larvae of the Douglas-fir beetle (RICHERSON and BORDEN, 1972). Fig. 2 shows the frequency distributions of relative angles of movement of the parasite within (top) and on (bottom) the periphery of the leaf. This angle was measured by the following method. First, points were dotted successively at intervals of 1.7 mm, i.e., the average distance per second at which the parasite walked on the leaf except for the mine (Table 1), along the path recorded on paper from the starting point to the host. Next, the relative angles between three successive such points were measured in turn in a clockwise direction. In Fig. 2 a relative angle of  $180^\circ$  is "straight ahead", while angles of  $0^\circ$  and  $360^\circ$  are "directly behind". Parasites turned within the periphery of the leaf with a frequency distribution of the relative angle similar to the circular normal one (SINIFF and JESSEN,

Table 1. MEAN WALKING VELOCITY OF THE PARASITE

On parts of a leaf	Velocity (mm/sec.)
Except mines	1.70 ± 0.75
On mines	1.08 ± 0.53

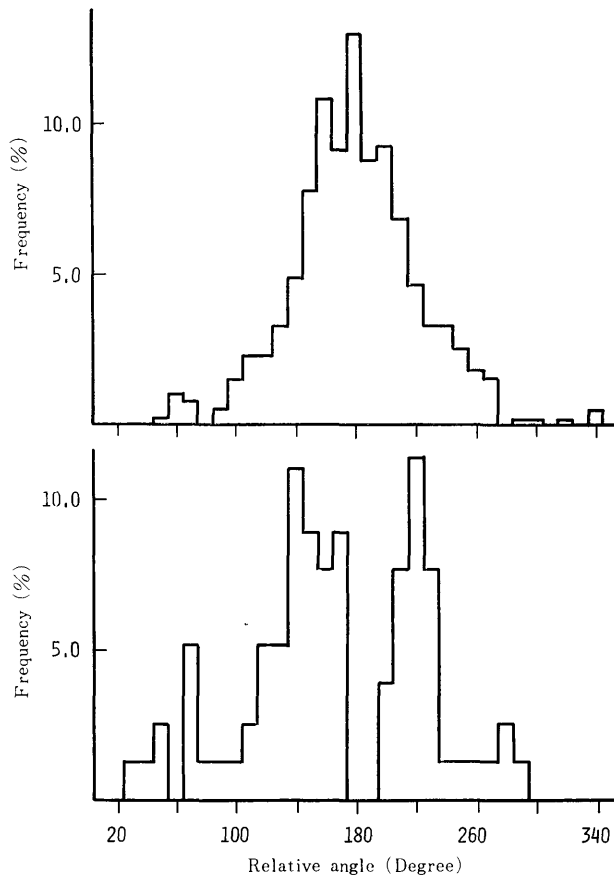


Fig. 2. Frequency distributions of relative angles taken by the parasite within (top) and on (bottom) the periphery of a leaf.

1969) with a mode of 180°. Almost all the angles were distributed between 100° and 260°. These results mean that it does not turn in all directions with equal probability. However, it turned at a much larger angle when it reached the periphery of the leaf (Fig. 2, bottom).

#### I-b. *Host-searching by the parasite*

A parasite which reaches a mine searches for the host larva in a characteristic manner. It walks along the mine beating frequently rhythmically on the mine surface

Table 2. RELATIONSHIP BETWEEN THE POSITION OF ANTENNAE ON A MINE AND THE NEXT ORIENTATION OF THE PARASITE

Position	Parasite turned to the center of the mine	Parasite left the mine
One antenna protruded beyond the mine	176	3
Both antennae protruded beyond the mine	25	31

alternately with each antenna. First, it was examined whether, after encountering a mine, the parasite would always advance along the mine towards the end of the mine at which the host larva was located. Although it walked along the mine towards the host larva in only 35 of 60 observations (about 60%), it seems reasonable to consider that the parasite selects either direction along the mine with almost equal probability ( $X^2 < X^2$  (0.05)). The antenna may play a significant role in this ingenious walk along the mine. In the course of the walk the relation of the positions of both antennae on the mine to the direction towards which the parasite next orientated was analyzed (Table 2). When a part of its body protruded beyond the mine, so that one antenna touched the leaf outside the mine, the parasite changed direction to advance towards the center of the mine. Such direction changes were observed along the mine throughout the course of host-searching, resulting in a zigzag movement along the mine. However, if both antennae touched the mine, the parasite could walk directly along the mine without any direction changes. If the parasite erroneously moved along the mine in the opposite direction from the host larva, sooner or later both antennae protruded beyond the mine because the width of the mine formed by the hatching larva was narrower than the distance between the antennae. In about half of such cases it then returned along the mine, beating the leaf surface very frequently with its antennae, and successfully reached the host larva at last. The above results suggest that the antennae play a significant role in the walk along the mine.

To determine whether the parasite walks exactly along the mine by the aid of its antennae alone, a parasite whose antennae had been removed was liberated on a leaf. In this case it could not walk exactly along the mine for any length of time (Fig. 3, left). The amputated parasite could arrive at hosts in only half of all trials, while an intact parasite could do so in almost all, as mentioned above (Table 3). This result also suggests that the walk along the mine is aided not only by the antennae but also by some other factors. A parasite was liberated on a transparent glass plate (1.5 mm thick) which covered an infested leaf. The glass plate was preliminarily washed well with water. The parasite could not walk along the mine as exactly as the antennaeless parasite (Fig. 3, right). It reached the host larva in a half of all trials (Table 3). This result suggests that sight may also play some role in the walk along the mine.

From the above results it may be concluded that the parasite can walk exactly along the mine by the aid of its antennae and also, in some degree, of its eyes.

#### I-c. *Searching efficiency of the parasite*

First, to study the efficiency of the mine-searching process two kinds of simulation were undertaken based upon some simple assumption.

I-c-1. *Efficiency of mine-searching.* As described above, the parasite searches for a mine

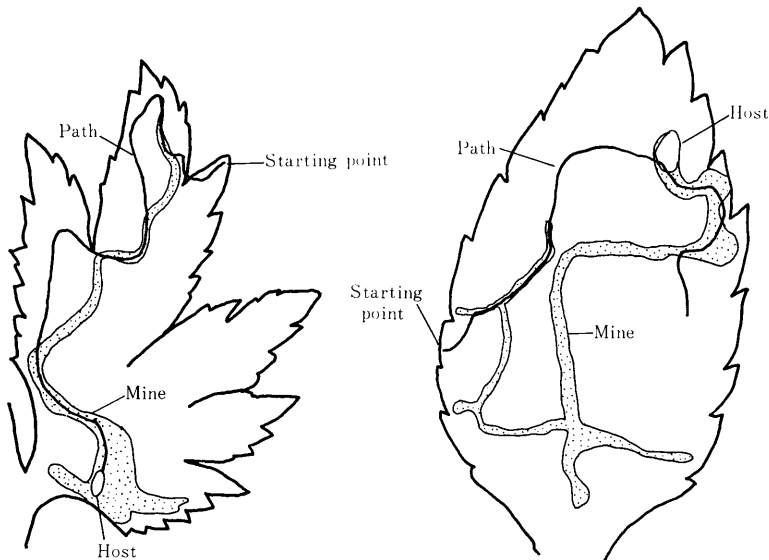


Fig. 3. Paths taken by antenna-less parasite (left) and by parasite liberated on a glass plate covering an infested leaf (right).

Table 3. HOST-FINDING BY INTACT (A) AND ANTENNA-LESS PARASITES (B) AND PARASITES LIBERATED ON A GLASS PLATE COVERING AN INFESTED LEAF (C)

Parasite	Finding hosts	Not finding hosts
A	29	3
B	13	14
C <sup>1</sup>	13	10

<sup>1</sup> In this case "host-finding" means that the parasite reached the point directly above the host on the glass plate.

by walking with a frequency distribution of relative angles similar to a circular normal one within the periphery of a leaf, and with larger relative angles appearing with high probabilities on the leaf periphery (Fig. 2). The parasite was therefore assumed, in Simulation Model 1, to walk with the observed distributions of relative angle within and on the periphery of a leaf. The parasite was also assumed, in Simulation Model 2, to walk with the uniform distribution of relative angle within, and with the observed one, on the periphery of the leaf. Because the parasite walked on the leaf at an average velocity of 1.7 mm/sec., this value was adopted as one step in both simulations. The simulations were manually run, based upon the Monte Carlo method (MIZE and Cox, 1968), by use of observed values of the starting point of the parasite walking on a leaf, the relative angle at the first step and the number of steps required for the parasite to encounter a mine, which was obtained by dividing the length of a path from the starting point to the mine by the average distance per second of the parasite's walk, (referred to as the required number of steps) on each of three leaf maps containing a

Table 4. RESULTS OF TWO KINDS OF SIMULATION OF THE PROCESS OF PARASITE ENCOUNTER WITH A MINE

Simulation	Leaf no.	Leaf size (cm. <sup>2</sup> )	Required no. of steps	No. of trials	No. of encountering hosts	Percent of encountering hosts
1	1	9.33	48	50	30	60.0
	2	9.33	30	50	11	22.0
	3	4.90	31	50	46	92.0
2	1	9.33	48	30	3	10.0
	2	9.33	30	30	0	0
	3	4.90	31	30	16	53.3

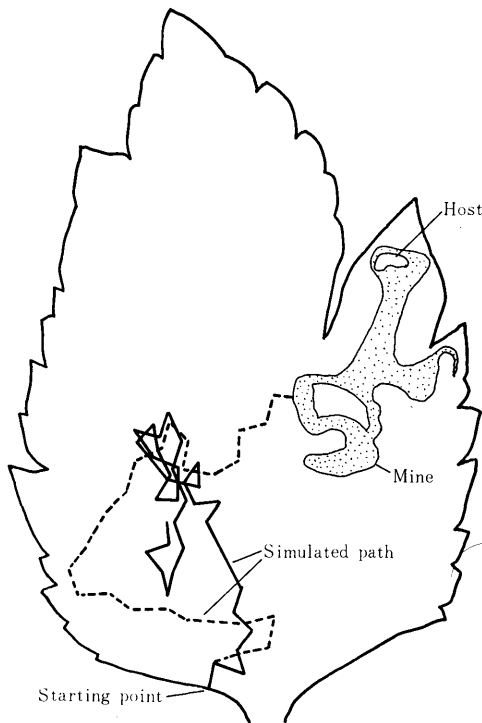


Fig. 4. Simulated movement patterns of the parasite on a leaf based on two kinds of hypotheses. Broken line: Simulation 1; solid line: Simulation 2.

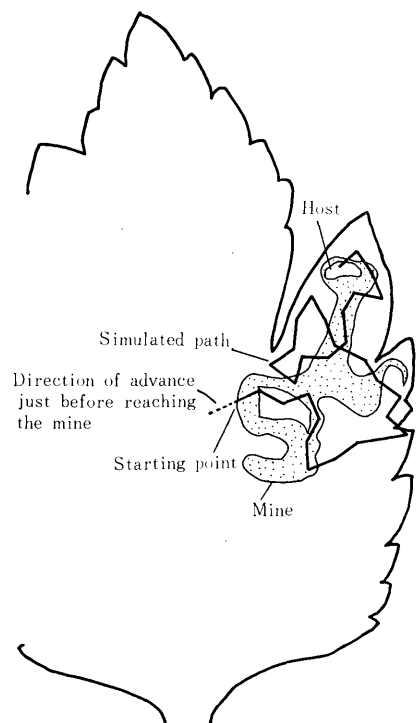


Fig. 5. Simulated movement pattern of the parasite based on the assumption that it searches for the host regardless of the mine. The broken line at the starting point shows the direction in which the parasite advanced just before it encountered the mine.

path and a mine. As shown in Table 4, 50 and 30 trials were performed in Simulations 1 and 2, respectively. Fig. 4 shows examples of simulated movement patterns obtained in Simulations 1 and 2. The path of walk is erratic with a noticeably clumped



Table 5. RESULTS OF SIMULATION FOR THE PROCESS OF HOST-FINDING BY THE PARASITE

Leaf no.	Required no. of steps <sup>a</sup>	No. of trials	No. of finding hosts	Percent of finding hosts
1	36	50	18	36
1	46	50	12	24
1	12	50	0	0
2	56	50	26	52
2	11	50	8	16
3	25	50	9	18

<sup>a</sup> Parasites were liberated on leaves 1 and 2 three and two times, respectively.

pattern in Simulation 2, while it becomes more linear in Simulation 1 as pointed out by SINIFF and JESSEN (1969) in the study of movement patterns in small woodland mammals. Table 4 shows that the parasite encounters the mine more successfully in Simulation 1 than in Simulation 2. It can encounter the mine more frequently if the leaf size is smaller or the required number of steps is larger. These results mean that the parasite can encounter the mine more successfully under conditions such that it can cover more of the area of the leaf.

I-c-2. *Efficiency of host-searching.* The searching efficiency in the host-searching process is discussed by comparing the observed searching behavior with that assumed on certain hypotheses. The latter was simulated assuming that the parasite searches for the host regardless of the mine at the average velocity of 1.7 mm/sec. and with the relative angular distribution which were observed in the first process. The required number of steps in this simulation was obtained by dividing the distance which the parasite actually walked along the mine until it arrived at the host by 1.7 mm (Table 1). Also the point at which the parasite actually encountered the mine for the first time was adopted as the starting point. The direction of advance at the first step was regarded as the direction in which the parasite had advanced immediately before it arrived at the mine (Fig. 5). Table 5 shows the probability (%) that a simulated parasite could find a host in a number of steps equal to or less than the required number of steps in each of six cases. It is natural that the parasite can find the host more successfully if the required number of steps is large compared to the leaf size. Searching efficiencies in the observed and assumed searching behaviors were compared by means of the Loss Index (referred to as LI).

$$LI = \frac{\text{The distance which the parasite actually walked along the mine to find the host}}{\text{The distance along the mine from the starting point to the host}} - 1$$

The numerator, of course, is not smaller than the denominator. If the parasite walks back and forth along the mine, in a waste of effort, LI is larger than zero. If it finds the host along the mine without any loss, LI is equal to zero. Fig. 6 shows the relation between LI calculated in each of six observations used in the simulation and the percentage of host-finding obtained by the simulation in each of those observations. A positive correlation is recognized between these two values ( $r=0.742$ ) because the larger values of LI mean a larger required numbers of steps used in the simulation compared to the distance from the starting point to the host larva along the mine.

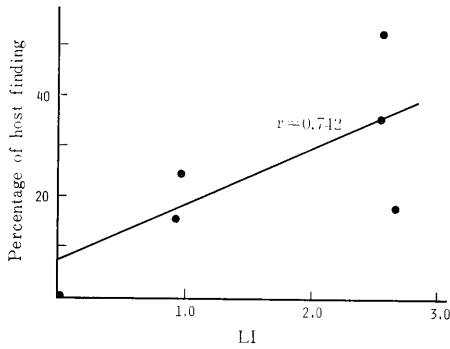


Fig. 6. Relationship of Loss Index (LI) to percentage of host-finding.

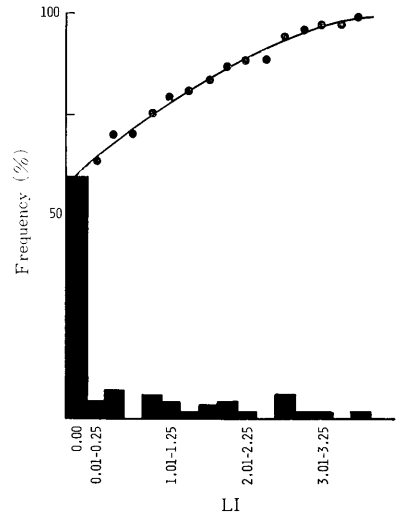


Fig. 7. Observed and accumulated frequency distribution of Loss Index (LI).

On the other hand, Fig. 7 shows an observed frequency and its accumulated frequency in LI. According to this figure, zero in LI occurred with a probability of about 60%. This probability means that the parasite can find the host without any loss in about six out of ten trials. Also LI is larger than 2.0 in some cases. Now, the observed frequency (%) accumulated below a certain value of LI is the probability that the actual parasite can find the host below that value of LI. As the numerator in LI is equivalent to the number of steps required for the parasite to find the host the frequency accumulated below a certain value of LI means the probability that the parasite can find the host in a number of steps equal to or less than the required number of steps corresponding to the numerator in that value of LI. After all, the frequency (%) accumulated below a certain value of LI means the percentage of host-finding by the parasite under the conditions of that value of LI. Therefore, the efficiency of the searching pattern in the present species may be discussed by comparing observed frequency accumulated below a certain value of LI (Fig. 7) with the percentage of host-finding which was obtained in the simulation (Fig. 6), at the same value of LI. The parasite can find the host by the actual searching pattern (60%) about nine times as successfully as by the assumed one (7%). Even if the parasite wastes its effort a little it can find the host by the former pattern three to four times as successfully as by the latter. In the data cited here, however, only 31 observations were made, and also the parasite, after it encountered the mine, walked along it towards the end at which the host larva was located with a probability of about 70%. On the other hand, as described in the first section, the parasite advanced towards this end of the mine in about 60% of 60 observations though the  $X^2$ -test proved that the parasite selected either direction along the mine with almost equal probability. Furthermore, the parasite very rarely left the mine without reaching the host larva, no matter how it walked along the mine. Therefore, it is reasonable to consider that the actual parasite can reach the host with slightly

Table 6. REACTIONS OF THE PARASITE TO THE COLOR AND THICKNESS OF OBJECTS SET IN A MINE<sup>a</sup>

	White	Yellow	Green	Red	Total
Colored paper	2 ov'	2 ov'	2 ov'	2 ov'	8 ov'
0.45 mm thick	1 p	0 p	1 p	0 p	2 p
Nylon film	1 ov'	1 ov'	0 ov'	0 ov'	2 ov'
0.1 mm thick	3 p	3 p	3 p	2 p	11 p
Total	2 ov'	3 ov'	2 ov'	2 ov'	10 ov'
	4 p	3 p	4 p	2 p	13 p
Cotton ball					9 ov'
					3 p

<sup>a</sup> ov': quick sting; p: host visit without any reactions.

lower efficiency than the one simulated above. The above analyses proved that the characteristic pattern of host-searching in the present parasite species is very effective and adaptive to searching for the host larva which forms a mine in a leaf.

## II. *Host recognition by the parasite*

It has been reported that a parasite recognizes its host by a species-specific procedure (DOUTT, 1959). In the present species the female walked to and fro beating the mine surface rapidly with the antennae for some time as soon as it arrived directly above the host in the mine. It then curved the tip of the abdomen perpendicularly to the mine surface and started drilling through the leaf epidermis with its ovipositor. The following experiments were undertaken to determine how the parasite recognizes the host in a mine. A rectangular piece (2—3 mm long, 0.6—1.0 mm wide) of colored paper (white, yellow, green or red; 0.45 mm thick) or nylon film (0.1 mm thick) or a cotton ball of the same size as the host larva was inserted into a mine through the lower epidermis of a leaf. It was ascertained under a microscope that the colored paper raised the upper surface of the mine a little, the cotton ball more so, and the nylon film less so. According to Table 6, the color seems to have no effect on either host recognition or ovipositor insertion. The parasite rarely inserted its ovipositor into a mine containing thin nylon film, but it did, in almost all cases, into those containing colored paper or a cotton ball. These results suggest that the parasite recognizes the host and receives stimuli for ovipositor insertion from the mine surface which is swollen by the host in the mine. In fact, it was frequently observed that the parasite inserted its ovipositor into the mine when it reached a part of the mine surface which was swollen for some other reason. Therefore, the present parasite seems to belong to the group of parasite species which recognize the host not by stimuli originated by the host itself, e.g., odor (BARTLETT and LAGACE, 1961) or infrared radiation (RICHERSON and BORDEN, 1972), but by changes in the host plant condition due to the host, e.g., the texture of the gall (BAIER, 1964).

## III. *Host-attack by the parasite*

On encountering the host, the parasite reacts through one of the following three

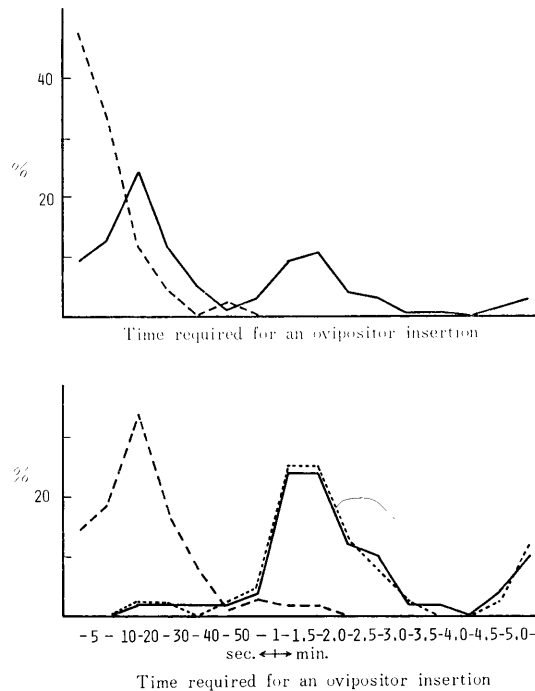


Fig. 8. Frequency distributions of time required for an ovipositor insertion. Top: time required for an ovipositor insertion into living (—) and dead (---) host larvae. Bottom: time required for an ovipositor insertion to kill the host larva (—), to parasitize it (---) or to sting it quickly with the ovipositor (quick sting) (---).

procedures: (1) it inserts its ovipositor into the host body through the leaf epidermis; (2) it leaves the host without inserting its ovipositor into the mine, after roaming over the host; or (3) even if it encounters the host, it passes it without any reaction. In the first procedure the parasite often killed the host by injecting venom into its body (CLAUSEN, 1962) but sometimes did not kill it despite inserting its ovipositor. In the former behavior the parasite lays an egg inside the killed host body, (internal parasitism), or sucks its body fluid at the opening in the leaf epidermis formed by its ovipositor. The latter behavior is called host-feeding (CLAUSEN, 1962). The parasite often performed both these behaviors on the same host. It will also insert its ovipositor into a dead host as well as into a living one. It seems not to lay eggs in dead hosts though it sometimes sucks their body fluids. Such oviposition and host-feeding are combined for convenience under the term "attack" in the present paper.

### III-a. Oviposition

To observe the attacking behavior of the parasite, a female was liberated on a leaf infested with both living and dead hosts. The parasite inserted its ovipositor into dead hosts for less than 20 minutes in almost all cases, but into living hosts for a longer time (Fig. 8, top). The frequency distribution of the time period in the latter case was

bimodal in shape. As pointed out above, the parasite may or may not lay any eggs in a host it has killed, or sometimes may not kill it in spite of inserting the ovipositor. Fig. 8 (bottom) shows the frequency distributions of the time period for ovipositor insertion into living hosts in these three categories. In the former two cases the frequency distributions agree well with each other and correspond quite well with the second mode in the frequency distribution in Fig. 8 (top). On the other hand, the pattern of the frequency distribution in the latter case corresponds well with the first mode in the bimodal frequency distribution. Oviposition insertion for a short period is called a "quick sting", through which the parasite seems to test the host suitability for oviposition (QUEDNAU, 1967). Therefore, the bimodal frequency distribution in Fig. 8 (top) reflects the effects of the quick sting and host killing. As the parasite almost always inserts its ovipositor into the host less than three times for egg laying (Fig. 9, left), it often stays on the host for 1 to 3 minutes and sometimes for longer than 7 minutes (Fig. 9, right).

### III-b. *Host-feeding*

Host-feeding consists of three processes, i.e., ovipositor insertion and withdrawal and sucking the host body fluid. Fig. 10 (A) shows the frequency distribution of time required in a set of these processes. Fig. 10 (B) shows the frequency of feeding by the parasite until it left the host. The parasite fed more frequently on living hosts than on dead ones. In some cases the parasite fed on the host more than twenty times before learning, regardless of whether the host was living or not. Fig. 10 (C) shows the frequency distribution of times spent in feeding on the host until the parasite left it; (the time period includes resting time). On the whole, the parasite tends to stay longer for host-feeding on a living host than on a dead one.

### III-c. *Attacking behavior and developmental stages of the host*

To determine how differences in the developmental stage of the host affect the attacking behavior of the parasite, a parasite was liberated on a leaf infested with living hosts in different instars. Parasites which had already attacked hosts several times shortly before the experiment were used, because a parasite isolated from hosts for a day has an extremely high probability of attacking the first host it finds when liberated on

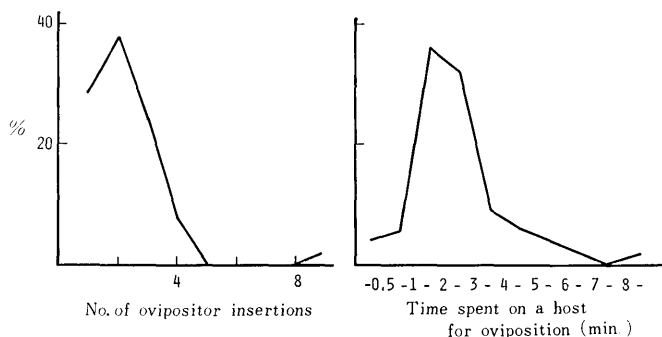


Fig. 9. Frequency distributions of number of ovipositor insertions (left) and of time spent on the host (right) for egg laying until the parasite left the host.

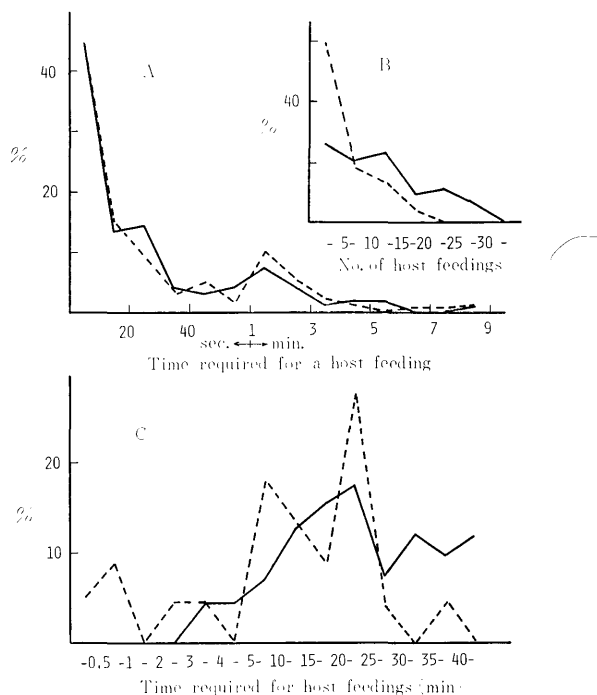


Fig. 10. Frequency distributions of time required for one host-feeding (A), number of host-feedings (B) and total time required for host-feedings (C) until the parasite left the host.

Table 7. NUMBER OF OBSERVATIONS IN THREE CATEGORIES OF VISIT OF THE PARASITE TO HOST LARVAE IN DIFFERENT INSTARS<sup>a</sup>

Host instar	No. hosts used	No. host-visits with attack	No. host-visits without attack	No. host visits without reaction
I	50	1 ( 2.0)	31 (62.0)	18 (36.0)
II	21	5 (23.8)	10 (47.6)	6 (28.6)
III	50	46 (92.0)	4 ( 8.0)	0 ( 0 )

<sup>a</sup> Numbers in parentheses indicate the percentages of parasite in respective visit.

the leaf. In this experiment parasites which found hosts performed three kinds of behavior, as shown in Table 7. In the table, "visit without reaction" means that the parasite left the host without inserting its ovipositor into the mine. The parasite rarely attacked first-instar larvae, but performed quick stings at best. This is attributed to the fact that the parasite has a lower chance of locating first instar larvae because the mines they form are narrower than the distance between the antennae of the parasite and the larva does not raise the mine surface sufficiently for the parasite to receive stimuli to insert the ovipositor into its body. On the other hand, the parasite attacked almost all of the third-instar larvae and some of the second-instar larvae within the limits

Table 8. PERCENTAGE MORTALITY AND PARASITISM IN HOST LARVAE IN DIFFERENT INSTARS<sup>a</sup>

Host instar	No. of hosts visited	No. of hosts killed	No. of hosts parasitized
I	21	3 (14.3)	0 ( 0)
II	72	44 (61.0)	16 (38.6)
III	128	79 (61.7)	49 (62.0)

<sup>a</sup> The numerals in parentheses show the percentage mortality and parasitism. The percentage of parasitism is calculated as (No. of parasitized hosts)/(No. of killed hosts) × 100.

of this experiment. This tendency in the relation of the attacking behavior to the developmental stage of the host corresponds well with observation in the field.

To determine how many of the attacked hosts are actually parasitized, on around the tenth day after the attack, the attacked host was examined for parasite eggs (Table 8). In this examination the ratio of attacked hosts was very different from those obtained in the above experiment; this difference seems due to a difference in the activity of adult parasites used. Table 8 shows that about 60% and about 50% of attacked hosts were parasitized in the third and in the second instars, respectively. The first-instar larvae were not parasitized at all within the limits of this experiment. Some first-instar larvae were killed by mutilation due to frequent quick stings. Close relationships between the attacking behavior and the developmental stage of the host were also observed in *Metaphycus helvolus* parasitizing the black scale, *Saissetia oleae*, (DEBACH, 1943) and in *Encarsia formosa* parasitizing the greenhouse whitefly, *Trialeurodes vaporariorum*, (BURNETT, 1962). The former parasite species killed more large-sized scales due to parasitism and more small-sized ones due to host feeding. The latter species killed more large-sized larvae due to parasitism and more small-sized ones due to unknown factors. BURNETT (1962) suggested that a large number of thrusts by the ovipositor resulted in mechanical injury and death to the host larva. In the present species, the difference between the numbers of killed hosts and of parasitized hosts in Table 8 is the number of hosts killed by host-feeding. This table shows that the third-instar larvae were more frequently parasitized and the second-instar ones, reversely, were more frequently fed on. Such a tendency in the present species agrees well with the preceding two species. Total parasite-caused mortality in the present host larvae was almost equal in the second- and third-instar larvae, as in the greenhouse whitefly larvae, though the mortality in larvae of the black scale increased with their size.

#### III-d. Host discrimination and oviposition restraint

Generally, in the field this parasite parasitizes a host larva singly. This observation suggests that this parasite species can distinguish between unparasitized and parasitized hosts and has a remarkable ability to limit laying to not more than two eggs per host. To determine how this parasite species distinguishes between unparasitized and parasitized hosts a female parasite was liberated on a leaf having both living hosts and others killed at different times (immediately, 1 or 2 days) before the experiment. The ratio of ovipositor insertion into the killed hosts was about half that into the living hosts regardless of the time lapse after the hosts were killed (Table 9). The parasite rarely attacked the killed hosts, compared with the living hosts: 1 or 2 days after they were killed, they were attacked about half as frequently as the living hosts. The para-

Table 9. NUMBERS OF OBSERVATIONS OF PARASITISM (ov), HOST-FEEDING (f), QUICK STING (ov'), AND HOST VISIT WITHOUT ANY ATTACKS (p) WITH TIME LAPSE AFTER THE HOSTS WERE KILLED

Time	Types of behavior	Living hosts	Dead hosts
Shortly after death	ov	26 (0.52) <sup>a</sup>	0 (0.05) <sup>a</sup>
	f	17 (0.63) <sup>b</sup>	7 (0.29) <sup>b</sup>
	ov'	9	30
	p	31	92
1 day after death	ov	9 (0.27) <sup>a</sup>	0 (0.16) <sup>a</sup>
	f	5 (0.45) <sup>b</sup>	13 (0.23) <sup>b</sup>
	ov'	9	6
	p	28	58
2 days after death	ov	4 (0.24) <sup>a</sup>	0 (0.13) <sup>a</sup>
	f	1 (0.39) <sup>b</sup>	5 (0.18) <sup>b</sup>
	ov'	2	2
	p	11	31

<sup>a</sup> The figures in parentheses show the ratios of no. of attacks to no. of visits, i.e.,  $(ov+f)/(ov+f+ov'+p)$ .

<sup>b</sup> The figures in parentheses show the ratios of no. of ovipositor insertions to no. of visits, i.e.,  $(ov+f+ov')/(ov+f+ov'+p)$ .

Table 10. PARASITE ATTACKS ON HEALTHY HOSTS AND ON HOSTS KILLED BY A STING THROUGH LOWER EPIDERMIS OF A LEAF<sup>a</sup>

Hosts	Host No.	No. of observations of attacking behavior			
Healthy hosts	1	2 ov	2 f	2 ov'	3 p
	2	2 ov	2 f	10 ov'	0 p
	3	1 ov	0 f	4 ov'	0 p
	4	1 ov	0 f	1 ov'	1 p
	5	1 ov	1 f	3 ov'	3 p
Killed hosts	1	0 ov	1 f	4 ov'	1 p
	2	0 ov	1 f	0 ov'	2 p
	3	0 ov	0 f	3 ov'	0 p
	4	0 ov	1 f	0 ov'	3 p
	5	0 ov	0 f	5 ov'	5 p
	6	0 ov	0 f	3 ov'	1 p
	7	0 ov	0 f	3 ov'	1 p

<sup>a</sup> ov: ovipositor insertion for a long time; f: feeding; ov': quick sting; p: host visit without reaction.

site fed on but did not lay any eggs in the killed hosts, and seemed to feed less on hosts which it had previously fed on once. These results suggest that the parasite can distinguish the hosts it visits without ovipositor insertion to some degree and with it to a considerable degree. Many parasite species can detect the host by marks they leave on its body (spoor effect) (SALT, 1937; FLANDERS, 1951; WILSON, 1961; HOKYO et al, 1966). In the first step of the host detection described above, however, the lower rate of ovipositor insertion by the present species into killed hosts than into living ones



Table 11. NUMBERS OF HOSTS ATTACKED BY A PARASITE IN ITS LIFE SPAN AND DAY<sup>a</sup>

Parasite No.	No. hosts tested	Life span <sup>b</sup>	Life span		Daily mean	
			No. hosts killed	No. hosts parasitized	No. hosts killed	No. hosts parasitized
1	372	26	158 (42.5)	79 (50.0)	6.1	3.0
2	195	15	95 (48.7)	68 (71.6)	6.3	4.5
3	416	25	206 (49.5)	95 (46.1)	7.9	3.7
4	459	32	287 (62.5)	148 (51.6)	9.0	4.6
5	246	16	167 (67.9)	127 (76.1)	10.4	7.9
Mean		22.8	182.6 (54.1)	103.4 (59.1)	8.0	4.8

<sup>a</sup> The figures in parentheses show percentage mortality and parasitism. The latter is calculated as (No. of parasitized hosts)/(No. of killed hosts) × 100.

<sup>b</sup> Days.

may be due not to the spoor effect but to the fact that the killed host larvae cause less swelling of the covering mine surface, due to the loss of extension in their bodies. In the second step the parasite may detect with its ovipositor a condition in the killed hosts which differs from that in the living ones, as in other species (DETHIER, 1947; NARAYANAN et al., 1954; WYLIE, 1965, 1970, 1971; ARTHUR, 1972).

To investigate what condition in the dead hosts the parasite can detect, a parasite was liberated on a leaf infested with some living hosts and some killed artificially with a sting through the lower epidermis of the leaf. Though the parasite quickly inserted its ovipositor into killed hosts many times, it did not lay any eggs in them (Table 10). These results suggest that the present parasite detects with its ovipositor not some substance previously injected into the host body by the ovipositor but some condition in the dead host which differs from that in the living one.

### III-e. *Attacking capacity of the parasite*

To determine the number of hosts the parasite attacks during its life span, each of five female parasites were liberated on several leaves infested with living hosts. These leaves were replaced by fresh ones infested with living hosts every day until the parasite died (Table 11). A parasite killed 182.6 host larvae during its life span, or 8.0 per day, and of these it parasitized 103.4 or 4.8 per day on an average. That is, 59.1% of killed host larvae were parasitized.

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