Learning of Plant's Contact Chemicals by
the Egg-Larval Parasitoid, *Ascogaster*
reticulatus WATANABE
(Hymenoptera: Braconidae)¹

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The females of *Ascogaster reticulatus* find the host egg mass using host kairomones located in wing scales and on the egg mass surface. Parasitoids were found to learn plant chemicals associated with the host egg mass. Each female was conditioned by releasing in a Petri dish with a host egg mass and the plant extract, and her behavior was observed until oviposition. Then the wasp was tested by releasing into a dish with only the plant extract. The wasp responded to the treated area with antennal palpation and walking along the extract line. Responses increased with corresponding increases in conditioning time and the memory remained for 24 h but disappeared in 48 h.

The patterns of parasitoid foraging are generally accepted as being determined by the interactions of genetic, physiological, environmental and experiential factors (VET et al., 1995). Compared to egg or pupal parasitoids of phytophagous insects, larval parasitoids may have a better chance to learn plant-derived factors for foraging hosts (VET et al., 1995), because chemicals are easily released by the action of host larvae feeding on plants. Learning of plant-derived chemicals for these parasitoids can solve the reliability-detectability problem (VET et al., 1991) and easily leads the parasitoids to their host. In fact, most of the examples of associative learning are on larval parasitoids (TURLINGS et al., 1992). However, even if to a lesser extent the presence of host eggs or pupae are related to specific plant cues, egg or pupal parasitoids could benefit from learning information about

the plants on which hosts are located.

Ascogaster reticulatus WATANABE is an egg-larval parasitoid of the smaller tea tortrix, *Adoxophyes* sp. (Lepidoptera: Tortricidae). The females find host eggs using host kairomones located in wing scales scattered around the host egg mass (KAINOH et al., 1990) and the egg mass surface kairomone stimulates oviposition behavior (KAINOH et al., 1982). In order to examine the learning ability of this species with respect to plant chemicals, I took mulberry leaves which are not a host food plant of *Adoxophyes* sp., and prepared an extract with ethanol for the following experiments.

MATERIALS AND METHODS

Insect cultures. The parasitoids and their hosts were from our stock culture based on the rearing method of KAINOH and TAMAKI (1982) and KAINOH (1986). Emerged adults were sexed and 10 to 20 of each were kept in a container (2 l) in which honey and water were provided. The rearing of insects and bioassays were conducted at $25 \pm 1^\circ\text{C}$ and $50 \pm 20\%$ RH with a 16L:8D photoperiod. The training and bioassay were conducted in the latter half of the light period when the parasitoids' searching activity is high (KAINOH, 1986).

Extract. Mulberry leaves (10 g) were homogenized in 10 ml EtOH with a mortar and a pestle, and the supernatant was subsequently filtered with a filter paper (Advantec, No. 2). The filtrate was kept in a refrigerator (5°C) until use.

Conditioning. In order to condition the wasps with the extract, we modified the method of KAINOH et al. (1991). Five microliters of the sample were applied in a 9-cm-long straight line along a red line drawn on outside of a 9-cm glass Petri dish across the center. After the solvent was air-dried, a host egg mass (1- to 5-day-old) was placed at the center and a 3- to 4-day-old female parasitoid was released into the dish, which was then capped. Her behavior was observed for a period of up to 3 min. After ca. 1 min from the start of oviposition, which was enough time for one egg-laying (KAINOH et al., 1982), the wasp was gently separated from the egg mass with an insect aspirator. These wasps were placed in a container (2 l) for 30 to 60 min before being used in the next bioassay.

Bioassay of learned response. The bioassay to measure the learned response to the plant extract was similar to the conditioning method. After the solvent had evaporated, a female parasitoid was introduced

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into the dish and her behavior was observed for 3 min. I measured the approximate total distance of intensive walking along the treated line with her characteristic antennating response.

Experimental procedure. Each of five females was first tested only with plant extract as a control. The female was then conditioned to the extract with the presence of an host egg mass, and was then subjected to bioassay. This conditioning followed by bioassay was repeated 3 times. Intervals between conditioning and bioassay were 30–60 min. On the next day (24 h after the initial training) and 2 days after (48 h), they were subjected to additional bioassays.

RESULTS AND DISCUSSION

When a female *A. reticulatus* was introduced into a Petri dish with leaf extract in the first bioassay, individuals did not respond to the treated area and passed over it. When the female walked close to a host egg mass placed at the center of the dish in the first training, she antennated the egg mass surface and began to oviposit. After the first training, parasitoids responded slightly to plant extract (avg. = 2 cm, Table 1). They walked a longer distance (avg. = 6.3 cm) after the 2nd training, but the difference was not significant (TUKEY test, 5%, Table 1). After the 3rd training, the wasp walked along the treated line for 21 cm (avg.), which was significantly further than in the 2nd bioassay (TUKEY test, 5%, Table 1). In *Microplitis croceipes*, when the experience was repeated 2 or 4 times, they responded to the odor of feces they had experienced (ELLER et al., 1992). The *A. reticulatus* females which had experienced oviposition 3 times without plant extract did not respond to the extract (KAINOH and HONDA, unpublished), which showed that this response was due to learning but not to "sensitization" (TURLINGS et al., 1992).

The fact that the female parasitoid responded to chemical(s) from a novel plant on which their host insect does not occur shows that *A. reticulatus* can learn novel plant chemical(s) which is not normally associated with host eggs. Once *A. reticulatus* learned the plant chemical(s) after 3 experiences, they responded to it after 24 h to a lesser degree (avg. = 5.3 cm), and did not respond at all after 48 h (Table 1). This short retention of memory may partly be due to the 5th bioassay without host on the next day, which was negative conditioning for females. In the case of *Microplitis croceipes*, a larval parasitoid of *Heliothis zea*, females responded to the odor of host frass 48 h after learning (LEWIS and MARTIN, 1990). Also in *Diaeretiella*

Table 1. Effect of conditioning with mulberry leaf extract associated with host egg mass on intensive walking response of female *A. reticulatus*

Bioassay number	Distance of walking on treated line (cm) ^a
1 (before Cd) ^b	0
2 (after 1st Cd)	2.0 ± 1.4 a
3 (after 2nd Cd)	6.3 ± 2.5 ab
4 (after 3rd Cd)	21.0 ± 9.0 b
5 (24 h later)	5.3 ± 2.4 ab
6 (48 h later)	0

^a Mean ± S.E. ($n = 5$). Means followed by the same letters are not significantly different (5%) as determined by TUKEY test.

^b Cd, conditioning.

rapae, plant experience lasted more than 2 days (SHEEHAN and SHELTON, 1989).

Response of experienced *A. reticulatus* females to plant extract was similar to the response when exposed to a glass surface treated with host egg mass kairomone. This behavior is thought to be in response to a contact chemical(s) in the plant extract, since the parasitoids seemed to show a random walking pattern before contacting the extract-treated area when released in a Petri dish. This is a unique example for parasitoids to learn contact plant chemical(s), since most of the work on parasitoid learning using olfactometers or wind tunnels has been restricted to volatile cues associated with the host (TURLINGS et al., 1992). The ability to learn plant contact chemical(s) associated with oviposition in *A. reticulatus* may help the female to make quick decisions as to whether to search for potential host or not after landing on a specific plant.

Currently, experiments are being conducted using the tea plant, which is one of the host plants of *Adoxophyes* sp. to test the ability of *A. reticulatus* to discriminate between different plant species.

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Temporal Regulation of *Bombyx mori* (Lepidoptera: Bombycidae) Nucleopolyhedrovirus DNA Polymerase Gene Expression¹

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Nucleopolyhedroviruses (NPVs), members of the family Baculoviridae, contain a double-stranded, covalently closed circular DNA genome of 80 to 200 kbp (ADAMS and MCCLINTOCK, 1991). NPV genes have been shown to be expressed in an ordered cascade in infected cells, and can be divided into four temporal classes, that is immediate early, delayed early, late, and very late genes. By definition, immediate and delayed early genes are expressed prior to the onset of viral DNA replication, while late and

very late genes are expressed after or concurrently with viral DNA replication. Although the role of NPV DNA replication in the temporal regulation of viral gene expression is largely unknown, studies using a DNA negative temperature-sensitive mutant and inhibitors of viral DNA synthesis suggest that expression of some of the late and very late genes is dependent upon viral DNA replication (KELLY and LESCOTT, 1981; GORDON and CARSTENS, 1984; RICE and MILLER, 1986). Analysis of the regulation mechanism for viral DNA replication will thus greatly facilitate our understanding of a possible relationship between viral DNA replication and coordinated viral gene expression.

Details of the NPV DNA replication mechanism are not well understood. Recent studies in *Autographa californica* NPV (AcNPV) identified six viral genes as essential and three viral genes as stimulatory for viral DNA replication (KOOL et al., 1994; LU and MILLER, 1995). DNA polymerase gene (*dnapol*) is among those genes that are involved in viral DNA replication, and possibly plays an important role in the determination of host range and pathogenicity of the viruses (cf., LIU and CARSTENS, 1995). Viral *dnapol* has been cloned and sequenced, and its transcriptional analysis has been performed in NPVs from *A. californica*, *Lymantria dispar*, *Bombyx mori*, and *Choristoneura fumiferana* (TOMALSKI et al., 1988; BJORNSEN et al., 1992; CHAEYCHOMSRI et al., 1995 a; LIU and CARSTENS, 1995). *B. mori* NPV (BmNPV) *dnapol*, as with AcNPV

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