Ovipositional Behaviour of the Parasitoid Fly, *Exorista japonica* (Diptera: Tachinidae), in the Laboratory: Diel Periodicity and Egg Distribution on a Host

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Ovipositional behaviour, diel periodicity of oviposition and egg distribution on the host of the parasitoid fly, *Exorista japonica* were studied in the laboratory. The hosts provided were larvae of *Mythimna separata*. The rate of adult fly emergence, and the number of eggs destroyed by hosts were also examined when one egg per host was laid on different body segments of a host. When provided with two hosts every 4 h from 22:00 on day 4 for 4 consecutive days, females laid more eggs in the morning and the rate of oviposition reduced with the passage of time towards evening; no eggs were laid during the period of darkness. The mean number of mature eggs, determined by dissection, showed a similar tendency to oviposition with a peak just after the end of the dark period and a subsequent decrease, although the decline was not statistically significant. When hosts were provided for the flies at 08:00 and 20:00 for 60 min each, no significant difference was found between the number of ovipositions at each time. Therefore, there is the possibility that the diel periodicity of oviposition of *E. japonica* was influenced by the number of mature eggs, which accumulated mainly during the night. The eggs laid on a host were concentrated on the head and thoracic segments when 1, 2 or 3 eggs were laid per host. When one egg per host was laid on different body segments, the rate of adult emergence followed a U-shaped curve, being lower on the 6th and 7th abdominal segments, which may have been mainly due to the host’s ability to remove or destroy eggs laid on its body. These results suggested that *E. japonica* females selectively oviposit on certain parts of the host’s body, an adaptation to the defence behaviour of the host.

*Key words:* Tachinidae, *Exorista japonica*, ovipositional behaviour, diel periodicity, egg distribution

**INTRODUCTION**

Parasitoids are considered to be very important agents for biological control of agricultural pests and the family Tachinidae is considered to contain potential biological control agents (Greathead, 1986). To achieve successes in biological control, it is very important to conduct fundamental studies on biology and ecology before going on to use the parasitoids as biological control agents (Grenier, 1988). However, tachinids have received relatively little attention in general parasitoid ecology compared to parasitic wasps (Belshaw, 1994).

*Exorista japonica* Townsend lays heavy-shelled macrotype eggs on the host cuticle, and the emerged first instar larvae penetrate the host integument after a few days’ incubation (Nakamura, 1994). This fly is often found on noctuids such as the common armyworm *Mythimna separata* Walker, the common cutworm *Spodoptera litura* Fabricius, and the cabbage armyworm *Mamestra brassicae* Linné (Oku and Kobayashi, 1974). Takahashi and Sawaki (1969) reported that *E. japonica* heavily parasitized *S. litura* larvae in the field and was
an important natural enemy. Although early notes on its biology and laboratory rearing methods were made by Nakamura (1994, 1996), there is still a lack of fundamental information on the ovipositional behaviour of E. japonica. This study was therefore conducted to achieve a better understanding of the insect's ovipositional behaviour such as the diel periodicity of oviposition, egg placement on the host, and its survival rate.

MATERIALS AND METHODS

Host and parasitoid. Larvae of M. separata were obtained from a stock culture and reared on an artificial diet according to Hattori and Atsusawa (1980). The E. japonica culture was initiated from parasitized last instar larvae of Hyphantria cunea Drury, collected at the National Institute of Agro-Environmental Sciences, Tsukuba, Ibaraki, in September, 1991 and maintained by the method of Nakamura (1996). A single mated female E. japonica was used for each experiment. The flies were individually caged in plastic cylinders (16 cm dia., 22 cm ht.) and provided with a sugar cube and distilled water. One day old (day 1) last instar larvae of M. separata were used as hosts and were placed in clear, plastic ice-cream cups (9 cm dia., 3.5 cm depth).

Patterns of ovipositional behaviour. To observe the ovipositional behaviour of E. japonica, each of 5 female flies was provided with a host for 6 h on days 3 and 4 in order to gain ovipositional experience, and then its behaviour was videotaped on day 5.

Diel periodicity of oviposition. To investigate the ovipositional activities of females at different times of day, the numbers of ovipositions or mature eggs were recorded as follows:

(1) 4 h intervals. Each of 20 female flies was provided with two hosts from 22:00 on day 4 for 4 consecutive days, both hosts being replaced with new ones every 4 h. Females did not lay eggs in the dark between 22:00 and 06:00 (see below) during the first two days of the experiment, so subsequently hosts were not provided during this dark period.

(2) 12 h intervals. Each of 10 female flies was provided with two hosts twice a day between 08:00 and 09:00, and between 20:00 and 21:00, for 4 consecutive days starting on day 5.

(3) Mature eggs. Since there was the probability that the number of mature eggs affects the diel periodicity of oviposition, five of 25 females, which had been treated as described for experiment (1), were dissected to determine the number of mature eggs at 06:00, 10:00, 14:00, 18:00 and 22:00 on day 5.

Before every experiment, a female fly was offered a host from 10:00 to 22:00 on days 3 and 4 in order to gain ovipositional experience.

Egg distribution on the host and its effect on parasitoid progeny survival. To study on which part of the host's body females laid eggs, the segments on which eggs were laid were recorded. Since host mortality reaches almost 100% when 3 eggs are laid per host (Nakamura, 1994, 1996), a host was offered to each of 40 female flies on days 3 to 14 and removed immediately after 1, 2 or 3 eggs (n = 100, respectively) had been laid per host.

To investigate the effect of egg deposition on different body segments of the host for adult fly emergence, a female fly (as above) was provided with a host and allowed to deposit one egg per host (n = 20 per segment); excess eggs were removed with forceps when necessary. The parasitized host larvae were kept individually in plastic Petri dishes (9 cm dia. × 2 cm depth) with a diet of armyworms and checked daily for eggs which had been destroyed by the host, maggots entering a host, and fly's adult emergence. The parasitized larva was often observed attempting to dislodge or destroy the fly's eggs or maggots with its
mandibles; I therefore examined the eggs on parasitized hosts to determine their fate. Eggs destroyed by hosts in this study were recognized by a clearly visible mark on the egg made by biting. Since it could not be determined whether eggs which dropped off the host had been removed by the host or detached by other factors, these eggs were counted as parasitoids which had not entered a host, which also included unhatched eggs. Whether or not a parasitoid larva had successfully entered a host was confirmed by observing an opening made by the parasitoid.

Every experiment was conducted at 25°C, 60% RH and with a photoperiod of 16L–8D (06:00–22:00 illuminated).

RESULTS AND DISCUSSION

Patterns of ovipositional behaviour

Female flies flew or walked to reach hosts, and sometimes appeared to respond to, and start approaching, hosts from a distance of about 20 cm under the experimental conditions. The time between placing a host in a cage and the fly’s reaching the host varied among individuals; some females responded to a host in 1 min but some took more than 60 min before approaching one. WESELOH (1980) divided ovipositional behaviour of Compsilura concinna into 3 phases including “encounter, examination and attack.” The ovipositional behaviour of E. japonica was similar to that of C. concinna. Once females of E. japonica came within about 5 mm of a host (an encounter), they began “examination” behaviour. This consisted of facing and touching the host with their front tarsi. Sometimes the fly moved completely around the host in one direction, but changes in direction were frequent. If the host was walking, the fly moved with it or chased it. When the host did not move, the fly stood still and faced the host during an examination which sometimes took more than 60 min before an attack occurred. An attack consisted of a rapid sequence of two events: the fly extended its ovipositor and attached an egg to the host cuticle. The fly tried to extend her ovipositor towards a host from the front, rear, and either side of the host’s body, and sometimes pounced on it. After encountering a host, a female laid eggs singly on the host cuticle. She often attacked the same host more than once, with each attack usually being preceded by a period of examination. When touched by a fly, host larvae were often observed to sway their head and thoracic segments and to try to bite the fly. Many reports suggest that host movement appears to increase the fly’s activity (e.g., ADAM and WATSON, 1971; BURKS and NETTLES, 1978; WESELOH, 1980; HEBERT and CLOUTIER, 1990). Similarly, E. japonica laid eggs only when hosts were moving.

After some attacks, female flies showed feeding behaviour in which they extended their proboscis and licked on or around a host. Although host feeding has been reported for many parasitic wasps (e.g., JERVIS and KIDD, 1986) and a tachinid, Eucelatoria bryani (NETTLES, 1987) which larviposits into its hosts, it was not observed in E. japonica. During feeding, females did not attack hosts even though the hosts walked in front of the flies or touched them. There are some reports for parasitic wasps that starved females preferred food to hosts (TAKASU and LEWIS, 1993) or did not show any ovipositional behaviour even when in contact with their hosts (TAKASU and HIROSE, 1991). With E. japonica females, feeding might also have priority over oviposition.

Diel periodicity of oviposition

Diel periodicity of oviposition has been studied in a few other tachinid species; in the
laboratory, Compsilura concinnata did not show any tendency to ward diel periodicity (Weseloh, 1980) but Parasitica silvestris, in the field, laid more eggs in the afternoon (Weseloh, 1976). Bonnetia comta responded to temperature, increasing its reproductive with temperature within a certain range. However, under constant temperature conditions, its reproductive activity increased gradually until midday and then decreased rapidly (Rubink and Clement, 1982). With E. japonica, the mean number of eggs laid per female per 4 h showed a different tendency, with a peak in the morning and a gradual decrease as time passed from morning to night (Fig. 1). Females did not lay any eggs during the dark period between 22:00 and 06:00; a similar phenomenon was observed with B. comta (Rubink and Clement, 1982). The mean number of mature eggs per female was highest just after the end of the dark period and decreased afterwards (Fig. 2), although the decrease was not statistically significant, probably because the number of replicates was not large enough and the number of mature eggs varies in different individuals. However, when hosts were provided for a female only twice a day at 08:00 and 20:00 to equalize the time intervals between ovipositions, no significant difference was found between the two times (Fig. 3, p > 0.05, paired t-test). Therefore, there was a possibility that diel periodicity of oviposition by E. japonica in the laboratory could be influenced by the time which had elapsed since the previous oviposition, which was reflected by the number of mature eggs which accumulated mainly during the dark period of each 24 h. This phenomenon suggests that time intervals between ovipositions rather than diel periodicity should be considered when ovipositional experiments of E. japonica are carried out in the laboratory.

Egg distribution on the host and its effect on parasitoid progeny survival

When female flies were allowed to lay one to three eggs on a host, they frequently oviposited on the head and thoracic segments (Fig. 4). Overall, 69.0, 66.0 and 68.0% of eggs were laid on these segments, and 75.0, 74.0 and 73.7% of eggs were deposited on these

Fig. 1. Mean number of eggs laid per E. japonica female when provided with two larvae of M. separata at different times of day. Vertical lines indicate standard error. Bars with the same letter are not significantly different by Tukey-Kramer's method at the 0.05 level.

Fig. 2. Mean number of mature eggs per E. japonica female when provided with two larvae of M. separata at different times of day. The number of eggs was counted after dissection of the female. Vertical lines indicate standard error. There was no significant difference among the mean numbers of mature eggs by Kruskal-Wallis test at the 0.05 level.
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Fig. 3. Mean number of eggs laid per *E. japonica* female when provided with two larvae of *M. separata* at 08:00–09:00 (AM) and 20:00–21:00 (PM). Vertical lines indicate standard error. There was no significant difference between AM and PM by paired t-test at the 0.05 level.

Fig. 4. Oviposition frequency of *E. japonica* on different body segments of *M. separata* larva when 1, 2 or 3 eggs were laid per host. A, abdominal segment; T, thoracic segment.

Fig. 5. Percentage of adult emergence per egg, eggs destroyed by the host and parasitoids not entering the host when one egg was laid on a different body segment of *M. separata* larva. A, abdominal segment; T, thoracic segment.
and the 10th abdominal segment (A10), when clutch sizes were 1, 2 and 3, respectively. Kumasawa (1933) reported that 70.7% of 188 eggs of E. japonica were placed on the head and thoracic segments of 105 cherry caterpillars (Phalera flavescens) in the field, although the clutch size in each case was not reported.

When one egg was laid on a host, survival of the egg varied among different parts of the host's body (Fig. 5). Host larvae attempted to dislodge or destroy eggs and newly emerged maggots with their mandibles. Egg destruction by hosts was observed only on the abdominal segments, not on the head and thoracic segments (Fig. 5). Since all eggs which disappeared were treated as parasitoids not entering a host, the same as other unhatched eggs, there is the possibility that eggs dislodged by host mandibles might have been counted as eggs which had disappeared, and the actual proportion eggs destroyed by a host could be higher than my estimate. The proportion of parasitoids not entering a host was relatively high on the head, but was highest on segment A6. Newly emerged maggots penetrate the host cuticle immediately in front of the egg (Nakamura, 1994); however, maggots emerging from an egg deposited on the head cannot pierce the head capsule of the host (except just after the host has moulted), so they must crawl to the first thoracic segment (T1) to get into the host. Due to some mortality factors operating during this process, the number of parasitoids failing to enter a host is relatively high at the head end. Graphs of the proportion of eggs destroyed by a host and of the proportion of parasitoids not entering a host show similar curves, although the former decreases on segments A5 and A6 rather than the latter. This phenomenon suggested that aggressive behaviour of the hosts may be effective against eggs and maggots as part of the host’s self-defence when eggs are laid on the abdominal segments.

As a result, the survival rate of the parasitoids increased when eggs were laid on the head, thoracic segments, or A10, and decreased when eggs were laid on A6 or A7. Female flies tended to concentrate ovipositions on the head and thoracic segments. There is the possibility that this concentration resulted from the fly’s behavioural response to deposit eggs on particular parts of the host’s body which moved more actively and frequently than other parts, since host movement appears to increase the fly’s activity (e.g., Adam and Watson, 1971; Burks and Nettles, 1978; Weseloh, 1980; Hebert and Cloutier, 1990). In some other tachinids that lay their eggs directly on the host, considerable concentration exists in the distribution of their eggs on the host body, and the parasitoids select distinct sites of a host for egg deposition (Chauthani and Hamm, 1967; Herrebout, 1969; Martin et al., 1989). Females of E. japonica showed similar behaviour, selectivity depositing eggs on particular segments of the host; this behaviour could be a counter strategy against the host’s defensive mechanisms.

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