Clutch Size Regulation and Host Discrimination of the Parasitoid Fly, *Exorista japonica* (Diptera: Tachinidae)

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The ability of *Exorista japonica* females to regulate clutch size in response to host density was investigated in the laboratory. On the assumption that the flies can estimate host density by the frequency of host encounters, the effect of time interval between host encounters and the number of hosts offered on the oviposition decision of female flies was studied, and host discriminatory ability was examined. A female fly was offered a cup (considered as a patch) containing host(s) at various time intervals. Clutch size decreased as the time interval between host encounters was shortened and as host density per patch increased. The number of eggs laid per patch during a single attack increased with increases in the time interval between host encounters was lengthened and as host density per patch increased, even at a constant time interval. Females discriminated between unparasitized and parasitized hosts when the parasitized host contained 10 eggs, but did not discriminate when the parasitized host had only 5 eggs. Moreover, the time interval between host encounters affected host discrimination. Females oviposited equally on an unparasitized and a parasitized host with 10 eggs when they encountered hosts at 24 h intervals, but they laid fewer eggs on the parasitized host when the interval between host provisions was 1 min. The results indicated that *E. japonica* females not only regulated clutch size in relation to host availability but also discriminated between unparasitized and parasitized hosts.

*Key words:* *Exorista japonica*, ovipositional behaviour, host discrimination, clutch size, Tachinidae

**INTRODUCTION**

The clutch size produced by a female parasitoid insect largely affects her reproductive success through survival rate and the body size of her progeny. Therefore, when a parasitoid female encounters a host while foraging, she must make very important decisions, such as how many eggs to lay per host, and in what sex ratio (Waage and Ng, 1984). These decisions are affected by many factors including the rate of host encounter, the size and stage of encountered hosts, and whether or not the hosts are already parasitized. There have been models which predict how a parasitoid female should behave so as to maximize its fitness, and there have also been observations which support the predictions of these models, although almost all the models and observations were based on hymenopterous parasitoids (reviews in Waage, 1986; Godfray, 1987, 1994). However, there has recently been increasing of interest in clutch size of parasitoid flies (Terkanian, 1993; Adamo et al., 1995; López et al., 1995; Nakamura, 1995; Reitz and Adler, 1995), which may serve as an impetus to further studies on the reproductive behaviour of this group of insects.

In some species of tachinids that oviposit on the host cuticle, females were said to be
unable to discriminate between parasitized and unparasitized hosts because superparasitism often occurred in the field (TURNOCK, 1978; TURNOCK and BILODEAU, 1992) or the numbers of eggs found on field collected hosts followed a Poisson (random) distribution (TERKANIAN, 1993). However, the occurrence of superparasitism or random egg distribution among hosts does not necessarily prove that a parasitoid lacks the ability to discriminate between parasitized and unparasitized hosts (GODFRAY, 1994; VAN ALPHEN and JERVIS, 1996). There are also some questions remaining about whether tachinids have the ability to regulate oviposition in response to host density and other exogenous stimuli.

Exorista japonica Townsend, a gregarious parasitoid of caterpillars which lays heavy-shelled macrotyle eggs on the host cuticle (NAKAMURA, 1994), sometimes deposits supernumerary eggs on a single host in the field (Takahashi and Sawaki, 1969). In a previous study (NAKAMURA, 1995), the author predicted that the optimal clutch size of E. japonica on its host, a last instar larva of the common armyworm Mythimna (= Pseudaelia) separata Walker, to achieve maximum fitness depends on available host density. The best strategy for the fly is to oviposit 1 egg on a host to gain maximum fitness per egg when host density is high; on the other hand, she should lay 10 eggs per host to maximize the fitness gain per host when host density is low and there is little chance of finding enough hosts to deposit all her eggs. In this study, to examine whether the flies really regulate clutch size according to changes in host density, the oviposition behaviour of E. japonica was observed when a host was given to the fly after different time intervals. Since the fly has a broad host range (listed in Yasumatsu and Watanabe, 1964) and some host species, such as the fall webworm Hyphantria cunea Drury, show gregarious behaviour in patches in the field (Watanabe and Umeya, 1968), the oviposition behaviour of the fly was also examined when two or more hosts were simultaneously provided at different time intervals, which was regarded as patch encounter rate in this study. Moreover, the fly's host discriminatory ability was also examined, since host discrimination by tachinids has rarely been reported (but see LÓPEZ et al., 1995) although it is believed to be a widespread phenomenon in dipteran parasitoids (VAN ALPHEN and JERVIS, 1996).

MATERIALS AND METHODS

Host and parasitoid. Larvae of M. separata were obtained from a stock culture and reared on an artificial diet after Hattori and Atsusawa (1980). One day old (day 1) last instar larvae of M. separata were used as hosts in all experiments. The E. japonica culture was initiated from parasitized last instar larvae of H. cunea, which were collected at the National Institute of Agro-Environmental Sciences, Tsukuba, Ibaraki, in September, 1993 and maintained after the method of NAKAMURA (1996). A mated and singly caged female E. japonica was given a host on days 3 to 5 in order to gain ovipositing experience. All experiments were conducted on day 6, 24 h after the previous oviposition, in a cage (plastic cylinder, 16 cm dia., 22 cm ht.) with a sugar cube and distilled water provided.

Effect of time intervals between patch encounters and host density per patch on oviposition. To examine how time intervals between patch encounters and host density per patch affect oviposition decisions of E. japonica females, a patch containing 1, 2 or 5 host larvae was given to an experienced fly at intervals of 1 min, 2 h or 24 h. The same number of hosts were presented to the female fly three times at each time interval. A clear, plastic ice-cream cup (9 cm dia., 3.5 cm depth) containing the host(s) was placed in the cage as a patch for oviposition. For each experiment, time spent in a patch, clutch size and the number of eggs
laid per patch were recorded. Ten adult females were used for each of 9 experiments combined with 3 time intervals and 3 different host densities. To determine whether egg distribution among the hosts in a patch was random, the goodness-of-fit of the mean number of eggs laid per patch when 5 hosts were provided at different time intervals to a binomial distribution was assessed with a chi square test.

It is sometimes very difficult to distinguish one bout from serial oviposition behaviour (GODFRAY, 1994). A female of *E. japonica* laid eggs singly on the host cuticle, and when more than one host was provided, she sometimes returned to, and oviposited on, a host on which she had previously laid eggs after egg laying on another host in the patch. When the fly left a patch, it usually walked some distance away, then flew to and stayed at the upper part of the cage. Therefore, a bout of oviposition in this study was defined as oviposition which occurred between the time of entering a cup and leaving it, and the end of an oviposition bout was defined as the time of leaving the cup. Clutch size was regarded as the number of eggs laid on a single host during a bout of oviposition.

![Fig. 1. Mean time in a patch during an oviposition bout (a); percentage parasitism in a bout (b); mean clutch size (calculated excluding unparasitized hosts) (c); and mean number of eggs laid per bout by *E. japonica* females (d), when the hosts were provided at intervals of 1 min, 2 h and 24 h, with host densities of 1, 2 and 5 hosts per patch. Vertical lines indicate standard error. Bars labelled with the same letter among host densities at each time interval (a-c) and among time intervals at each host density (x-z) are not significantly different at the 0.05 level ((a), (c) and (d): multiple comparison test, based on TUKEY-KRAMER’s method; (b): $\chi^2$-test).](image-url)


Host discrimination. To determine whether *E. japonica* has the ability to discriminate between parasitized and unparasitized hosts, and to investigate the effect of time interval between host encounters on host discrimination, experiments were conducted as follows.

(A) An unparasitized and a parasitized host were simultaneously placed in the ice-cream cup for an experienced fly for 60 min, and the number of eggs laid per host during this period was recorded. In this experiment, to examine whether females also distinguish hosts parasitized by themselves from those parasitized by others, parasitized hosts were considered as those on which eggs had previously (2 to 3 h before the experiment) been laid either (1) by the same fly or (2) by another conspecific female. In order to subject both flies to similar preoviposition conditions in experiments (1) and (2), another unparasitized host was given to the female in experiment (2) for 1 h, 2 to 3 h before the experiment. Each experiment was repeated 3 times at 24 h intervals.

(B) After the fly's host discriminatory ability was revealed, the effect of time intervals between host encounters on host discrimination was examined. The number of eggs laid per host in a single bout was recorded when a parasitized host (on which a conspecific female had oviposited 2 to 3 h before the experiment) and an unparasitized host were simultaneously placed in the ice-cream cup and offered to an experienced female at intervals of 1 min or 24 h. A female fly was provided with a pair of hosts 3 times at each time interval. Since the optimal clutch size to gain the maximum fitness per host (=the Lack clutch size, see, SKINNER, 1985; GODFRAy, 1994; van ALPHEN and JERVIS, 1996) of *E. japonica* was regarded as 10, as mentioned above, parasitized hosts in both experiments (A) and (B) were divided into 2 categories—hosts with 10 eggs and hosts with 5 eggs. When necessary, excess eggs were removed with forceps.

In all experiments, a cup containing hosts was immediately removed from the cage at the end of each oviposition bout except for experiment (A). New hosts placed in a cup were then offered to a female fly 3 times for each selected time interval. Ten female parasitoids were used for each treatment and direct observation was made throughout all experiments except for the host discrimination experiment (A). All experiments were conducted at 25°C, 60% RH and with a photoregime of 16L–8D.

RESULTS

Effect of patch encounter rate and host density per patch on oviposition

Comparison within the same host densities revealed that the parasitoids tended to remain in a patch for a shorter time when the interval was 1 min than when it was 2 h or 24 h (Fig. 1(a), Tukey-Kramer's method, *p* < 0.05). On the other hand, the time spent in a patch when the interval was 1 min increased with host density, though there was no significant difference in the time spent in a patch at other intervals as the host density increased.

The flies parasitized 100% of the hosts at all time intervals when given only one host per patch (Fig. 1(b)). When given 2 or 5 hosts, the percentage of parasitism increased as the time interval increased, but decreased as the host density increased (*χ*²-test, *p* < 0.05), so that the flies left the patch while some hosts remained unparasitized.

Since the number of unparasitized hosts increased as host density per patch increased, mean clutch sizes were calculated excluding unparasitized hosts (Fig. 1(c)). Clutch sizes at every host density when the time interval was 1 min were smaller than those when the intervals were 2 h and 24 h (Tukey-Kramer's method, *p* < 0.05). Although there was no
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Fig. 2. Comparison between the frequency of observed clutch size of *E. japonica* females and that predicted by the binomial distribution when hosts were provided at intervals of 1 min (a); 2 h (b); and 24 h (c) with host density of 5 per patch (see text). There was a significant difference between the frequencies of observed and predicted clutch sizes at the 0.05 level ($\chi^2$-test) in (c).

![Graph showing clutch size distribution](image)

Fig. 3. Mean number of eggs laid by *E. japonica* on unparasitized and parasitized hosts in Experiment (A). The parasitized hosts bore 5 and 10 eggs oviposited by the same or another conspecific female. One unparasitized and one parasitized host were simultaneously provided for each female for 60 min. Numbers in the bars indicate the number of eggs previously deposited on each parasitized host. Vertical lines indicate standard error. The asterisks indicate significant differences between unparasitized and parasitized hosts at the 0.05 level (GENMOD, SAS Institute (1993)).

![Bar chart showing number of eggs per host](image)

Fig. 4. Mean clutch size of *E. japonica* on unparasitized and parasitized hosts in Experiment (B). The parasitized hosts bore 5 and 10 eggs oviposited by conspecific females. One unparasitized and one parasitized host were simultaneously provided for each female at intervals of 1 min or 24 h. Numbers in the bars indicate the number of eggs previously deposited on each parasitized host. Vertical lines indicate standard error. The asterisk indicates a significant difference between unparasitized and parasitized hosts at the 0.05 level (GENMOD, SAS Institute (1993)).

![Bar chart showing clutch size per time interval](image)

significant difference among clutch sizes at three different host densities with an interval of 1 min, clutch size decreased as host density per patch increased with intervals of 2 h and 24 h (Tukey-Kramer's method, $p<0.05$). The number of eggs laid per patch increased not only as the time intervals increased but also as the host density per patch increased (Fig. 1(d), Tukey-Kramer's method, $p<0.05$).

The mean numbers of eggs laid per patch at a host density of 5 were 2.2, 4.3 and 5.6 at time intervals of 1 min, 2 h and 24 h, respectively (Fig. 1(d)). Data from experiments in
which the parasitoids oviposited 2 \((n = 25)\), 4 \((n = 8)\) and 5 eggs \((n = 9)\) at intervals of 1 min, 2 h and 24 h, respectively, were used for analysis of egg distribution among hosts in a patch. The results revealed random distribution when the time intervals were 1 min and 2 h, and aggregated distribution at 24 h intervals (Fig. 2, \(\chi^2\)-test, \(p < 0.05\)).

**Host discrimination**

In experiment (A), results obtained both in experiments which used hosts previously parasitized by the same female and those parasitized by a conspecific fly showed the same tendency (Fig. 3). A significant difference in the mean number of eggs laid was found only between unparasitized and parasitized hosts with 10 parasitoid eggs (generalized linear model = GENMOD, SAS Institute (1993), \(p < 0.05\)) but not between unparasitized and parasitized hosts with 5 parasitoid eggs. No particular difference was found between experiments which used hosts previously parasitized by the same female and those parasitized by a conspecific fly under these experimental conditions. Furthermore, the rate of host provision affected host discrimination by the parasitoids (Fig. 4). *E. japonica* females did not discriminate against parasitized hosts with 5 or 10 eggs when the host encounter interval was 24 h, and there was no significant difference between the number of eggs laid on a parasitized and an unparasitized host. However, the flies laid smaller clutches on the parasitized host when both hosts were offered at intervals of 1 min, although the difference was significant only when the parasitized host had 10 eggs (GENMOD, \(p < 0.05\)).

**DISCUSSION**

Models which consider fitness gain per unit time (CHARNOV and SKINNER, 1984, 1985; IWASA et al., 1984; PARKER and COURTNEY, 1984; SKINNER, 1985) predict that parasitoids should vary clutch size in response to the rate of host finding and their oviposition ability. In accordance with these models, if the host density and the rate of host encounter are low, or the number of eggs the parasitoid has is very large relative to the number of hosts available, she should lay the clutch size which would maximize her fitness per host. On the other hand, if hosts are abundant and the host encounter rate is high, or if eggs are in short supply, the female should produce the clutch size which maximizes her fitness per egg. Experiments with parasitoid wasps supported this prediction (IKAWA and SUZUKI, 1982; IKAWA and OKABE, 1985; WAAGE and NG, 1984), and results of the present study also upheld the prediction of the models. As the time interval at which hosts were offered was shortened, the females of *E. japonica* laid smaller clutches, and they conversely deposited larger clutches as the time interval was lengthened (Fig. 1(c)). However, the largest clutch size observed was 3.4 on an average when one host was offered with intervals of both 2 and 24 h, which was less than the predicted Lack clutch size of 10. Although the present data offer no firm answer to explain this discrepancy, there are some reports on wasps that claim clutch sizes were smaller than predicted (CHARNOV and SKINNER, 1984, 1985; WAAGE and NG, 1984; WAAGE and GODFRAY, 1985) and HARDY et al. (1992) discussed that the more likely explanation for the discrepancy was a gross underestimation of the fitness penalties of small adult size, which is a possible explanation for the findings of the present study.

Hosts were offered to a female fly not only at different time intervals but also at different host densities in this study, because some host species of *E. japonica* such as the fall webworm live gregariously in nature (WATANABE and UMeya, 1968). The flies laid more eggs per patch as host density increased, even when the interval at which they encountered
patches remained constant (Fig. 1(d)). When a parasitoid encounters patches at short intervals, there is a possibility that oviposition is limited by egg shortage, but not by the ability to recognize local host density. However, our results in Fig. 1(d) revealed that *E. japonica* females regulated deposition of the number of eggs and left the patch with mature eggs when host density was low. This indicates that the flies are able to regulate the number of eggs laid in a bout because they have ability not only to recognize the rate of patch encounter but also the host density.

Although the ability of host discrimination in parasitoid wasps appears to be widespread (e.g., van Lenteren, 1981; Waage, 1986), the existence of this ability was not positively reported in parasitoid flies until very recently (Askew, 1971; Weiseth, 1976; Turnock, 1978; Turnock and Bilodeau, 1992; Törkanian, 1993). However, López et al. (1995) found that the tachinids *Myiophorus doryphorae* and *M. aberrans*, which are solitary and larvipositing into their hosts (Colorado potato beetles, *Leptinotarsa decemlineata*), were able to discriminate between parasitized and unparasitized hosts. The results of the present study showed that *E. japonica* also had the ability of host discrimination (Figs. 3 and 4). Females of some hymenopterous parasitoids are able to discriminate between hosts parasitized by themselves and hosts parasitized by other conspecific females, and oviposit less in the former when they superparasitize (Hubbard et al., 1987). However, no clear difference was found in *E. japonica* females in discrimination between self superparasitism and conspecific superparasitism in this study (Fig. 3). The time interval of host provision affected host discrimination, as the female flies avoided ovipositing on hosts with 10 eggs only when the interval was 1 min, not when it was 24 h, but they did not avoid hosts parasitized with 5 eggs at intervals of both 1 min and 24 h (Fig. 4). Many models predict that superparasitism is an adaptive strategy for parasitoids when the rate of encounter with hosts is low and avoidance of superparasitism is adaptive when sufficient unparasitized hosts can be found (van Alphen and Visser, 1990; Godfray, 1994). *E. japonica* seems to follow this theory and to change oviposition behaviour on parasitized hosts according to the rate of host provision (Fig. 4). However, the question remains as to why the flies avoid oviposition only on hosts parasitized with 10 eggs and not on hosts with 5 eggs when the interval is 1 min. Further investigations that clarify how the flies recognize the parasitized hosts should help to solve this question.

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