

Host spatial pattern influences induction of parasitization by *Trichogramma* (Hymenoptera: Trichogrammatidae)

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Abstract. The influence of host intrapatch spatial distribution on parasitoid host acceptance behavior was investigated with *Trichogramma principium* parasitizing eggs of grain moth, *Sitotroga cerealella*. Single females were placed in Petri dishes, each containing 60 host eggs arranged either as a compact patch or partitioned into 60 or 12 clusters each consisting of 1 or 5 eggs, respectively. Partitioned patches provoked parasitization more often than compact patches. The percentage of ovipositing females (i.e., females parasitizing at least one of 60 host eggs) increased with the number of clusters, while it was independent of the intercluster distance over intervals of 2.5–15 mm. The mean number of eggs parasitized by ovipositing females during 48 h was almost independent of the host egg spatial pattern. As a result, the rate of parasitization was higher when the hosts were sparsely distributed within a patch than when they were aggregated.

INTRODUCTION

Spatial variability and irregularity of spatial distribution are an integral part of any natural biological system. Population density is generally acknowledged to be the most effective tool for describing spatial heterogeneity. Particularly, in parasitoid-host interactions, “host density in a patch” is commonly used. The term “patch” in this instance means a distinct spatial subunit of the foraging area in which an aggregation of hosts may occur. The size of this subunit is also dependent on the peculiarities of the searching behavior of the parasitoid, “a patch is what a parasitoid perceives as a patch” (Hassell & Southwood, 1978; Alphen & Vet, 1986; Li et al., 1992). In both laboratory and natural conditions, host number per patch significantly influences the attack rate in numerous species of insect parasitoids (Hassell, 1982; Stiling, 1987, 1988; Walde & Murdoch, 1988). In most previous studies, host number per patch (or per unit area) was used as a variable, while the host distribution within a patch was uniform. However, certain experiments have shown that the host distribution within a patch can also affect parasitism (Burnett, 1958; Madden & Pimentel, 1965; Walde & Murdoch, 1988).

For several years, we have been studying the phenomenon of “refusal to parasitize” eggs of grain moth, *Sitotroga cerealella* Oliv. by various *Trichogramma* species. It is known that some host species are readily accepted for oviposition by certain of *Trichogramma* females, while other females of the same line refused to parasitize (Zaslavsky & Mai, 1982; Smith & Hubbes, 1986; Hohmann et al., 1988a, b; Fleury & Boulétreau, 1993; Pavlík, 1993; Bouchier et al., 1994; Schmidt, 1994; Liu et al., 1995; Losey & Calvin, 1995; Glenn et al., 1997). We have shown that in *Trichogramma* females refusing to parasitize, a sequence of behavioral reactions resulting in parasitism is interrupted at the arrestment and host recognition stage (Reznik & Umarova, 1990). A high number

of mature eggs in the ovaries of females refusing to parasitize suggests that this refusal is not connected with female sterility or with low egg load, and should therefore be considered as egg retention (Reznik et al., 1997). Hence, *Trichogramma* females may be in one of the two alternative states: the parasitism state (ovipositing females) or the egg retention state (refusing females).

Earlier (Reznik & Umarova, 1991), it was demonstrated that the percentage of *Trichogramma* females parasitizing at least one host (i.e., ovipositing females), directly depends on the number of grain moth eggs per test tube. Our main objective in the present study was to investigate the influence of intrapatch spatial pattern on the likelihood of attack (i.e., on the percentage of ovipositing females) and on the mean fecundity of ovipositing females. Different experiments on the effect of the host spatial pattern on parasitization rate have been done by various authors. However, only a few of these studies concerned intrapatch spatial arrangement and no investigations were conducted where the influence of host spatial pattern on the percentage of females ovipositing, and on their mean fecundity, were separately investigated.

MATERIAL AND METHODS

Insects

In all experiments, a laboratory line of *Trichogramma principium* Sug. et Sor. collected in Chimkent district (Kazakhstan) from Noctuidae eggs and reared for more than 250 generations on grain moth eggs, was used.

General methods

All studies were conducted at 20°C under a light regime of 17L : 7D. Emerging females were allowed opportunities to mate for 12–24 h. Then single unfed females were placed in Petri dishes. The bottom of each dish was lined with filter paper with grain moth eggs pasted onto it with a non-toxic water-soluble polyvinyl-based glue. Parasitoids were not exposed to honey or other carbohydrate food during the experimental period. Host eggs were offered for 48 h, then the egg sheets were incubated

until larval development was completed. The number of darkened eggs of grain moth was taken to be the number of *Trichogramma* eggs laid. Two characteristics were used to describe the result of the experiment: the percentage of ovipositing females (the number of females laying at least one egg divided by the total number of females in the replicate) and the mean number of eggs laid by ovipositing females.

Experimental procedures

In all variants of our experiments, the total number of hosts in the foraging area was the same (each Petri dish contained 60 grain moth eggs). The following characteristics of the host spatial pattern were used as variables.

1. Host number per cluster. Host patch was either compact (one cluster of 60 eggs) or partitioned into 60 or 12 clusters each consisting of 1 or 5 eggs, respectively. In partitioned patches, clusters were regularly placed at sites of a right-angled lattice (8×8 or 3×4).

2. Distance between neighboring clusters in partitioned patches was either 2.5, 5, 10 or 15 mm. Within a cluster (as well as within a compact patch) eggs were spaced densely, i.e., the distance between the neighboring eggs did not exceed 0.5 mm. Note that the radius of perception of *Trichogramma* for *S. cerealella* eggs is cca 1.8 mm (Laing, 1938).

3. Dish size. Petri dishes of two sizes were used, namely small (3.8 cm in diameter) and large (8.6 cm in diameter), 11 and 75 cm³ in volume, respectively.

Due to the considerable amount of work involved in preparing artificial egg patches and because of some natural restrictions (for example, long distances between 60 clusters are unattainable in a small Petri dish), not all combinations of variables were tested. Every tested treatment (combinations of variables) was replicated 5–10 times, each involving 20–25 females. In each replicate, the females used in different treatments were randomly selected from the same generation. The percentage of ovipositing females and the mean number of eggs laid by ovipositing females were separately calculated for each replicate of each treatment and these values were used for statistical analysis.

Statistical analysis

Student's t-tests and Tukey HSD tests with Tukey-Kramer adjustment were used for statistical treatment of the results (Wilkinson, 1990).

RESULTS

First experiment

In the first experiment, 60 eggs of *S. cerealella* were offered to individual *T. principium* females either as a compact patch (one cluster of 60 eggs), or as 12 clusters containing 5 eggs, or as 60 clusters each containing one egg. The intercluster interval was 5 or 10 mm. The experiment was conducted in small and in large Petri dishes. Preliminary treatment of the results has indicated no significant differences in the percentage of ovipositing females or in their mean fecundity between trials with intercluster intervals of 5 and 10 mm. Therefore, in the following analysis of the results from the first experiment, these two intercluster intervals were treated together.

From Fig. 1a we note that the percentage of ovipositing females sharply decreases with an increase in the number of eggs per cluster, i.e., with a decrease in the number of clusters (note that in all variants of our experiments, the total number of host eggs was the same). In addition, *Trichogramma* females more often refused to parasitize grain moth eggs in large than in small Petri dishes (Student's t-test revealed that the difference was significant in case of a compact patch: $t = 3.8$, $df = 8$, $p < 0.01$, and in case of five-egg clusters: $t = 3.0$, $df = 28$, $p < 0.01$).

The mean number of hosts parasitized by ovipositing females was essentially independent of the number of eggs per cluster (the slight decrease noticeable in Fig. 1b is not significant). By averaging over three cluster sizes and two intercluster intervals the mean number of hosts parasitized by ovipositing females was slightly (t-test: $t = 2.5$, $df = 58$, $p < 0.05$) higher in small (36.9 ± 0.9) than in large (34.0 ± 0.8) Petri dishes.

Second experiment

Sixty host eggs were offered to individual females either as a compact patch or as 12 clusters each containing 5 eggs. Clusters were placed at intervals of 2.5, 5, 10 or 15 mm, in small and in large Petri dishes. Analysis using the Tukey HSD test showed that the difference in the percentage of ovipositing females was significant only be-

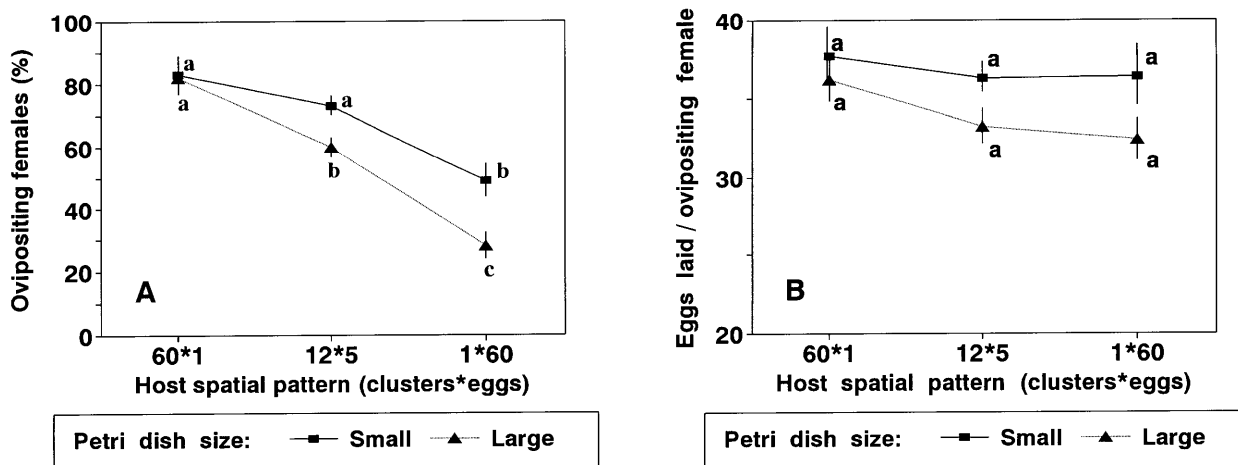


Fig. 1. The effect of the host spatial pattern (number of clusters * number of eggs per cluster) on the percentage of ovipositing females (A) and mean number of eggs laid by ovipositing females (B). Given are the means, vertical bars indicate \pm SE. Same symbols accompanied with different letters are significantly different at the $p < 0.05$ level (Tukey HSD test).

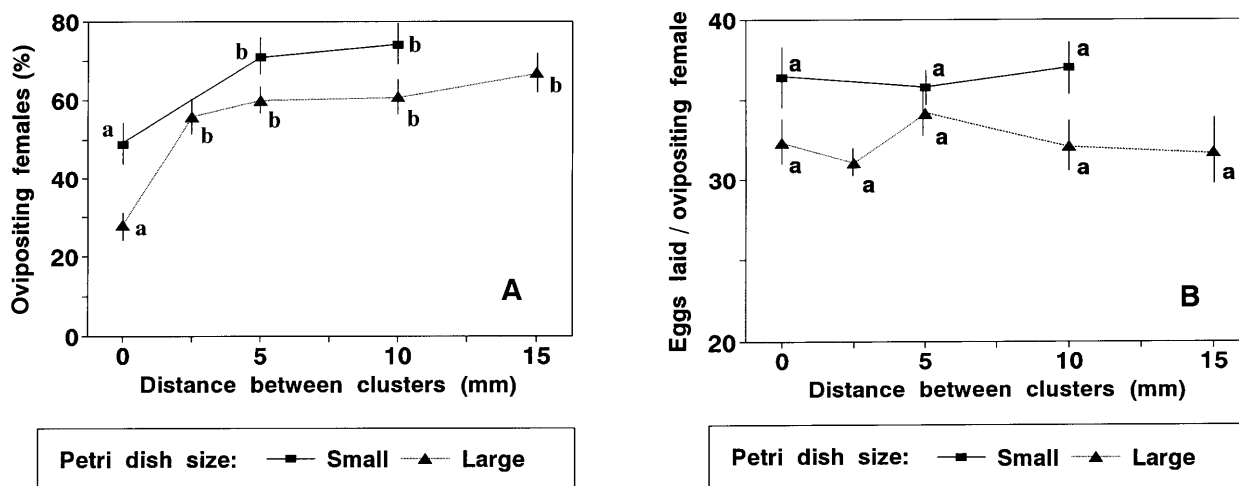


Fig. 2. The effect of the distance between 12 clusters each containing 5 eggs on the percentage of ovipositing females (A) and mean number of eggs laid by ovipositing females (B). Given are the means, vertical bars indicate \pm SE. Same symbols accompanied with different letters are significantly different at the $p < 0.05$ level (Tukey HSD test).

tween compact (zero distance) and partitioned patches (Fig. 2a). As in the first experiment, the mean number of host parasitized by ovipositing females was independent of the distance between host egg clusters. By averaging overall intervals, the mean number of parasitized hosts was slightly but significantly (t-test: $t = 3.2$, $df = 58$, $p < 0.01$) higher in small (36.4 ± 0.9) than in large (32.3 ± 0.7) Petri dishes.

DISCUSSION

The density and spatial patterns of hosts are usually considered to influence parasitoid efficiency (the number of hosts parasitized), rather than host acceptance (Burnett, 1958; Madden & Pimentel, 1965; Hassell, 1982; Stiling, 1987; Walde & Murdoch, 1988). However, the number of hosts per patch also proved to be an important factor in the induction of parasitism. In egg parasitoids, host acceptance and/or host discovery (the probability of parasitizing at least one host egg within a given patch) usually increases with the number of host eggs per patch, with the result that single eggs and small patches are often undiscovered or rejected (Morrison et al., 1980; Morrison, 1986; Hohmann et al., 1988b; Collaza et al., 1991; Reznik & Umarova, 1991).

Our experiments reveal that induction of parasitism depends not only on the amount of host eggs, but on their spatial pattern as well. The correlation between number of eggs per cluster and parasitization is negative. In other words, 60 host eggs positioned singly at 5–10 mm intervals provoke parasitization twice as often as the same number of the same eggs positioned as a compact patch in the same volume, while 12 clusters each containing 5 eggs induce an intermediate reaction (Fig. 1a). At a constant mean number of eggs laid by one ovipositing female (Fig. 1b), this obviously results in the negative relationship between the total rate of parasitization and the number of eggs per cluster. In contrast to these data, in most other parasitoids studied to date, an aggregated host distribution within a patch resulted in a higher rate of parasitization than that found with a uniform distribution of

hosts (Burnett, 1958; Madden & Pimentel, 1965). This discrepancy may be related to the fact that, in the case of *Trichogramma* females parasitizing grain moth eggs, the total rate of parasitization is heavily dependent on the percentage of wasps that manifest egg retention behavior and hence lay no eggs. In contrast, in most of the previous studies, conducted with preferred hosts, refusal to oviposit was not recorded.

The mechanism of the positive correlation between the number of intrapatch clusters and the percentage of ovipositing females at constant total number of host eggs per patch is not yet clear. Schmidt & Smith (1987) demonstrated that the number of eggs laid by *Trichogramma minutum* Riley into a single host decreased with reduced interhost distance, and suggested that the wasps can measure time or distance between host encounters. It was also found that *Trichogramma* females refusing to oviposit still moved about and came into contact with the host, although that might be inevitable in a small test tube or Petri dish (Reznik & Umarova, 1991). In the present experiments, with the assumption of random search, we supposed that the frequency of *Trichogramma* contacts with the host in the case of 60 host eggs positioned singly is higher than in the situation of a compact patch of 60 eggs. It is possible that the probability of the induction of parasitism increases with the number or frequency of contacts with hosts. Nordlund et al. (1981) reported that *T. pretiosum* exhibited success motivated searching, i.e. that oviposition results in an increased search rate. One may suppose that frequent contacts with the host caused by dispersed host distribution or small arena size can also provoke further intensive searching and/or parasitism. However, this hypothesis needs testing.

Laing (1938) demonstrated that the frequency with which *Trichogramma* females found neighboring eggs and the rate of finding those eggs was negatively correlated with the distance between *S. cerealella* eggs in the range from 1.25 to 10 mm. Stochastic simulation modeling of the walking patch of female *Trichogramma* suggested that host spatial pattern may influence the number

of hosts attacked during a given period (Bruins et al., 1994). However, in our study the mean number of eggs parasitized by ovipositing females during 48 h was independent of the host egg spatial distribution. The possible reasons are the relatively small arena size and the relatively long duration of our experiments. If this is so, the number of mature ovarian eggs (oogenesis rate), rather than searching or handling time limits the number of hosts parasitized by ovipositing females. In other words, if oviposition is triggered, a search for hosts in a Petri dish does not create any problem, because the number of hosts occasionally encountered during 48 h is much higher than the number of mature ovarian eggs in a typical *Trichogramma* female.

It was earlier demonstrated that an increase in leaf surface area (in natural conditions) or in searching arena size (in the laboratory), produces an inverse response in the likelihood of host location by *T. nubilale* Ertle and Davis, as may be expected assuming that *Trichogramma* females search in a random way (Need & Burbutis, 1979). In these experiments, a significant decrease in the mean percent of host location in laboratory tests with individual females was observed in relatively large arenas (up to 5 liter). Thorpe & Dively (1985) also tested the effect of arena size on the rate of parasitism by *Trichogramma* females in large (from 1.8 to 4,000 l) cages. In our study (Figs 1a and 2a) the influence of arena size on the percentage of ovipositing females was demonstrated in Petri dishes, where at least incidental contacts with the host are practically inevitable.

In summary, the results of the present study reveal that, in contrast to other studies published to date, an aggregated host distribution within a patch results in a lower rate of parasitization than that found with a uniform distribution. A possible reason for this effect is the positive correlation between the number of intrapatch clusters and the percentage of ovipositing females, at constant total host numbers per patch. Non-ovipositing females manifested egg retention throughout the duration of the experiment. With ovipositing females, the mean number of parasitized hosts was essentially independent of host spatial pattern. We conclude that in further investigation of the effect of host spatial pattern on the rate of parasitization not only the mean or total percentage of hosts parasitized needs to be recorded, but also the percentage of ovipositing females, particularly in the case of a non-preferred host.

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