Host preference of *Trichogramma dendrolimi* (Hymenoptera: Trichogrammatidae) on its native host, *Mamestra brassicae* (Lepidoptera: Noctuidae) after 12 continuous generations on a factitious host

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(Received 2 June 2000; Accepted 17 November 2000)

Abstract

Factitious hosts are needed for mass-propagation in crop pest management. Egg parasitoids are considered as effective biocontrol agents of lepidopterous pests because they kill host eggs and can usually be easily mass propagated on factitious hosts. However, previous studies indicated that parasitoids which had been reared on a factitious host changed host preference and did not parasitize the native host. In the present report, *Trichogramma dendrolimi* were collected from egg masses of *Mamestra brassicae* in cabbage fields maintained without insecticides. *T. dendrolimi* was maintained in the laboratory for 12 successive generations on *Ephestia kuehniella*. After the 12 successive generations, the parasitoid females still preferred *M. brassicae* to *E. kuehniella* for oviposition. *T. dendrolimi*, that emerged from *M. brassicae* eggs were larger in size and laid twice as many eggs as those from *E. kuehniella* during their lifetime. These results suggest that *T. dendrolimi* parasitizes the native host without losing host preference even after 12 successive generations on a factitious host.

Key words: Biological control, host preference, *Trichogramma dendrolimi*

INTRODUCTION

Chalcidoid wasps of the genus *Trichogramma* are extensively used as biological control agents because they attack eggs of several lepidopterous species that cause serious damage to many kinds of crops (Hassan et al., 1988; Ciochia, 1991; Nikonov et al., 1991; Greenberg et al., 1998a). In inoculative or augmentative release programs for the control of pests, natural enemies should be mass propagated (Stinner, 1977; Sun et al., 1990). In many cases, factitious hosts have been widely used for the mass production of *Trichogramma* species to reduce costs. However, mass-rearing programs have often resulted in adaptation of the insect to laboratory conditions. Changes in oviposition preference of parasitoids after rearing on factitious hosts have been reported. Van Bergeijk et al. (1989) reported that *Trichogramma maidis* had reduced acceptance to its natural host eggs of the European corn borer, after 3 generations of continuous rearing on eggs of the Mediterranean flour moth, *E. kuehniella*. Bautista and Harris (1997)

also reported that insectary rearing of *Diachasmimorpha longicaudata* for >160 generations may modify the parasitization behavior.

Trichogramma dendrolimi Matsumura is a candidate for an effective agent to control the population density of lepidopterous pests on cruciferous crops (Sun et al., 1990; Hamada, 1992; Hirai et al., 1996). In this study, we examined whether or not the oviposition preference of *T. dendrolimi* against *Mamestra brassicae* eggs changes after successive rearing for 12 generations on *E. kuehniella* eggs, and the allocation of the number of progeny on two different host species, as well as sex allocation in relation to age of the female wasp.

MATERIALS AND METHODS

Host. *M. brassicae* were collected in cabbage fields maintained without insecticides at Kagami-hara, Gifu Prefecture. A stock culture was continuously maintained using an artificial diet (Insecta LF[®], Nihon-Nohsan, Kanagawa) under a 16L:8D photoperiod at 25±2°C. Ten each of newly

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emerged male and female adult moths were put in a plastic cylinder (16 cm in diameter $\times$ 20 cm in height), the inside of which was covered with a thick paper, and fed on 5% sucrose solution. The egg-mass attached on the inside paper was cut off with a suitable size of paper and used for experiments. A stock culture of *E. kuehniella* was also continuously reared using wheat flour under the same conditions as described above. The eggs of *E. kuehniella* were trapped on a 250 μ m mesh after being passed through a 500 μ m mesh, then attached on a thick paper with double-sided sticky tape and used for parasitization.

Parasitoid. A *T. dendrolimi* colony was also established by collecting the *M. brassicae* parasitized eggs from the same field as the host, and maintained for 12 successive generations with eggs of *E. kuehniella* as a factitious host. Adult wasps which emerged from host eggs were maintained in glass tubes at $25 \pm 0.5^\circ\text{C}$, and fed on 10% honey solution. To avoid cannibalism by hatched larvae, *M. brassicae* or *E. kuehniella* eggs were killed by ultraviolet light within 24 h after oviposition and used for experiments as described by Takada et al. (2000). Female wasps which had mated within 24 h after emergence were used for parasitization. Parasitization was performed by leaving the female wasps until their death in the glass tube in a conditioned room at $25 \pm 2^\circ\text{C}$. The parasitized eggs were reared at $25 \pm 0.5^\circ\text{C}$ until emergence of the parasitoid.

Host preference of *T. dendrolimi* after several successive generations. One hundred eggs of each of two different host species, *M. brassicae* and *E. kuehniella*, were attached to both ends of a strip of paper (60 mm in length $\times$ 10 mm in width), and placed in a glass tube (16 mm in diameter $\times$ 180 mm in length). A small piece of paper absorbed with 10% honey solution was placed in the glass tube as food for the wasp. To exclude the location-effect of food, the wasp's food was placed beside each host's eggs in the glass tube in half of the total replicates. A female wasp, which had mated within 24 h after emergence, was introduced into the tube. This experiment was performed at the 7th and 13th generations. Thirteen replicates for the 7th generation and 30 replicates for the 13th generation were prepared. At the 7th generation, host eggs were exposed to a wasp for 24 h for parasitization. The number of progeny wasps which

emerged from each of the host eggs was counted. Furthermore, for the 13th generation, to determine which of the host species was preferred, the location where the female wasp searched for host eggs was recorded every 30 min for 3 h under $25 \pm 0.5^\circ\text{C}$. At the 13th generation it was released for 3 h for parasitization. To avoid the effect of differences in light intensity in the tube on the behavior of the female, the glass tube was laid parallel to the light for 3 h.

Difference in body size and number of eggs laid by wasps which emerged from two different hosts after 12 successive generations. *T. dendrolimi*, which emerged from the *E. kuehniella* eggs after 12 successive generations on *E. kuehniella*, were forced to parasitize *M. brassicae* eggs at the 13th generation. Then, the effect on body size and number of progeny of restoring to the native host after continuous rearing on a factitious host was examined. Each of 25 female wasps which emerged from *M. brassicae* eggs was compelled to parasitize either a *M. brassicae* or an *E. kuehniella* egg at the 14th generation until the wasp's death. Furthermore, each of 25 females emerged from each host was forced to parasitize the respective host's eggs at the 15th generation to ascertain whether or not the restoring effect of the host continued. The number of progeny wasps were counted and their tibia lengths were measured.

Lifetime fecundity of a parasitoid wasp reared on different host species. The mean longevity of adult *T. dendrolimi* females is 5 days (Takada et al., 2000). To clarify the allocated number and sex ratio of progeny in relation to female age, 240 eggs of *E. kuehniella* were offered to a female wasp daily for 5 days. Parasitized eggs were maintained at $25 \pm 0.5^\circ\text{C}$ under continuous daylight. The number of progeny emerging from the parasitized eggs and their sex ratio were examined.

RESULTS

Host preference of *T. dendrolimi* after 12 continuous generation

In a choice test at the 13th generation, most parasitoid wasps preferred the eggs of *M. brassicae*; two wasps stayed still or searched on the *E. kuehniella* eggs for 3 h (Fig. 1). Consequently, more progeny emerged from *M. brassicae* eggs than from *E. kuehniella* eggs. However, no signifi-

Fig. 1. Location of *T. dendrolimi* females in a choice test between *M. brassicae* and *E. kuehniella* eggs. Thirty replicates were observed every 30 min until 3 h. Dashed bar indicates the number of wasps searching to oviposit on *M. brassicae* host eggs; dotted bar: on *E. kuehniella* host eggs; and open bar: on other places. There is a significant difference between the three places at 0.5 h ($0.01 < p < 0.05$) and from 1 h to 3 h ($p < 0.01$) by χ^2 test.

Table 1. Host preference of *T. dendrolimi* after 12 successive generations on *E. kuehniella*

Host species	Number of wasps examining hosts ^a	Total number of progeny emerged	Number of progeny-wasps emerged per female (average $\pm$ SD)	
<i>E. kuehniella</i>	3 a	79	26.3 $\pm$ 5.1	NS ^b
<i>M. brassicae</i>	27 b	1,084	40.1 $\pm$ 18.4	

^aThirty replicates were observed. Each 100 eggs of two different hosts, which were attached to both ends of a strip of thick paper, were presented to a female wasp for 3 h. Different letters mean significant difference between the two host species by Binominal test at $p < 0.01$.

^bNo significant difference was observed between the two host species. Statistical analysis was performed by Student-*t* test ($p = 0.2125$).

cant difference was found in the number of eggs laid per female wasp (Table 1). Similarly, a choice test of the wasps at the 7th generation for the two different host species indicated that more females preferred to oviposit in *M. brassicae* than in *E. kuehniella* eggs, although one female wasp oviposited only in *E. kuehniella* (Fig. 2).

After *T. dendrolimi* was restored to the native host *M. brassicae* at the 13th generation, the parasitization of *E. kuehniella* had no effect on hind tibia length (HTL) of progeny wasps between the 14th and the 15th generations ($p < 0.01$, one-way

Fig. 2. A choice test of the wasps between *M. brassicae* and *E. kuehniella* was performed at the 7th generation. Each of two host egg clusters was cut off after parasitization for 24 h and kept at $25 \pm 0.5^\circ\text{C}$ until emergence. The number of progeny emerged from each host's eggs was counted. There is a significant difference between *E. kuehniella* and *M. brassicae* in all columns ($p < 0.01$, Binominal test).

ANOVA) (Table 2). The HTL of *T. dendrolimi* which emerged from *M. brassicae* eggs was longer than that from *E. kuehniella* eggs at any of the three generations mentioned above. On the other hand, after being restored to the *M. brassicae* eggs *T. dendrolimi* allocated a greater number of progeny to the *E. kuehniella* host at the 14th generation, which was almost the same number of progeny as that to the *M. brassicae* host. However at the 15th generation, the number of progeny from *E. kuehniella* eggs decreased by half (Table 2). These results suggested that the progeny females which emerged from *M. brassicae* have many more eggs than do those from *E. kuehniella*.

Lifetime fecundity of *T. dendrolimi* reared on different host species

The longevity of *T. dendrolimi* was 4–5 days. Do the females allocate a similar number of progeny through out the lifetime? Lifetime fecundity differed between the two host species. Female wasps that parasitized *M. brassicae* hosts allocated twice as many progeny as those that parasitized *E. kuehniella* only on the 1st day after emergence (Fig. 3). Two days after emergence the female wasps laid only a small number of eggs regardless of which host species they parasitized (Fig. 3). The rate of female progeny was about 80% throughout the lifetime of the female wasp regardless of host species (Fig. 4).

Table 2. Number and hind-tibia length of progeny wasps of *T. dendrolimi* which emerged from *M. brassicae* eggs at 13th generation

Host species ^a	Generation	Number of wasps examined	Hind tibia length ^b (average $\pm$ SD μ m)	Number of progeny per female ^b (average $\pm$ SD)
<i>M. brassicae</i>	13th	32	186.7 $\pm$ 5.1 a	—
	14th	25	181.2 $\pm$ 11.9 ab	113.1 $\pm$ 14.8 ab
	15th	25	177.2 $\pm$ 11.3 b	104.0 $\pm$ 18.4 b
<i>E. kuehniella</i>	14th	25	140.1 $\pm$ 9.5 c	128.9 $\pm$ 25.6 a
	15th	25	134.7 $\pm$ 6.5 c	54.7 $\pm$ 14.6 c

^a *T. dendrolimi* which was reared on an *E. kuehniella* host for 12 continuous generations, parasitized *M. brassicae* eggs at the 13th generation. Each of 25 wasps which emerged from *M. brassicae* oviposited in each host species at the 14th generation. Furthermore, each of 25 wasps emerged from each host's eggs parasitized the respective host eggs at the 15th generation.

^b Different letters mean significant difference on the same column detected by one way ANOVA at 0.01 and by means separation using Fisher's PLSD test (StatView, Abacus Concepts, CA, 1996).

Fig. 3. Change in number of progeny with female aging when *M. brassicae* (dotted line) or *E. kuehniella* (solid line) was used as a host.

Fig. 4. Sex allocation (female ratio) of progeny wasps which emerged from each host species in relation to female age. There is a significant difference between columns with different alphabetical letters at $p < 0.01$ by one way ANOVA with Fisher's PLSD.

DISCUSSION

T. dendrolimi appears to act as an effective control agent of *M. brassicae* populations in cabbage fields maintained without insecticides in Kagami-hara, because the parasitization rate of *M. brassicae* egg-masses was 60–80% and the *M. brassicae* population was kept at a low density for three years (1997 to 1999, unpublished data). When an augmentative biological control program is promoted in cabbage fields, *T. dendrolimi* should be reared continuously over several generations before releasing. However, if host preference for the native host changes, the cabbage field will suffer severe damage.

The finding of this study suggested that host preference of *T. dendrolimi* to *M. brassicae* as a native host did not change even after 12 successive generations on *E. kuehniella* as a factitious host, although the preference of the parasitoid wasp was not examined when the successive rearing of *T. dendrolimi*, which we obtained from *M. brassicae* eggs collected in the field was started. However, the facts that three in 30 females preferred the *E. kuehniella* host and never went to a *M. brassicae* host at the 13th generation (Fig. 1, Table 1), and that some females preferred to oviposit in *E. kuehniella* eggs at the 7th generation (Fig. 2), might indicate a change of host preference. Rojas et al. (1999) reported that after 10 generations of continuous rearing on *Callosobruchus maculatus* as a factitious host, *Catolaccus grandis* females switched host acceptance, and 1 generation of rear-

ing back on the native host, *Anthonomus grandis*, could not restore acceptance of the natural host to the original level. However, some strains of *Trichogramma* (including *T. dendrolimi*) did not lose their preference for the native host despite the fact that they were reared on a factitious host for several years (Pavlik, 1993; Lui et al., 1998). Hassan (1989) also reported that 17 strains of *T. dendrolimi* had the highest fecundity and showed a nearly equal preference between the target pests *Cydia pomonella*, *Adoxophyes orana* and the factitious host *Sitotroga cerealella* after rearing for at least 2 generations. Host preference of *T. dendrolimi* may not change easily even after continuous rearing on a factitious host.

E. kuehniella eggs that are used as a factitious host are smaller than *M. brassicae* eggs. Female wasps emerged from the smaller hosts were less fecund and lived shorter than those from the larger ones (Klomp and Teerink, 1967; Marston and Ertle, 1973; Bigler et al., 1987; Hohmann et al., 1988; Bai et al., 1992; Greenberg et al., 1998b). The size of progeny emerged from the *M. brassicae* host was larger than that from *E. kuehniella* among the 13th, 14th and 15th generations (Table 2). When the host species was changed from *M. brassicae* to *E. kuehniella*, the number of progeny emerging from *E. kuehniella* was not different from that emerged from the *M. brassicae* host (Table 2). These findings suggested that the size of the progeny depended on the host species, and that the female wasps emerging from larger hosts are more fecund than those from smaller ones.

Furthermore, female wasps within 1 day after emergence produced the greatest number of progeny during their lifetime (Fig. 3). Female wasps within 1 day after emergence should, therefore, be used for mass production. On the contrary, this may be a limiting factor when *T. dendrolimi* is mass propagated.

ACKNOWLEDGEMENTS

The authors wish to express their thanks to Kazuo Hirai for the classification technique of *Trichogramma* male genitalia and invaluable advice.

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