

The influence of previous host age on current host acceptance in *Trichogramma*

S. Ya. Reznik, T. Ya. Umarova & N. D. Voinovich
Zoological Institute, 199034, St. Petersburg, Russia

Accepted: July 25, 1996

Key words: host age, host acceptance, oviposition, behavior, parasitoid, *Trichogramma*, *Sitotroga*, Hymenoptera, Trichogrammatidae

Abstract

Trichogramma principium Sug. & Sor. females were sequentially offered two portions of the grain moth (*Sitotroga cerealella* Oliv.) eggs, either young (1-day old) or old (eggs that had developed 6 days at a temperature of 20 °C). The probability of host acceptance depended not only on current host age, but also on the age of the previously offered host. Particularly, *Trichogramma* females more often oviposited in old host eggs when previously offered young eggs (35–45% of *Trichogramma* females laid eggs) compared to females which were sequentially offered two portions of old eggs (15–20% of *Trichogramma* females laid eggs). In other words, parasitization by *Trichogramma* was stable even when transferred from young (preferred) to old (usually rejected) eggs. Dissections showed that refusing females had significantly more mature eggs than ovipositing females, independent of host age. Among ovipositing females, wasps provided with young hosts had fewer mature ovarian eggs than wasps provided with old hosts. Supposedly, *Trichogramma* females offered old hosts require a higher motivation to oviposit and have a correspondingly higher egg load than females offered young (preferred) hosts.

Introduction

It is known that host age is one of the most important factors determining host acceptance in insect parasitoids (Vinson, 1985). In particular, acceptability of various host species by *Trichogramma* females depends usually on the age of the host. In most species of *Trichogramma*, the number of parasitized hosts decreases with increasing development of the host's embryo. Parasitoids usually show a significant preference for young (less than 2 days old) over old (less than three days before hatching) host eggs, and some *Trichogramma* females refuse to parasitize old eggs (Pak, 1986; Pak et al., 1986; Reznik & Umarova, 1990; Hintz & Andow, 1990; Lopes & Parra, 1991; Li & Henderson, 1993; Smith & Strom, 1993). In the majority of the preceding investigations, females were offered host eggs at one definite stage of development. In some studies, females had the opportunity to choose between young and old eggs offered simultaneously. As far as we know, in none of the previous experiments were

females offered two different ages of host eggs sequentially.

In certain *Trichogramma* species, discrimination between parasitized and unparasitized hosts may depend on their prior ovipositional experience (Klomp et al., 1980; van Alphen & Dijken, 1988; Miura et al., 1994). The ability to learn to discriminate previously parasitized host eggs has been shown in numerous insect parasitoids (Vinson, 1985) and a similar reaction might be expected in their ability to discriminate between host eggs of different ages.

In our previous investigations, we have shown that *Trichogramma* females offered 6- or 8-days old eggs of the grain moth, *Sitotroga cerealella* Oliv. exhibit the phenomenon of 'refusal to parasitize' (Reznik & Umarova, 1990). 'Refusing' females were not sterile, they moved actively, incidentally contacting the host, and yet their parasitization behaviour was interrupted at the stage of arrestment and host recognition. Furthermore, *Trichogramma* females showed a tendency to behave in the same manner (i.e., to parasitize

or to refuse) when sequentially offered two portions of young (1-day old) host eggs (Reznik & Umarova, 1991). During these studies we noted that few females would move from a parasitization state to a refusal state, although a reverse sequence (from refusal to parasitization) was rather frequent.

Previously, we investigated *Trichogramma* reaction to young and/or old eggs offered simultaneously (Reznik & Umarova, 1990) and *Trichogramma* reaction to young eggs offered sequentially (Reznik & Umarova, 1991). In this study females were offered young and/or old eggs sequentially. We addressed one main question: does the host acceptance depend on the age of the previous host eggs as well as the current host egg?

Materials and methods

Insect culture and general methods. In all experiments, we used a laboratory line of *Trichogramma principium* Sug. et Sor. collected in Chimkent district (Kazakhstan) from Noctuidae eggs and reared for 15 years (ca. 250 generations) in a thermostatic chamber (20 °C, L17:D7) on young eggs of the grain moth. *T. principium* was identified by the authors of this species, Dr. E.S. Sugonjaev (Zoological Institute, St. Petersburg, Russia) and Dr A. P. Sorokina (All-Russian Institute of Plant Protection, Pushkin, Russia). Type specimens are stored in the insect collection of the Zoological Institute (St. Petersburg, Russia). All studies were conducted at 20 °C under a light regime of L17:D7. Young (less than 1 day old) *Trichogramma* females were placed individually into small (40 × 5 mm) test tubes enclosed on one side by a cotton cloth and containing a strip of paper with 80–100 host eggs pasted onto it with non-toxic water soluble glue. Pure honey was streaked on the glass to feed the wasps.

Experimental design. Females were offered sequentially two portions of grain moth eggs, either young (1-day old) or old (eggs that had developed 6 days at a temperature of 20 °C). Each age was offered for two days, followed by the second age for a consecutive two days. In the first treatment, females received sequentially two portions of young eggs, in the second treatment – first young and then old eggs, in the third treatment – first old and then young eggs, in the fourth treatment – two portions of old eggs. In each replicate, 50 females were selected randomly for each of the four treatments. *Trichogramma* oviposition in the grain

moth eggs almost always results in laying a single egg (Hassan & Guo, 1991). Therefore, when *Trichogramma* development was completed, the number of darkened eggs of the grain moth was taken to be number of *Trichogramma* eggs laid. Oviposition during the first and second period of the experiment was recorded for each female separately. Females which died during the experiment were excluded from the data analysis. In total, 2200 *T. principium* females were studied in 11 replicates of the experiment. On completion of the experiment, 25–35 females per each treatment were dissected.

Our previous work (Reznik & Umarova, 1991) suggested that *Trichogramma* females will tend to behave in a similar manner during the first and second host exposures (i.e., to parasitize or not to parasitize). To estimate the degree of this stability, we used two special indices: stability of parasitization, $SP = (C/(C + F)) \times 100\%$, and stability of refusal, $SR = (R/(R + S)) \times 100\%$, where C is the number of ‘continuing’ females which oviposited during both the first and second host-age exposure, F is the number of ‘finishing’ females which oviposited only during the first host-age exposure, S is the number of ‘starting’ females which oviposited only during the second host-age exposure, R is the number of ‘refusing’ females which did not oviposit in either age of host eggs.

Statistical analysis. Two-way ANOVA test was used to analyze percentage of ovipositing females and stability of parasitization and refusal state. Means of percentage of ovipositing females, stability of parasitization and refusal state, and number of mature ovarial eggs per female were compared among treatments by Tukey HSD test with Tukey-Kramer adjustment (Wilkinson, 1990).

Results

T. principium females offered young eggs oviposited much more often than females offered old eggs. As expected, the percentage of females that oviposited during the first period of the experiment depended on the age of the first portion of host eggs, regardless of the age of the second portion of eggs (Tables 1, 2). However, the percentage of females that oviposited during the second period of the experiment depended significantly both on the age of the first and the second portions of host eggs. Females oviposited more often

in old host eggs when previously offered young eggs (treatment 2) compared to females which were sequentially offered two portions of old eggs (treatment 4). The opposite reaction, i.e., reduction of acceptability of young eggs after contact with old eggs was not significant (Table 2).

The stability of the refusal state (SR) depended significantly on the age of the second portion of grain moth eggs (Table 1). Females showed much higher SR when offered old eggs in the second period of experimentation rather than young eggs (Table 2). The parasitization state stability (SP) was only slightly (not significantly) higher in females provided with young eggs than old eggs (Tables 1, 2).

Dissections conducted at the end of the experiment showed that refusing females had significantly more mature eggs than ovipositing females (Table 2). Among ovipositing females, wasps provided with young hosts during the second period of the experiment, i.e. during two days before dissection (treatments 1 and 3) had fewer mature ovarian eggs than wasps provided with old hosts (treatments 2 and 4). The difference in the number of mature ovarian eggs between the different treatments was insignificant for females which refused to parasitize (Table 2).

Discussion

Our previous experiments showed that *Trichogramma* females tend to maintain parasitization when sequentially offered two sets of young host eggs (Reznik & Umarova, 1991). In the present study, parasitization behaviour was stable even when young (preferred) and old (usually rejected) eggs were offered in sequence. The mechanism of this parasitization stability is not clear. It is known that oviposition experience may influence host acceptance. Based on oviposition experience, the threshold for acceptance of hosts may be changed. Particularly, given young eggs, initial host acceptance may lower the threshold for subsequent acceptance of old eggs. However, learned behavior is typically not persistent (Vinson, 1985; Godfray & Waage, 1988; Vet & Groenewold, 1990; Papaj & Lewis, 1992). Recently, Ruberson & Kring (1993) investigated the influence of previous ovipositional experience on the selection of different ages of host eggs in *Trichogramma pretiosum*. Their study revealed that experienced and inexperienced females differed little in their response to various host ages. Another possible mechanism is host feeding: possibly, young *S. cerealella* eggs are a better source

of adult nutrition than old eggs. However, Leatemia et al. (1995) have shown that protein food did not increase progeny production in *Trichogramma*. Carbohydrate food (honey) was offered in all treatments of our experiment. Consequently, it is unlikely that adult feeding underlies the mechanism of the previous host age influence on the probability of acceptance of the current host.

It is known that the nervous system is only part of the mechanism which controls oviposition behaviour in insects. Responsiveness of insects to specific stimuli is modulated by endogenous factors (Barton Browne, 1993). Usually, egg-laying is subject to twofold control, nervous and neuroendocrine. Endocrine control of oviposition was confirmed by experiments conducted with different insect species (Raabe, 1986). Possibly, the stability of the parasitization state in *Trichogramma* is based on endocrine mechanisms: presence of an oviposition-stimulating neurohormone which was released during the first period of the experiment may influence female behaviour during the second period. However, this is nothing more than a hypothesis which is difficult to verify by usual endocrinological methods because of the minute size of the wasps.

A high number of mature eggs in the ovaries of wasps refusing to parasitize suggests that refusal is not connected with female sterility and should rather be considered as egg retention. Ovipositing females generally have a low number of mature eggs in their ovaries upon dissection (Pak et al., 1985; Pavlik, 1993; Bai et al., 1995). The difference in the number of ovarian eggs between ovipositing females provided with young host eggs and ovipositing females provided with old host eggs may be explained by the fact that in most insect parasitoids, a higher egg load results in low selectivity of parasitism (Mangel, 1989; Minkenberg et al., 1992). Supposedly, *Trichogramma* females offered old hosts need a higher motivation to oviposit and correspondingly a higher egg load than females offered young (preferred) hosts. In such a situation, an ovipositing female offered old hosts never lays her next egg until a relatively high number of mature ovarian eggs becomes available.

We conclude that the probability of host acceptance depends not only on current host age, but also on the age of previously offered hosts. In particular, *Trichogramma* females much more readily accept old eggs when previously allowed to oviposit in young eggs of the same host species. Obviously, under natural conditions, parasitoids may sequentially contact patches

Table 1. The effect on parasitization when *T. principium* females were sequentially offered two different ages of *Sitotroga cerealella* eggs. H1 – host age at the first period, H2 – host age at the second period of experiment (two-way ANOVA test, n = 44)

Dependent variable	Source	DF	Mean square	F	P
Percentage of ovipositing females at the first period	H1	1	7947.6	33.2	<0.001
	H2	1	51.4	0.2	0.65
	H1 × H2	1	298.9	1.3	0.27
Percentage of ovipositing females at the second period	H1	1	2427.7	8.8	0.005
	H2	1	5612.5	20.5	<0.001
	H1 × H2	1	389.6	1.5	0.24
Stability of parasitization state	H1	1	172.8	0.4	0.55
	H2	1	595.5	1.3	0.26
	H1 × H2	1	66.1	0.1	0.71
Stability of refusal state	H1	1	266.4	1.1	0.30
	H2	1	8963.9	37.1	<0.001
	H1 × H2	1	16.5	0.1	0.80

Table 2. The effect on parasitization when *T. principium* females were sequentially offered two different ages of *Sitotroga cerealella* eggs. Means ± SEM are shown. Means followed by the same superscript letter in the same column are not significantly different at 0.05 level (Tukey HSD test)

Treatment	Host age sequence		Ovipositing females (%)		Stability of parasitization state	Stability of refusal state	Number of mature ovarial eggs per female	
	First period	Second period	First period	Second period			Ovipositing females	Refusing females
1	Young	Young	37.8 ± 5.8 ^a	56.3 ± 5.8 ^a	89.2 ± 2.0 ^a	65.9 ± 7.1 ^a	4.1 ± 1.2 ^a	20.8 ± 2.1 ^a
2	Young	Old	45.2 ± 5.7 ^a	39.7 ± 5.0 ^a	79.4 ± 5.8 ^a	93.2 ± 1.5 ^b	9.9 ± 1.7 ^b	27.1 ± 2.0 ^a
3	Old	Young	16.1 ± 2.5 ^b	47.4 ± 5.6 ^a	82.8 ± 5.7 ^a	60.0 ± 5.8 ^a	3.3 ± 0.9 ^{ac}	23.9 ± 2.1 ^a
4	Old	Old	13.1 ± 3.8 ^b	18.9 ± 3.1 ^b	78.0 ± 9.1 ^a	89.5 ± 1.5 ^b	10.0 ± 2.9 ^{abc}	23.9 ± 1.6 ^a

of host eggs of different ages. Therefore, the results of the present study should be taken into account in further investigations on *Trichogramma* ecology and behaviour.

Acknowledgements

We thank two anonymous reviewers for helpful comments on the manuscript. The research described in this publication was made possible in part by Grant R4Z000 from the International Science Foundation and by Grant R4Z300 from the International Science Foundation and Russian Government.

References

- Alphen, J. J. M. van & M. J. Dijken, 1988. Host discrimination: the learning hypothesis revisited. *Colloques de l'INRA* 48: 35–36.
- Bai, B., S. Cobanoglu & S. M. Smith, 1995. Assessment of *Trichogramma* species for biological control of forest lepidopteran defoliators. *Entomologia Experimentalis et Applicata* 75: 135–143.
- Barton Browne, L., 1993. Physiologically induced changes in resource-oriented behavior. *Annual Review of Entomology* 38: 1–25.
- Godfray, H. C. J. & J. K. Waage, 1988. Learning in parasitic wasps. *Nature* 331: 211.
- Hassan, S. A. & M. F. Guo, 1991. Selection of effective strains of egg parasites of the genus *Trichogramma* (Hym., Trichogrammatidae) to control the European corn borer *Ostrinia nubilalis* (Lep., Pyralidae). *Journal of Applied Entomology* 11: 335–341.

- Hintz, J. L. & D.A. Andow, 1990. Host age and host selection by *Trichogramma nubilale*. *Entomophaga* 35: 141–150.
- Klomp, H., B. J. Teerink & Wei Chun Ma, 1980. Discrimination between parasitized and unparasitized hosts in the egg parasite *Trichogramma embryophagum*: a matter of learning and forgetting. *Netherlands Journal of Zoology* 30: 254–277.
- Leatemia, J. A., J. E. Laing & J. E. Corrigan, 1995. Effects of adult nutrition on longevity, fecundity, and offspring sex ratio of *Trichogramma minutum* Riley (Hymenoptera: Trichogrammatidae). *Canadian Entomologist* 127: 245–254.
- Li, S. Y. & D. E. Henderson, 1993. Response of *Trichogramma* sp. nr. *sibericum* (Hymenoptera: Trichogrammatidae) to age and density of its natural hosts, the eggs of *Rhopobota naevana* (Lepidoptera: Tortricidae). *Journal of the Entomological Society of British Columbia* 90: 18–24.
- Lopes, J. R. S. & J. R. P. Parra, 1991. Efeito da idade de ovos do hospedeiro natural e alternativo no desenvolvimento e parasitismo de duas espécies de *Trichogramma*. *Revista de Agricultura (Piracicaba)* 66: 221–244.
- Mangel, M., 1989. Evolution of host selection in parasitoids: does the state of the parasitoid matter? *American Naturalist* 133: 688–705.
- Minkenbergh, O. P. J. M., M. Tatar & J. A. Rosenheim, 1992. Egg load as a major source of variability in insect foraging and oviposition behavior. *Oikos* 65: 134–142.
- Miura, K., S. Matsuda & M. Kobayashi, 1994. Discrimination between parasitized and unparasitized hosts in an egg parasitoid, *Trichogramma chilonis* Ishii (Hymenoptera, Trichogrammatidae). *Applied Entomology and Zoology* 29: 317–322.
- Pak, G. A., I. van Halder, R. Lindeboom & J. J. C. Stroet, 1985. Ovarian egg supply, female age and plant spacing as factors influencing searching activity in the egg parasite *Trichogramma* sp. Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent 50: 369–378.
- Pak, G. A., 1986. Behavioural variations among strains of *Trichogramma* spp. A review of the literature on host-age selection. *Journal of Applied Entomology* 101: 55–64.
- Pak, G. A., H. C. E. M. Buis, I. C. C. Heck & M. L. G. Hermans, 1986. Behavioural variations among strains of *Trichogramma* spp.: Hostage selection. *Entomologia Experimentalis et Applicata* 40: 247–258.
- Pavlik, J., 1993. Variability in the host acceptance of European corn borer, *Ostrinia nubilalis* Hbn. (Lepidoptera, Pyralidae) in strains of the egg parasitoid *Trichogramma* spp. (Hymenoptera, Trichogrammatidae). *Journal of Applied Entomology* 115: 77–84.
- Papaj, D. R. & A. C. Lewis, 1992. *Insect Learning: Ecological and Evolutionary Perspectives*. Chapman and Hall, London, 398 pp.
- Raabe, M., 1986. Insect reproduction: regulation of successive steps. *Advances in Insect Physiology* 19: 29–154.
- Reznik, S. Ya. & T. Ya. Umarova, 1990. The influence of host's age on the selectivity of parasitism and fecundity of *Trichogramma*. *Entomophaga* 35: 31–37.
- Reznik, S. Ya. & T. Ya. Umarova, 1991. Host population density influence on host acceptance in *Trichogramma*. *Entomologia Experimentalis et Applicata* 58: 49–54.
- Ruberson, J. R. & T. J. Kring, 1993. Parasitism of developing eggs by *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae): host age preference and suitability. *Biological Control* 3: 39–46.
- Smith, S. M. & K. B. Strom, 1993. Oviposition by the forest tent caterpillar (Lepidoptera: Lasiocampidae) and acceptability of its eggs to *Trichogramma minutum* (Hymenoptera: Trichogrammatidae). *Environmental Entomology* 22: 1375–1382.
- Vet, L. E. M. & A. W. Groenewold, 1990. Semiochemicals and learning in parasitoids. *Journal of Chemical Ecology* 16: 3119–3155.
- Vinson, S. B., 1985. The behavior of parasitoids. In: G. A. Kerkut & L. I. Gilbert (eds.), *Comprehensive Insect Physiology, Biochemistry and Pharmacology*. Pergamon Press, Oxford. Vol. 9, pp. 417–469.
- Wilkinson, L., 1990. SYSTAT: The System for Statistics. Systat, Inc. Evanston, IL, USA., 632 pp.