

# Temperature Effects on Induction of Parasitization by Females of *Trichogramma principium* (Hymenoptera, Trichogrammatidae)

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**Abstract**—The effects of temperature on induction and stability of parasitization of less preferred factitious host (the Angoumois grain moth, *Sitotroga cerealella*) eggs by *Trichogramma principium* females were studied under laboratory conditions. The percentage of parasitizing females at 15°C was significantly lower than that at 20, 25, and 30°C. At constant temperatures of 15, 20, 25, and 30°C, mean durations of pre-oviposition periods were 6.5, 2.1, 0.9, and 0.6 days, respectively (differences were significant between all regimens). In most of females, parasitization induced by high temperature (25°C) continued after the temperature decrease to 15°C. These results suggest that although emerged females are ready to lay eggs, rather prolonged pre-oviposition period may precede parasitization of less preferred hosts, and the duration of this period may depend on temperature.

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Parasitization specificity is one of the main ecological characteristics of insect parasitoids determining both their role in ecosystem and practical importance. Significant intraspecific variability of host search, selection, and parasitization behavior was revealed in most of the species studied (Victorov, 1976; Vinson, 1985; Papaj, 1993; Roitberg, 1993).

Egg parasitoids of the genus *Trichogramma* are widely used for biological control of different insect pests in agriculture and forestry (Smith, 1996) and also as model insects for various laboratory and field researches. As repeatedly demonstrated, females of various *Trichogramma* species offered eggs of non-preferred hosts delayed parasitization for several days or even permanently refused to parasitize (see references in Reznik, 1995; Reznik *et al.*, 2001a, 2001b).

Our previous investigations (Reznik *et al.*, 1998, 2001a, 2001b) showed that *Trichogramma principium* Sug. et Sor. females offered with the Angoumois grain moth, *Sitotroga cerealella* Oliv. eggs delayed parasitization for 2–10 days. The main behavioral difference between parasitizing and refusing females was that refusing females did not react to the host. Further experiments with sequentially offered standard portions of the host eggs have demonstrated that the switch from “egg retention state” to “parasitization state” was practically irreversible. Moreover, in certain experiments a tendency for parasitizing was stable when

eggs at different stages of the embryo development or even eggs of different host species were sequentially provided and, particularly, when a less preferred host was provided after more preferred hosts (Reznik *et al.*, 1997). In all these experiments, host characteristics (species, stage of embryo development) were changed, but environmental conditions were constant.

The aim of the present study was to investigate the influence of temperature on the induction and stability of “parasitization state.”

## MATERIALS AND METHODS

In our experiments, we used the laboratory strain of *T. principium*, which was cultivated for many generations on the eggs of the Angoumois grain moth, *S. cerealella*. *Trichogramma* developed under constant conditions (L : D=18 : 6 and 20°C). Two experiments were conducted to estimate the induction and stability of parasitization state at different temperatures.

At the beginning of the first experiment, emerged adults of *T. principium* were allowed opportunities to mate during 12–24 h and then were individually placed in small (8 mm in diameter, 45 mm in length) test tubes. Honey (50% water solution) was streaked on the glass as a food source. In each tube 50–60 *S. cerealella* eggs pasted to a paper card were placed. During the first 4 days of the experiment, paper strips with host eggs were replaced daily, and then, every

2nd–3rd day until death of the female. When larval development was completed, the number of hosts parasitized during each exposure (period between host replacements) was determined. As *T. principium* females usually lay only one egg in each egg of the grain moth, the number of darkened eggs of the grain moth was taken to be the number of eggs laid by *Trichogramma* female. Thus, for each exposure of each experiment, the percentage of parasitizing females (i.e., those which laid at least one egg) was calculated. For each parasitizing female, its total fecundity and duration of pre-oviposition period, i.e. period of time from first contact with the host till laying of the first egg were determined. The time of laying of the first egg was approximated by the middle of corresponding exposure.

Four treatments of the first experiments were conducted at temperatures of 15°, 20°, 25°, or 30°C. Note that *T. principium* females are able to oviposit under these temperatures (Adashkevich and Umarova, 1985). Five replicates of the first experiment were conducted; each replicate included 200 *Trichogramma* females (50 females per each temperature regimen).

The method used in the second experiment was based on the fact that parasitizing females usually had in their oviducts not more than 10–15 eggs, while those refusing to parasitize, had not less than 15–20 eggs. This method, although it is not perfectly precise, was repeatedly used both for *Trichogramma* (Honda and Luck, 2000; Mansfield and Mills, 2002), and for other insects (e.g., Veenstra and Byrne, 1998). Based on the results of our previous work with *T. principium* (Reznik *et al.*, 1998), threshold level of 15 eggs/female was selected. Then 100% of parasitizing females and 23% of non-parasitizing (refusing) females fell below threshold level (Reznik *et al.*, 1998; fig. 2). Thus, percentage of ovipositing females in a sample could be approximated from the formula:  $P_0 = P_{15} - 0.3(100 - P_{15})$ , where  $P_0$  is the percentage of ovipositing females and  $P_{15}$  is the percentage of females with less than 15 mature eggs.

For the second experiment, four groups, each including 300–500 females, were selected from a generation of laboratory strain of *T. principium*. These females were individually placed in test tubes (3 cm in diameter, 10 cm in length) and offered eggs of the grain moth according to parasitoid : host ratio of about 1 : 20. In the first and the second treatments of the second experiment, *Trichogramma* females during four days were allowed to parasitize two portions of

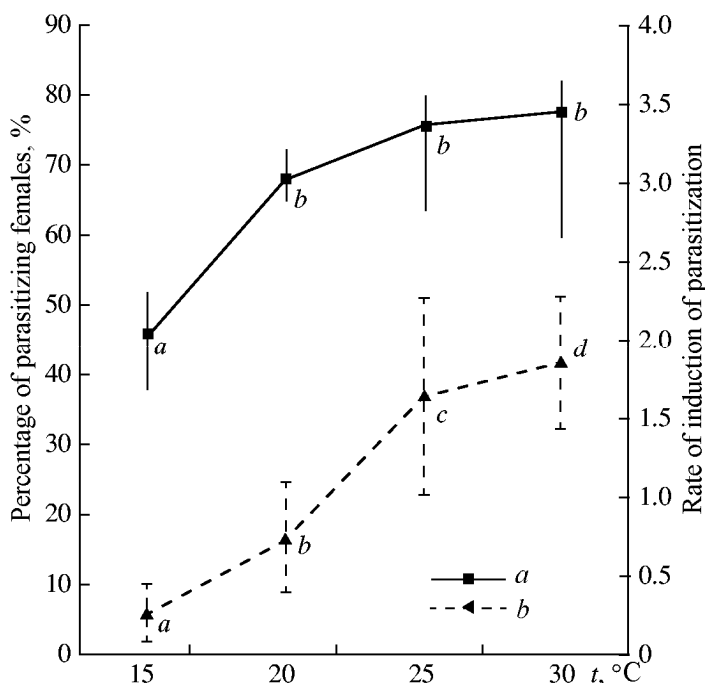
the grain moth eggs, cards with eggs were replaced after the 2nd day of the experiment. The first treatment was conducted at 15°C and the second treatment, at 25°C. In the third treatment, females were kept at 25°C during one day and at 15°C during three days, cards with host eggs were replaced simultaneously with the temperature switch. In the fourth treatment, the temperature regimen was the same as that in the third treatment (i.e., one day at 25°C and three days at 15°C), but the grain moth eggs were provided during the 1st day only and therefore till the end of the second exposure parasitizing females were absent. This treatment was a negative control. Honey solution was permanently provided in all treatments of all experiments. In all treatments, females were simultaneously dissected at the end of the 4th day of the experiment.

Preliminary statistical treatment of the results of the first experiment showed that differences in fecundity of ovipositing females and in duration of egg retention period between replicates of the same treatment were insignificant. Hence, before the final statistical analysis, data on these characteristics were pooled for all replicates. The percentage of ovipositing females was much more variable (see also Reznik *et al.*, 1996) and thus it was calculated separately for each replicate of the first experiment. The percentage of ovipositing females was averaged with medians and quartiles, while means and SD were used for fecundity, duration of egg retention period, and rate of induction of parasitization. The Kolmogorov-Smirnov test was used for paired comparison of treatments. Temperature dependence was described by means of regression analysis. All calculations were performed using SYSTAT 10.2.

## RESULTS

### *Rate of Induction of Parasitization at Different Temperatures*

In the first experiment (Fig. 1), the final cumulative percentage of ovipositing females was practically equal at 25° and 30°C, insignificantly ( $p = 0.3$ ) lower at 20°C, and significantly ( $p < 0.05$ ) lower at 15°C. However, the duration of pre-oviposition period markedly decreased with increasing temperatures. At temperatures of 15°, 20°, 25°, and 30°C, the duration of preoviposition period (mean  $\pm$  SD) was:  $6.5 \pm 4.5$ ,  $2.1 \pm 1.7$ ,  $0.9 \pm 0.8$  и  $0.6 \pm 0.5$  days, respectively. The difference between the data obtained at 25° and 30°C is significant at  $p = 0.04$ , the differences between other



**Fig. 1.** The influence of temperature on the final cumulative percentage of parasitizing *Trichogramma principium* females and on the rate of induction of parasitization. Abscissa: temperature; ordinate: [(a) left axis] final cumulative percentage of parasitizing females (%), [(b) right axis] rate of induction of parasitization (means and SD). Symbols on the same line accompanied with different letters are significantly different at the  $p < 0.05$  level (Kolmogorov-Smirnov test).

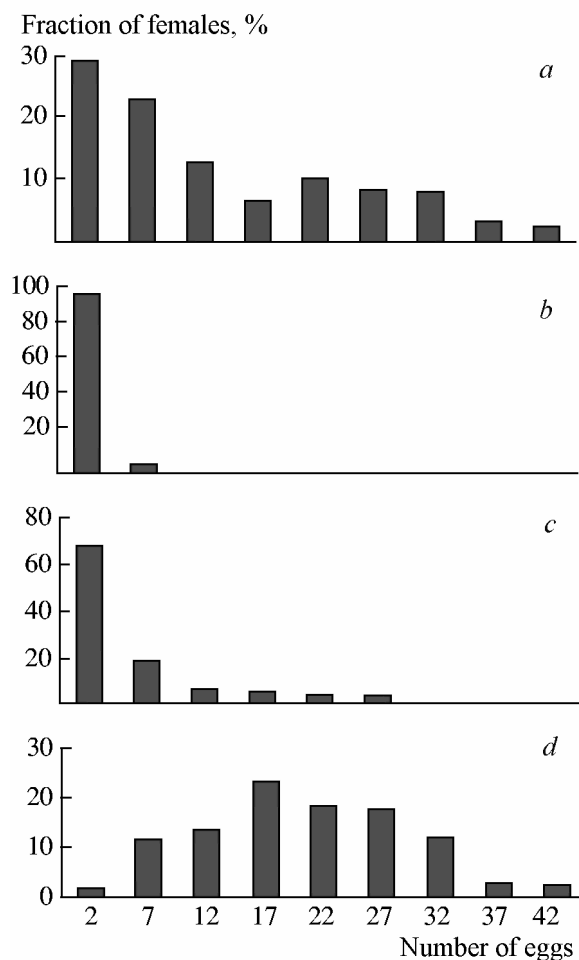
temperature regimens, at  $p < 0.001$ . Consequently, the rate of induction of “parasitization state” (the reciprocal of the duration of preoviposition period) was also significantly different between all studied temperatures. The pattern of dependence (Fig. 1) suggests linear relationship with a threshold of about 12–13°C. Regression analysis of pooled data for all females has also revealed a significant linear dependence:  $Y = 0.113X - 1.42$  ( $r = 0.79$ ,  $n = 595$ ,  $p < 0.001$ ). Attempts at approximation with quadric and cubic parabola does not significantly increased the correlation coefficient ( $r = 0.79$  and  $r = 0.81$ , respectively). Hence linear regression adequately describes the temperature dependence for the temperatures studied.

The mean total number of eggs laid during the experiment by ovipositing females at 15° was  $36.3 \pm 18.9$  which is significantly ( $p < 0.001$ ) lower than that at 20°, 25° и 30°C ( $50.1 \pm 24.1$ ,  $52.3 \pm 23.3$  и  $49.3 \pm 24.5$ , correspondingly). Differences in fecundity between three last regimens were insignificant ( $p > 0.5$ ).

#### Stability of the Parasitization State

The first two treatments of the second experiment (Figs. 2a, 2b) showed that at 15°C almost half of the females had a relatively high number (more than 15)

of mature eggs in their oviducts, while at 25°C such females were absent. The Kolmogorov-Smirnov test showed high ( $p < 0.001$ ) significance of the difference between two distributions. As shown above (Materials and Methods), the pattern of frequency distribution of females by number of mature ovarian eggs can be used to estimate the percentage of parasitizing individuals. Judging from the number of mature eggs in oviducts, at 15° about 50% of females refused to parasitize, while at 25°C all dissected individuals were parasitizing females. The stability of the parasitization state was estimated in the third treatment, when eggs of the grain moth were offered to tested females during one day at 25°C and then during 3 days at 15°C. From Fig. 2c it is clearly seen that in this treatment the percentage of females with a high number of mature eggs (i.e., those refusing to parasitize) is much lower ( $p < 0.001$ ) than that at a constant temperature of 15°C. In the third treatment, the proportion of parasitizing females indirectly estimated by the number of eggs ready to be oviposited was more than 80%. In the fourth treatment, females that have started oviposition were forced to terminate it because of the absence of host eggs. As expected, this caused a sharp increase in the number of eggs ready to be laid (Fig. 2d).



**Fig. 2.** Effects of temperature and host on frequency distribution of *Trichogramma principium* females with different numbers of mature ovarian eggs. Abscissa: numbers of eggs (means for classes); ordinate: fraction of females in each class (%). Treatments (in parentheses, sample size is given): (a) constant temperature of 15°C ( $n = 220$ ), (b) constant temperature of 25°C ( $n = 198$ ), (c) one day at 25°C and 3 days at 15°C ( $n = 347$ ), (d) one day at 25°C and 3 days at 15°C in the absence of hosts ( $n = 211$ ).

## DISCUSSION

The first and the second experiments have clearly demonstrated that the rate of induction of parasitization increased with temperature. The differences in percentage of parasitizing females between the first and the second experiments were due to the different numbers of host eggs which averaged 50–60 eggs of the grain moth in experiments with individual females and several thousand eggs, in experiments with groups of females. Earlier (Reznik and Umarova, 1989) we have demonstrated that an increase in the number of host eggs led to increased stimulus to oviposition.

Linear dependence of the rate of development on temperature is typical of insects (Honěk and Ko-

courek, 1990; Lactin *et al.*, 1995; Chernyshev, 1996). However as far as we know, such a dependence has not been earlier described for the rate of induction of parasitization. Correlations between temperature and the number of hosts parasitized were repeatedly investigated in various insect parasitoids. Different authors have demonstrated that temperature influenced parasitization by *Trichogramma* females (Sorokina, 1978; Zaslavskiy and May Phu Qui, 1982; Atamirzaeva, 1991; Pavlik, 1992; Scott *et al.*, 1997; Wang and Ferro, 1998). However, in our experiments the **final cumulative** percentage of ovipositing females was almost independent of temperature within the optimum range and significantly decreased only at 15°C, while the differences in the **rate of induction** were significant between all studied temperatures (Fig. 1). Note that we have observed just behavioral changes and not acceleration of oogenesis, as females of different *Trichogramma* species emerged from the host chorion with a considerable (averaging 15–20) number of mature eggs ready to be laid (Bower and Stern, 1966; Reznik *et al.*, 1998; Takada *et al.*, 2000).

In the second experiment, the stability of the parasitization state induced by high temperature was estimated. In the third treatment, when eggs of the grain moth were offered to tested females during one day at 25°C and then during 3 days at 15°C, about 80% of females parasitized at the moment of dissection, while at the first treatment, when the grain moth eggs were offered during 4 days at 15°C, about 50% of females parasitized (comp. Figs. 2a and 2c). Evidently, in most of females parasitization was started at 25°C and was continued after the decrease in temperature. However, the difference between distribution patterns recorded in the second and the third treatments (comp. Figs. 2b and 2c) was also significant ( $p < 0.01$ ). Consequently, a decrease in temperature still resulted in termination or delay in oviposition in certain females. As noted above, the fourth treatment of this experiment was conducted as a “negative control:” it is this pattern of distribution that would be recorded in the third treatment if the decrease in temperature caused termination of oviposition in all females (comp. Figs. 2c and 2d). In this treatment, the proportion of parasitizing females was estimated as 3%, which may be explained by inaccuracy of the method (in fact, parasitizing females were absent).

Thus, the duration of pre-parasitization period in *T. principium* females offered the grain moth eggs markedly decreased with increasing temperature. Para-

sitization induced by high temperature was usually continued after the temperature decreased. As applied to using *Trichogramma* for insect pest biocontrol, our results suggest that although *Trichogramma* females emerge with a number of mature ovarial eggs and may oviposit shortly after emergence, a rather prolonged pre-oviposition period may precede parasitization of non-preferred host eggs. The length of this pre-oviposition period may depend both on the mean temperature, and on the pattern of temperature variations.

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