

The Role of Endogenous and Exogenous Factors in Regulation of Synchronous Emergence of *Trichogramma embryophagum* Hartig and *T. principium* Sug. et Sor. (Hymenoptera, Trichogrammatidae)

S. G. Karpova

Zoological Institute, Russian Academy of Sciences, 199034 St. Petersburg, Russia

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Abstract—The interaction of exogenous and endogenous factors affecting the daily rhythm of emergence of adult parasitoids *Trichogramma embryophagum* and *T. principium* from laboratory strains was studied. Under a constant temperature of 20°C and different photoperiods (photophase of 12, 16, or 20 h), both species demonstrated a distinct rhythm; the majority of adults emerged during 4 first hours after light-on. The fraction of individuals emerged during this period depended on the photoperiod and was different in both the species studied. Experiments with non-periodic regimes (constant light or constant darkness, 20°C) demonstrated that the rhythm is based on the endogenous circadian oscillations that are synchronized with the external photoperiod. The time of emergence, however, was not rigidly “programmed” and could be modified by the direct influence of the light and temperature. Light-on and/or temperature increase (from 20° up to 30° C) induced the emergence of a certain percentage of individuals virtually at any time of the 24-h-long cycle. The magnitude of the response, estimated as the fraction of adults emerged during 2 h after the stimulation, changed daily under the control of the circadian oscillation. Thus, the plasticity of the daily dynamics of emergence was controlled (to a certain extent) by the endogenous rhythm of sensitivity to the stimuli. The interspecific differences in the relative sensitivity to light-on and temperature increase were revealed. When light and temperature stimuli were applied simultaneously, they caused a synergistic effect, exceeding a simple sum of separate impacts. These results demonstrate an important role of exogenous factors in control of the emergence rhythm in *Trichogramma* and can also be used in elaboration of methods of regulation and synchronization of emergence in these parasitoids.

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Numerous species of egg parasitoids of the genus *Trichogramma* Westw. (Hymenoptera, Trichogrammatidae) are widely used in biological plant protection. During laboratory and industrial breeding of *Trichogramma*, it is frequently necessary to obtain the greatest number of emerged adult insects in certain hours of the day. Firstly, it is associated with the optimization of the use of parasitoids: it is necessary to release the greatest amount of insects only under favorable weather conditions; otherwise, efficiency of pest infestation would be significantly decreased (Forsse et al., 1992). Secondly, groups of insects of the same age are used in some laboratory experiments. Studies of regularities defining the daily rhythm of emergence are essential for the elaboration of methods timing mass emergence of the *Trichogramma* from host eggs to a certain hour and also for acceleration or deceleration of this process.

A great number of publications is devoted to the studies of the daily dynamics of the emergence of *Trichogramma*. It was demonstrated that in the majority of *Trichogramma* examined, including *T. embryophagum* Hartig and *T. principium* Sug. et Sor., adult insects emerge from host eggs strictly synchronously during the first hours after the light-on (Forsse and Smith, 1991; Forsse et al., 1992; Dahiya et al., 1993; Corrigan et al., 1995; Pompanon et al., 1995; Zaslavski et al., 1995; Reznik et al., 1998). The emergence dynamics of parasitoids greatly depends on light and temperature conditions. The distinct rhythm of emergence of *Trichogramma* is observed under a photoperiod (at constant temperature) and also under a temperature rhythm (at constant light) (Zinovjeva et al., 1996; Zaslavski et al., 1995; Reznik et al., 1998). When photo- and thermoperiods are combined, dynamics of emergence depends on the position of the

thermophase in the photoperiodic cycle (Zinovjeva et al., 1996). Average temperature of the experiments is also significant. For example, the distinct character of the rhythm decreases under lower temperatures in *T. embryophagum* and *T. principium* (Karpova and Reznik, 2003).

Endogenous and exogenous factors are known to participate in the regulation of all the daily rhythm patterns (Tshernyshev, 1984; Saunders, 2002). On the one hand, the rhythm observed is controlled by the circadian oscillators ("biological clock"), generating spontaneous oscillations with a period constituting about 24 h; on the other hand, exogenous factors also affect this rhythm. Changes in illumination and temperature serve as "time detectors," synchronizing the biological clock with circadian cycles in the environment, and can also directly influence the degree of animal activity, stimulating or depressing the latter.

Properties of congenital rhythmic processes are well studied in *Drosophila melanogaster*, by the example of daily rhythms of its emergence from pupae (Pittendrigh, 1966, 1984; Saunders, 2002). It is believed that an endogenous period exists, when emergence is "permitted" (the so-called "emergence gates") (Pittendrigh, 1966); in other time periods, it is blocked.

On the basis of the ideas mentioned, some authors studying the distinct rhythm of emergence in *Trichogramma*, assumed the existence of a rigid endogenous control of this process (Rounbehler and Ellington, 1973; Forsse and Smith, 1991). This assumption, however, was not confirmed experimentally. More detailed investigations, on the contrary, testify to the greater significance of direct reaction of insects to exogenous factors. For example, light-on stimulus or the increase of the temperature resulted in the emergence of a certain fraction of individuals from host eggs in three species of *Trichogramma*; this reaction could be observed virtually at any moment of the 24-h cycle (Zaslavski et al., 1999; Karpova and Reznik, 2002).

Thus, mechanisms of control of the daily rhythm of emergence of *Trichogramma* are studied insufficiently, which hampers development of methods of its regulation.

The goal of the present work included the study of interaction of endogenous rhythmic processes with exogenous factors providing regulation of the daily rhythm of emergence in *T. embryophagum* and *T. principium*.

The following goals were proposed: (1) to study the influence of different light regimens on the daily rhythm of emergence; (2) to prove the existence of the endogenous rhythm experimentally; (3) to determine the degree of possible modification of the dynamics of emergence under a single change in light and temperature conditions.

MATERIALS AND METHODS

Two laboratory strains of *Trichogramma* were used in experiments: a parthenogenetic strain of *T. embryophagum* Hartig¹ from Moscow Province and a bisexual strain of *T. principium* Sugonjaev and Sorokina from Chimkent Province (Kazakhstan). The parthenogenetic strain of *T. embryophagum* is spread in Russia (westwards from Transbaikalia inclusively), Estonia, Latvia, Lithuania, Belarus, Ukraine, Moldavia, Kazakhstan, and Western and Middle Europe (Sorokina, 2001). *T. principium* is found in Turkmenistan, Uzbekistan, southern Kazakhstan, and also in Ukraine and Bulgaria (Sorokina, 2001); this species possesses high tolerance to high temperatures (up to 30°C) and low humidity in comparison with the majority of European species of *Trichogramma* (Adashkevich and Umarova, 1985).

Both strains were maintained in the laboratory for more than 10 years (20° or 25°C and 18 h photoperiod) and were reared on eggs of *Sitotroga cerealella* Oliv. (Gelechiidae).

For parasitization, eggs of *S. cerealella* were glued to cardboard cards (2 × 2 cm) (about 500 eggs on each card) and left in a chamber with females of *Trichogramma* for 24 h, after which cardboard pieces were put into glass tubes and placed under experimental conditions. Development of species from laboratory strains occurred in thermostatic boxes with automatically regulated photoperiod (chambers designer G.M. Shkarlat); chambers were illuminated with luminescent lamps (50 lux).

The daily pattern of emergence was recorded starting from the second day after the emergence of the first *Trichogramma* individuals from host eggs. Individuals emerged before switching off the light were moved from the glass tubes simultaneously. In the majority of experiments, adult insects were recorded since the light-on moment in the given experiment; all adults emerged in darkness were recorded. Further

¹ Some authors regard the parthenogenetic strain of *T. embryophagum* as a separate species *T. telengai* Sorokina (Sorokina, 1987).

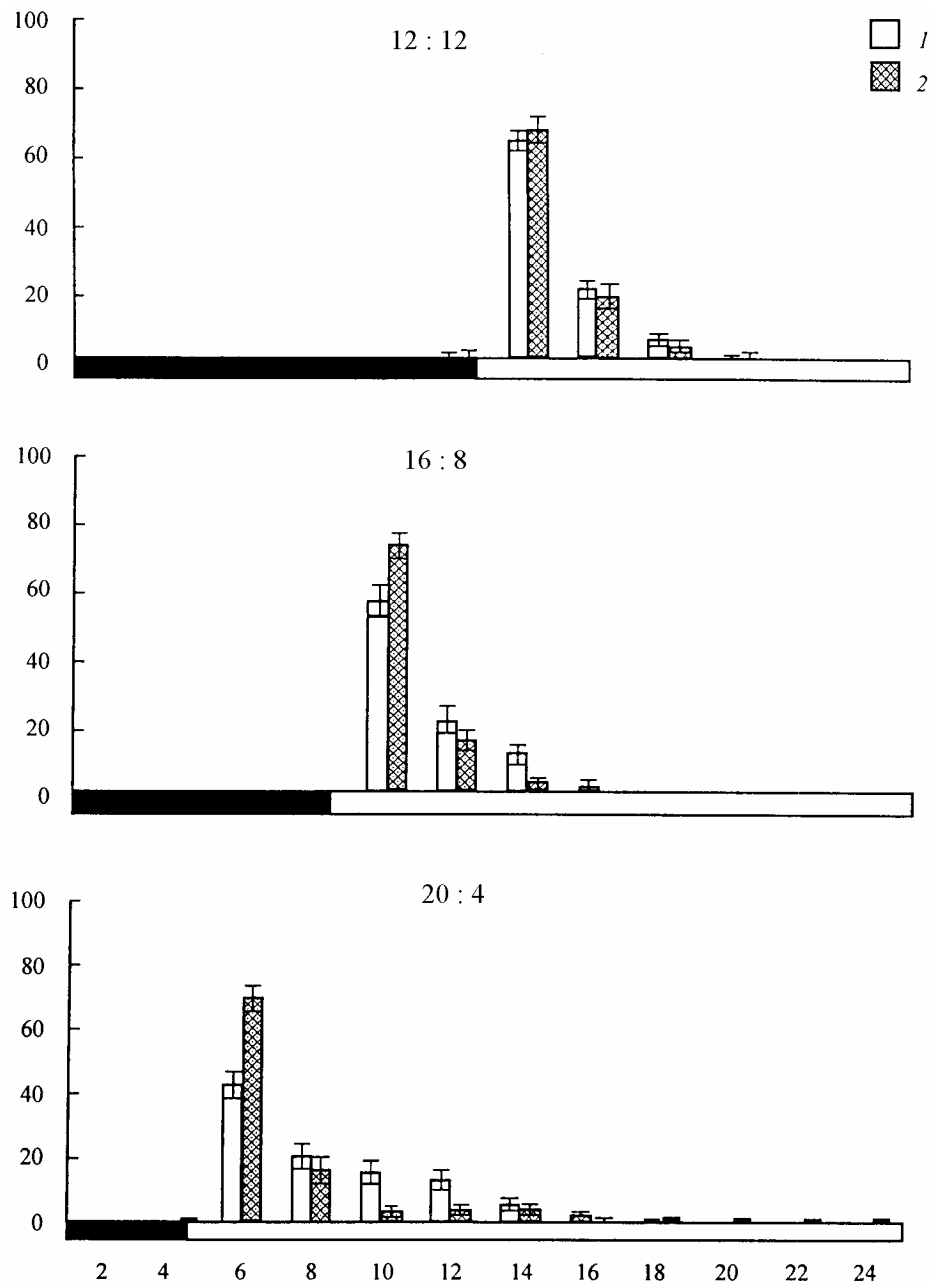


Fig. 1. Daily dynamics of emergence of *Trichogramma* under different photoperiods and constant temperature of 20°C: (1) *Trichogramma embryophagum* Hartig; (2) *T. principium* Sug. et Sor. Abscissa: time (h after light-on); ordinate: fraction of specimens emerged (%); (12 : 12, 16 : 8; 20 : 4) variants of the photoperiod. Period of darkness is designated by black color. Error scales demonstrate confidence intervals ($p = 0.05$).

recordings were performed after each 2 h. The fraction of specimens emerged during the single 2-h interval was calculated as a percentage of the total number of adult specimens. Experiments lasted from 24 to 170 h in different variants. The time intervals were recorded since the light-on moment.

Each experiment was performed in no less than three replicas, with no less than 100 individuals emerged in each replica. When significant differences

between replicas were absent, their results were summarized during the analysis.

1. The Influence of the Photoperiodic Regimen on the Daily Rhythm of Emergence

Specimens maintained under a constant temperature of 20°C and different variants of the photoperiod, differing in the ratio (hereinafter, designates the number of light and dark hours per 24-h cycle), namely, under

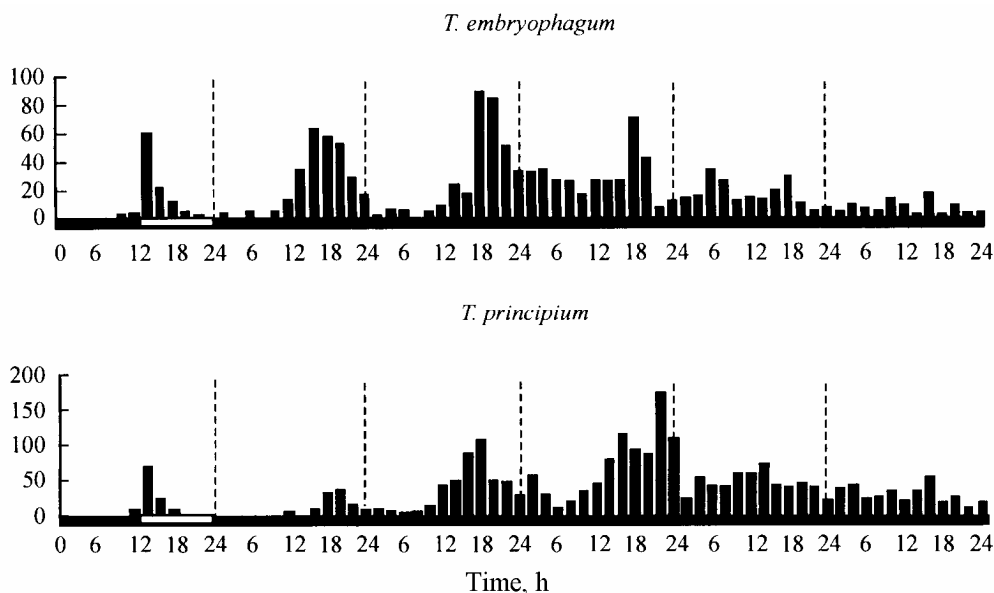


Fig. 2. Daily dynamics of emergence of *Trichogramma* in darkness (20°C). During the first day of the experiment, insects were maintained under the L : D = 12 : 12 photoperiod; the last five days, in darkness. Ordinate: number of specimens. Dotted lines separate the days of observation.

the L : D = 12 : 12, 16 : 8, or 20 : 4 photoperiods, were examined. Recordings were performed under similar conditions during 2–3 days at the time of the photophase.

2. The Rhythm in Conditions of Isolation of the Exogenous Time Signals

In these experiments, the ability of *Trichogramma* to manifest emergence rhythm without exogenous time signals was studied. All the specimens developed under a constant temperature of 20°C and L : D = 12 : 12. On the first day of recording, eggs of *S. cerealella*, infested with *Trichogramma*, were placed in constant darkness (at the usual light-off time) or in around-the-clock weak illumination (7 lux) (at the usual light-off time). The subsequent dynamics of emergence of trichogrammas was recorded during five days under a constant temperature of 20°C. In experiments in darkness, recordings were performed under weak red light.

3. The Influence of Single Light and/or Temperature Stimuli on the Synchronicity of the Emergence

Before the experiment, all the specimens developed under a constant temperature of 20°C and L : D = 12 : 12 photoperiod.

On the day of emergence (18–19th day after parasitization), the light was switched off at the usual time and, with different time intervals (varying from 2 to 24 h with an interval of 2 h), the reaction to the fol-

lowing stimuli was studied: (A) light-on (50 lux); (B) short-time heating (2h under 30°C); or (C) simultaneous light-on and short-time heating. Intensity of the reaction was estimated as the fraction of individuals, emerged during 2 h after the beginning of the stimulation (percentage of the total number of specimens emerged during recordings). In variants with a short dark period (less than 12 h), recordings were stopped 24 h after light-off, and in variants with longer dark period, 36 h after light-off. With such duration of experiments, a single pronounced peak of emergence was observed in each experimental variant, allowing correct comparing of variants.

After light-on, glass tubes were left under around-the-clock light (in the B variant, light was switched on after termination of the temperature stimulus). Temperature changes were performed by transfer of glass tubes into chambers with different temperatures.

Recording of emerged adults started from the light-on moment in certain experiments; the number of all the adults, emerged in darkness, was recorded. However, in the experiment, where glass tubes were warmed before the light-on, the first recording was performed under a weak red light 2 h before the end of the period of darkness. Then, the material was placed into a chamber with a temperature of 30°C and left there for 2 h. After that, light was switched on, the number of emerged adults was recorded and the glass tubes were returned to the chamber with 20°C.

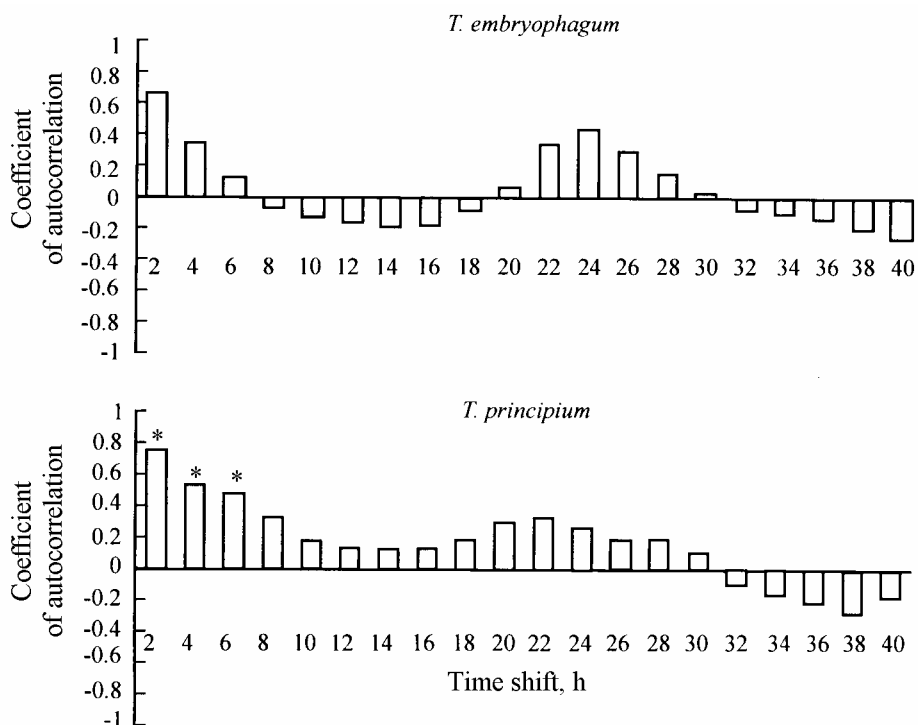


Fig. 3. The autocorrelation plot of the emergence dynamics of *Trichogramma* under constant darkness. The graph demonstrates dependence of the coefficient of correlation on the value of the time lag. Ordinate: Coefficient of autocorrelation; (*) $p < 0.05$.

Thus, the response of specimens to a single impact of light and/or rise of temperature was investigated at different times of the 24-h cycle.

For determination of significance of differences between the fraction of specimens emerged during certain time intervals, we used the Fisher's criterion with the ϕ -transformation (Ivanter and Korosov, 1992). For comparison of distribution of specimens by the time of emergence, the Kruskal-Wallis's and Kolmogorov-Smirnov's tests were used; calculations were performed with a Statgraphics software package.

RESULTS

1. The Influence of the Photoperiodic Regimen on the Daily Rhythm of Emergence

In all the variants of photoperiodic conditions (at 20°C), both species demonstrated a distinct daily rhythm of emergence (Fig. 1). The majority of adults emerged during 4 h after light-on; only solitary specimens emerged during the dark period or at the end of the photophase. Thus, the peak of emergence was associated with the time of the "dawn."

Under a photoperiod with L : D = 12 : 12, *T. embryophagum* and *T. principium* did not differ in the

daily dynamics of emergence ($p > 0.05$, Kruskal-Wallis's and Kolmogorov-Smirnov's tests). In variants with longer daylight, (16 and 20 h), *T. principium* demonstrated a similar distinct rhythm as under the 12 : 12 regimen. Mass emergence was observed during the first 4 h of the photophase; not more than 15% of adults emerged during the rest of the day. In *T. embryophagum*, by contrast, the fraction of specimens emerged at the beginning of the photoperiod, decreased; as a result, the rhythm became less distinct. In variants with 16 and 20 h, interspecific differences were significant ($p = 0.001$, Kruskal-Wallis's and Kolmogorov-Smirnov's tests).

2. The Rhythm under Conditions of Isolation of the Exogenous Time Signal

On the second day after placing of specimens into constant darkness, mass emergence in both species began later than under the L : D = 12 : 12 photoperiod. The maximum emergence was observed 16–18 h after light-off, whereas under the photoperiod it was observed after 14 h (Fig. 1, L : D = 12 : 12). During the next 2 days, peaks of mass emergence regularly were repeated. In *T. embryophagum*, maximum emergence was observed after each 18 h, whereas these peaks

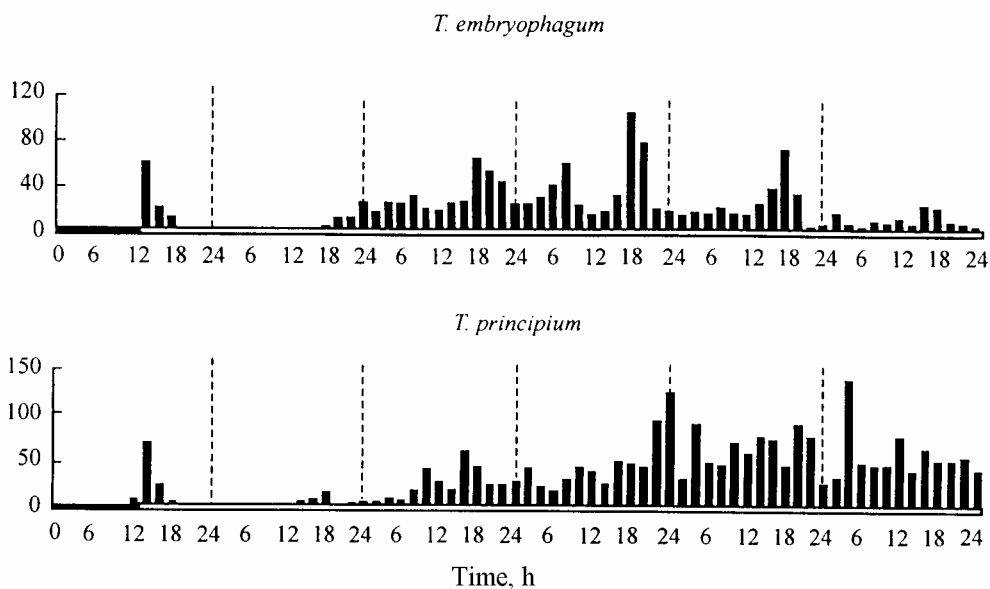


Fig. 4. Daily dynamics of emergence of *Trichogramma* under constant light (20°C). On the first day of recording, specimens were maintained under the L : D = 12 : 12 photoperiod; the last five days, in constantly illuminated chamber. Ordinate: number of specimens. Dotted lines separate the days of observation.

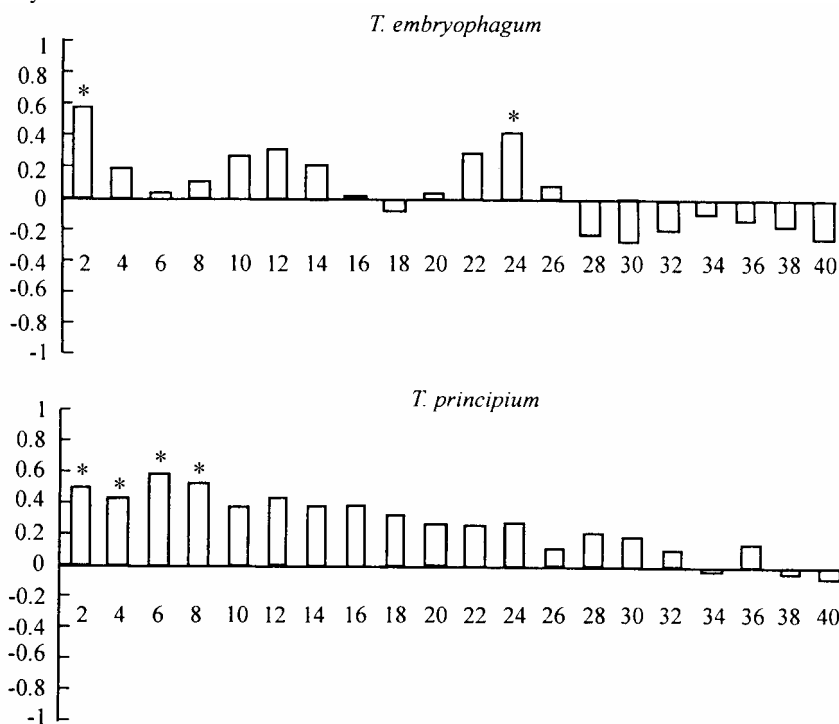


Fig. 5. The autocorrelation plot of the emergence dynamics of *Trichogramma* under constant light. The graph demonstrates dependence of the coefficient of correlation on the value of the time lag; (*) $p < 0.05$.

were less distinct in *T. principium*; however, the average peak of the emergence (on the time interval between 2 and 24 h) was also associated with 18 h. At the 4th and 5th day, emergence of *Trichogramma* from eggs occurred already rather uniformly, without any distinct rhythm.

In order to reveal the cyclic character of the process studied, autocorrelation analysis was performed (Ivanter and Korosov, 1992). Coefficient of autocorrelation, shown in Fig. 3, demonstrates the cyclic character of the emergence of *T. embryophagum* in darkness. Shifting of the time series by 24 h, the coefficient was

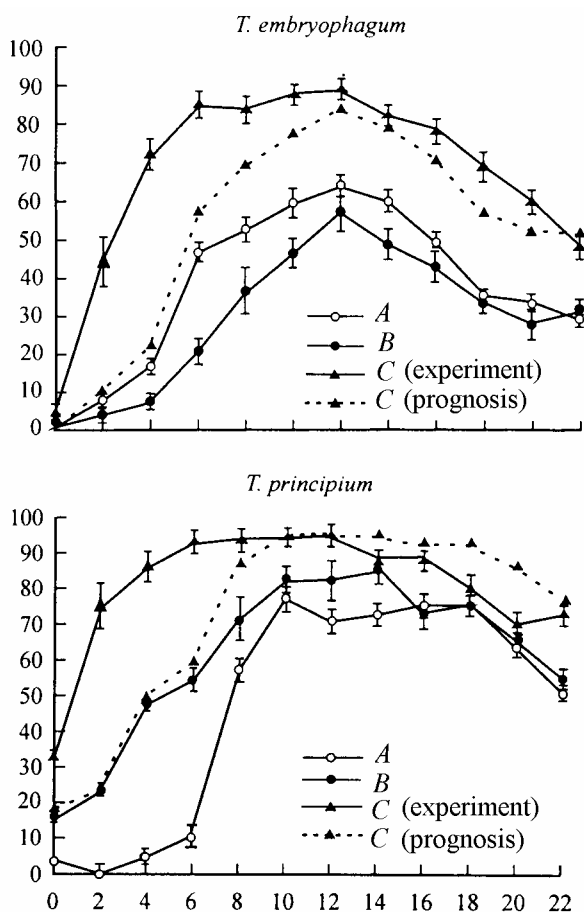


Fig. 6. Daily changes in sensitivity of *Trichogramma* to light and/or temperature stimuli. (A) response to light-on (50 lux); (B) response to temperature increase in darkness (from 20 to 30°C); (C (experiment)) experimental data; (C (prognosis)) theoretical calculation of the response to simultaneous impact of two stimuli, when they affect independently. Error scales demonstrate confidence intervals ($p = 0.05$). Abscissa: time of the beginning of stimulation, h after light-on; ordinate: fraction of specimens emerged during 2 h after the beginning of stimulation (%).

maximally positive ($p < 0.05$), i.e., the period of the rhythm corresponded to 24 h. Analysis of the data on the emergence of *T. principium* also demonstrated the increase of the autocorrelation coefficient, when the series was shifted by 22 and 24 h (Fig. 3), but the values of the coefficient were not significant with $p = 0.05$.

In experiments with around-the-clock illumination, depression of the emergence during the first day of the experiment was noted (Fig. 4). During the next 4 days, *T. embryophagum* emerged rhythmically; peaks of mass emergence were observed after each 18 h and coincided with those observed in specimens emerged at constant darkness. According to the autocorrelation analysis, the period of the rhythm constituted 24 h (Fig. 5). In *T. principium*, emergence under constant

light occurred without distinct periodicity (Fig. 4), which is confirmed by a graph of autocorrelation function: no correlation was observed between the points on the time scale separated by an interval of more than 8 h (Fig. 5).

3. Influence of Single Light and/or Temperature Stimuli on the Synchronicity of Emergence

The increase of illumination and/or temperature stimulated immediate emergence of *Trichogramma* at any time of the day. The intensity of the reaction (the fraction of specimens emerged during the first 2 h after stimulation) increased during the first 12–14 h of darkness and then decreased again (Fig. 6). In *T. embryophagum*, the average time of maximal sensitivity to light and temperature stimuli coincided with 12.6 ± 0.8 and 11.2 ± 0.8 h, respectively; in *T. embryophagum*, these peaks were observed at 12.7 ± 0.7 and 13.0 ± 1.0 h, respectively (mean between replicas, \pm SEM).

At the same hour of the day, the light-on stimulated emergence more than the increase of the temperature in *T. embryophagum*. *T. principium*, on the contrary, responded to the temperature stimulus more intensively, at least in experiments with the period of darkness lasting less than 16 h. On the whole, the latter species was characterized by a greater synchronicity of the emergence. For example, the light-on or the increase of the temperature in the time interval between 10 to 18 h resulted in the immediate emergence of the majority (more than 75%) of *T. principium* specimens. Besides, in *T. embryophagum*, the effect of these stimuli did not exceed 65% at any time of the day.

Simultaneous light-on and temperature increase stimulation (Fig. 6B, experiment) resulted in a significantly stronger response in comparison with the effect of the independent impact of each factor. In order to check whether this more pronounced reaction was the result of a simple summation of two above stimuli, some additional calculations were performed. The sum of fractions of specimens, emerged during 2 h after the increase of temperature in darkness and after light-on at constant temperature, was calculated for each point of the time scale by a standard formula of percentage addition: $P(t + l) = P(t) + P(l) (100 - P(t)) / 100$, with $P(t)$ designating percentage of specimens emerged after the increase of temperature; $P(l)$, percentage of specimens emerged after light-on; and $P(t + l)$, percentage of specimens, whose emergence was expected

after simultaneous independent impact of these factors.

The results are shown in Fig. 6 (dotted lines). The data convincingly testify to the fact that the joint action of the light-on and the temperature increase is significantly more effective than it could be expected for the independent action of these factors. Evidently, we observe a super-additive effect, which is most pronouncedly expressed during the first 10 h after the beginning of the darkness period.

DISCUSSION

1. Synchronization of the Rhythm by the Photoperiod

The ability of adjustment to the cycles of the exogenous factors is one of the characteristic features of the biological rhythm. This property allows an insect to associate its rest and activity periods with the most favorable time of the day.

Our results demonstrated that the rhythm of emergence of *T. embryophagum* and *T. principium* in laboratory changed its phase in correspondence with the light-darkness conditions of the development of specimens. For example, shortening of the "night" period resulted in the shift of the peak of emergence to earlier hours (Fig. 1). A similar correlation between the peak of emergence and the photoperiod was noted earlier in *Trichogramma semifumatum* Perkins (Rounbehler and Ellington, 1973); in these experiments, development of specimens and recording of emergence from host eggs maintained under two photoperiods (L : D = 10 : 14 and L : D = 14 : 10) were studied. It was found that emergence of the majority of adults was approximately equally distributed between the end of the darkness period and the beginning of the photophase under both light-darkness regimens; i.e., the position of the maximum was associated with the light-on moment. Similar changes in the rhythm, depending on the light regimen, are well-known. In the majority of insects, the time of the greatest activity is correlated (by a certain phase) with the time of the dawn or the sunset (see reviews by Tshernyshev, 1984, and Saunders, 2002).

The emergence from pupae in morning hours is typical of many diurnal insects (Saunders, 1982; Lankinen, 1986; Ruberson et al., 1988; Pompanon et al., 1995). Such distribution of the emergence during the day can be treated as an adaptation. Firstly, in morning hours, when air humidity is higher than in the middle of the day, environmental conditions are evidently more favorable for the emergence of insects from pu-

pae, because water losses through the soft cuticle of a newly emerged adult are very significant (Lankinen, 1986). Secondly, the emergence at the beginning of the daylight period allows parasitoids to start immediate reproductive activity, decreasing the risk of their death before oviposition (Pompanon et al., 1995).

Interspecific differences revealed in the rhythm of emergence at L : D = 16 : 8 and L : D = 20 : 4 photoperiods are especially interesting. In particular, in *T. principium*, a distinct peak of emergence after the light-on was observed under all the variants of the photoperiod, whereas in *T. embryophagum*, the synchronicity of emergence decreased with the increase of the photophase (Fig. 1).

The dependence of the distinct character of the daily rhythm on the duration of the daylight period was earlier revealed in other insect species. In *Calliphora vicina*, the maximal amplitude of the locomotor rhythm was observed under the 16 h photophase (Zotov et al., 1990). In *Drosophila*, the most synchronous rhythm of emergence from pupae was observed at the 12 h photophase (Tshernyshev, 1984). Comparative analysis of the influence of the duration of the photophase on the rhythm in closely related species was not conducted earlier. The question on possible reasons of interspecific differences remains open and needs further experimental investigations.

2. The Development of the Endogenous Rhythm in the Absence of Exogenous Time Signals

The development of the circadian rhythm under conditions of isolation from periodical changes of exogenous factors (light and temperature) testifies to its endogenous nature (Tshernyshev, 1984; Saunders, 2002). Thus, retaining of the rhythm of emergence, noted by us in both species maintained at constant darkness (Fig. 2), confirms the existence of the endogenous circadian rhythm controlling emergence.

The expression of the rhythm in darkness differed in *T. embryophagum* and *T. principium*: repeated peaks of mass emergence were less distinct and "diffuse;" as a result, the autocorrelation analysis of the data confirmed the presence of the rhythm in *T. embryophagum*, but gave no significant evidence of the periodicity of emergence in *T. principium* (Fig. 3).

The species investigated also differed in the ability to develop the rhythm of emergence after replacement into constant light conditions: adult *T. embryophagum* continued their emergence with the circadian periodicity, whereas emergence became arrhythmic in *T. prin-*

cipium (Fig. 4). Vanishing of the rhythmic character under the constant light impact was described in many animals, including insects (Ashoff, 1984; Saunders, 2002). It is known that light intensity, invoking this phenomenon, differs in different species (Hong and Saunders, 1994).

Thus, data were obtained on the control of the emergence of *Trichogramma* by the endogenous circadian oscillators; however, the ability to maintain the autonomous rhythm in the absence of exogenous time signals differed in the two species.

The question concerning the relative role of the endogenous rhythm in regulation of the daily periodicity of the emergence deserves discussion. In many insects, the rhythm of emergence from pupae is rigidly controlled by endogenous factors. For example, the emergence of *Drosophila melanogaster* demonstrates distinct periodicity during ten days after placement into constant darkness (Pittendrigh, 1967). Such investigations were never performed in *Trichogramma*. The data on the rhythmic character of the emergence of *T. evanescence* in darkness (Dahiya et al., 1993) are the only exception. However, the authors performed recordings only during the first two days; therefore, it is impossible to find out, whether this periodicity was retained longer. In our experiments, the periodicity of the emergence in darkness lasted for no more than three days; after that, the rhythm vanished. It means that the role of the endogenous control of emergence in *T. embryophagum* and *T. principium* is rather insignificant in comparison with the majority of the other species examined.

3. The Role of Direct Reactions to Changes of Exogenous Factors in the Daily Rhythm of Emergence

The ability of the rhythm of emergence, synchronized by the L : D = 12 : 12 photoperiod, to change simultaneously with sudden changes of exogenous factors, was studied in several sets of experiments. For this purpose, single changes in illumination and/or temperature were performed at different times of the day. It was found that the rhythm was rather flexible in both species because *Trichogramma* species are able to respond directly to exogenous factors irrespective of the time of the day. The significant role of the "rapid" response of *Trichogramma* species to light and temperature changes was earlier noted in *Trichogramma pintoii* and *Trichogramma* sp. (Zaslavski et al., 1999) and also in *T. embryophagum* (Karpova and Reznik, 2002). In the present study, comparative analysis of

the influence of light and temperature regimens on the emergence of two closely related species of *Trichogramma* was studied for the first time.

The results of our study demonstrated that the intensity of the response to the direct action of signal stimuli (light-on or temperature increase) was determined by several factors.

First of all, the effect of the signal depended on the time of its impact. Immediately after the light-off, specimens were insensitive to stimuli; the intensity of reaction gradually increased till the moment of the usual light-off (12 h) and then it decreased again (Fig. 6). In our previous studies (Karpova and Reznik, 2002) with *T. embryophagum* as an example, the control of daily changes in the sensibility (in relation to light in particular) by endogenous rhythmic processes was confirmed experimentally.

Dependence of the light-on reaction on the time of the day was noted earlier by other entomologists, with daily rhythms of locomotor activity or emergence from pupae in some insects as an example (Zotov, 1983; Morris and Takeda, 1994; Germ and Tamioka, 1998). Evidently, daily changes in sensitivity of insects to exogenous factors are very important adaptations, because they prevent undesirable responses to occasional changes of exogenous factors at the time periods "unfavorable" for the activity (Tshernyshev, 1984).

The intensity of the reaction also depends on the character of an exogenous stimulus. Comparative analysis of the data on the emergence of *T. embryophagum* and *T. principium* demonstrated that these species differed in the relative sensitivity to both light and temperature. As shown in Fig. 6, the light-on (50 lux) caused stronger response in *T. embryophagum*, in comparison with temperature increase (from 20° to 30°C) at the same time of the day. The opposite tendency was revealed in *T. principium*.

Interspecific differences in the degree of sensitivity to experimental rising of the temperature can be associated with climatic conditions of natural habitats of *Trichogramma*.

In the continental climate, where *T. principium* is found, the increase of illumination in morning hours occurs rapidly and virtually synchronously with the temperature increase. Under such conditions, warming is a rather informative signal detecting the beginning of the light period of the day. Besides, in the dry and hot climate, high temperature is frequently correlated with low humidity. Such combination of environ-

mental factors can be disastrous for such small insects as *Trichogramma*, because of rapid water losses. For example, experiments demonstrated that high temperature (33°C) and low humidity (30–35%) resulted in the absence of oviposition and the death of *T. principium* females during the first day after their emergence (Sorokina, 1978). Evidently, immediate response to heating provides a rapid and synchronous emergence of the majority of specimens in morning hours, when humidity is relatively high and the temperature is far from pessimal values. In such a way, in the day hours, when probability of the death of young insects from overheating and desiccation is high, their emergence is limited.

In the temperate climate, where the *T. embryophagum* strain was collected, the air slowly warms up at dawn and temperature increase is delayed in comparison with the increase of the degree of illumination; therefore, increase in illumination unassociated with the heating can serve as an effective signal for the beginning of emergence. Sensitivity of *Trichogramma* to the light stimulus is evidently associated with peculiarities of the daily activity of these insects. According to field observations on the behavior of different species of *Trichogramma* in the temperate zone (Shchepetilnikova, 1962; Kolmakova, 1978), parasitoids start searching for host in the morning hours, as soon as the air temperature rises up to 12–13°C. Their reproductive period is limited to a rather short time interval, because mobility of females decreases after the evening fall of temperature; it is also frequently depressed in the midday hot hours. This “warning behavior,” i.e., emergence from host eggs at dawn even before the rise of the temperature is apparently very important for such short-living insects as *Trichogramma* because it distributes all the events of ontogenesis in time very efficiently: young adults have time to get stronger and can start searching activity immediately under favorable environmental conditions.

The effect of the joint action of two signal factors attracts special attention. The response to the light stimulus pronouncedly increased when the light-on coincided with the increase of the temperature from 20° to 30°C. The effect caused by the simultaneous impact of two factors should be considered a superadditive one, because it is not a simple sum of efficiency of each of the factors, but is significantly more efficacious (Fig. 6). This phenomenon testifies to the fact that the light-on and increase of the temperature stimulated emergence of adults from host eggs not inde-

pendently; on the contrary, their action integrated at a certain stage of the reaction. Similar conclusions on the interaction of two factors were made earlier, as the result of the study of locomotion rhythm in fruit flies and bees (Ikeda and Tomioka, 1993; Moore and Rankin, 1993; Tomioka et al., 1998).

4. The Relative Role of Exogenous and Endogenous Factors in Regulation of the Emergence Rhythm

The data obtained testify to the fact that the synchronous emergence of adult *T. embryophagum* and *T. principium* at the beginning of the light period of the day is a “trigger” response. *Trichogrammas* possess an inborn rhythm that changes the readiness of individuals to emerge from host eggs, responding to the increase of temperature or illumination. The endogenous rhythm is adjusted by the photoperiod in such a way that the highest sensitivity coincides with the moment of the dawn. In this time of the day, such signals as light-on or heating cause mass synchronous emergence.

It should be noted that no distinct time periods “prohibiting” or “permitting” emergence were revealed in *Trichogramma*; the existence of such periods is assumed in other insects (Saunders, 2002). Observations, by contrast, testify to the fact that circadian oscillators control the process gently; they only set gradual changes of the potential readiness of individuals for emergence. Such method of “flexible” endogenous control was described for the rhythms of individual activity of insects (Tshernyshev, 1984; Zotov, 2001); however, its applicability to circadian rhythms of emergence of adults is proved experimentally for the first time.

A significant role of exogenous effects (direct reactions to changes in the environment) and relatively weak influence of endogenous circadian processes, in comparison with the majority of other insect species investigated, is the characteristic feature of regulation of the daily emergence rhythm in *Trichogramma* (see a review by Saunders, 2002). High sensitivity of parasitoids to daily temperature and illumination changes is an evident adaptation. Firstly, rapid reaction to light-on and increase of the temperature result in a very synchronous emergence of individuals at the beginning of the light period of the day. Advantages of emergence of *Trichogramma* from host eggs at dawn has been already discussed in the present publication and preceding studies (Forsse et al., 1992; Dahiya et al., 1993; Pompanon et al., 1995; Karpova and Reznik, 2002). Secondly, significant plasticity of the

daily emergence rhythm and sensitivity to the increase of the temperature and illumination evidently allows parasitoids to emerge and start host search immediately under favorable environmental conditions. Such maximally effective temporal organization of ontogenetic events is very significant for *Trichogramma*, taking into account the short lifespan of these insects (Pompanon et al., 1995).

The knowledge of regularities of the daily emergence rhythm can be used in the selection of regimens of mass rearing of *Trichogramma*. For example, a 12-h daylight period is expedient for obtaining a maximally synchronous emergence of *T. embryophagum* and the L : D = 16 : 8 photoperiod, for the breeding of *T. principium*. Directly at the day of emergence, the time of the mass emergence of adults can be regulated by the use of advanced or delayed (in comparison with the usual one) light-on. The maximal effect should be expected when the light-on is accompanied by the increase of temperature. According to our data, it is possible to determine the period when such stimulation will result in the emergence of the majority of individuals (more than 75% of insects emerged during 2 h after light-on). At 20°C, this period lasts from 6 to 16 hours after light-off in *T. embryophagum* and from 2 to 22 hours after light-off in *T. principium* (Fig. 6). The strongest effect was observed 12 h after light-off; during this period, simultaneous temperature and illumination increase resulted in the emergence of 90.2 and 95.9% of *T. embryophagum* and *T. principium* individuals, respectively.

CONCLUSIONS

(1) In the laboratory (20°C, daylight periods 12, 16, or 20 h), mass emergence of *T. embryophagum* and *T. principium* occurs during the four first hours of the photophase. Interspecific differences in the degree of the synchronicity of the emergence at different photoperiodic regimens were revealed.

(2) The control of the time of the emergence by the inborn rhythmical processes was demonstrated. The importance of the endogenous control in the regulation of the rhythms in the species examined is, however, rather low in comparison with the majority of other insects. The time of mass emergence is not fixed rigidly, because changes in the environment (light-on and/or temperature increase) can stimulate mass emergence of *Trichogramma* at any time of the day. Nevertheless, the plasticity of emergence dynamics is limited to a certain degree, because sensitivity to the ex-

ogenous stimuli is controlled by the endogenous rhythm.

(3) Interspecific differences in the relative sensitivity of *Trichogramma* to light and temperature stimuli were revealed.

(4) It was found that the light and the increase of temperature stimulate emergence of adults from host eggs not independently and their affect is integrated at certain stages of the response. Elaboration of methods of regulation and synchronization of the emergence of *Trichogramma* is impossible without taking into account the synergism of the effect of both factors.

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