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Temperature dependency of the daily pattern of adult eclosion in two *Trichogramma* species (Hymenoptera, Trichogrammatidae)

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Many aspects of insects physiology and behaviour exhibit daily periodicity, with certain biological events being restricted to a particular time of the day or night. There are, for instance, daily rhythms of locomotion, feeding, pupation, oxygen consumption, etc. The mechanisms of such periodic alternations in various aspects of insects biology are particularly well studied by the example of adult eclosion rhythm. It is well known that an exhibited daily rhythm comprises endogenous and exogenous components (Saunders, 1982; Edery, 2000). On the one hand, it is governed by internal biochemical oscillators, or "biological clocks". These endogenous oscillations are circadian (with a period of about 24 hours) and self-sustained, persisted in isolation from any external periodical information, for instance, under darkness and constant temperature. On the other hand, an exhibited rhythm may be affected by external factors, mostly by light and temperature changes, in two different ways. First, environmental fluctuations can cause direct responses in individuals, strongly inhibiting or increasing their activity. Second, photoperiod and daily temperature cycles synchronise "biological clocks" and as a result an observed rhythm achieves a particular phase relationship with environmental periodicity.

As far light and temperature conditions regularly change in accordance with latitude, the question arises: How do insects adjust their rhythms to a new temporal structure during adaptation to environmental conditions? It was repeatedly reported that the daily patterns of activity in insects species from high latitudes are more flexible, less distinct and also have another phase relation with photoperiod, in comparison with southern species (Corbet, 1966; Muller, 1973; Tshernyshev, 1984). However, most studies concerning geographical variation in the rhythms included only field observations. The lack of experimental investigations makes it difficult to reveal the mechanisms underlying the observed latitudinal dependency of circadian rhythms.

The most comprehensive comparative analysis of different geographical populations was made with species of Drosophilidae family by the example of adult eclosion rhythm (see Lankinen, 1993 and Riihimaa, 1996 for the references). The results signified that some inherited characteristics of endogenous clocks in these insects are latitude dependent. In the northern strains of most *Drosophila* species studied so far an endogenous oscillation governing eclosion had shorter period, early phase and lower amplitude. As a result of this variation, under the same photoperiod the eclosion occurred early in the morning in the higher latitudes. On the basis of these findings and some model constructions (Pittendrigh *et al.*, 1991) it was suggested that latitudinal variation in overt daily rhythms in insects can be explained by a change of period and amplitude of endogenous biochemical oscillations (the essential inherited properties of "biological clocks"), as a result of natural selection. However, other characteristics of eclosion rhythm have not been examined carefully in the aspect of geographical variation. For example, a temperature dependency of the insects timing system has not been ever compared between northern and southern species.

Like *Drosophila*, a minute hymenopteran parasitoid *Trichogramma* is an ideal model for investigation of insect rhythmicity. All species studied so far were proved to possess distinct eclosion rhythmicity. The rhythm can be synchronised with the external photo- or thermoperiod so that the maximum of emergence usually occurs near the onset of the photo- or thermophase (Tavares & Voegele, 1991; Dahiya *et al.*, 1993; Corrigan *et al.*, 1995; Pompanon *et al.*, 1995; Zaslavski *et al.*, 1999). The comparative analysis of 9 strains of 7 *Trichogramma* species collected from different geographical sites has revealed interspecies differences in the overt rhythm (Reznik *et al.*, 1998). No correlation, however, was observed between the daily pattern of eclosion and climatic conditions of the sites from which the strains originated. As far these experiments have been conducted only under two variants of conditions (a photoperiod under constant temperature 25 °C and a thermoperiod under constant light), we suggest to investigate interspecies variation further under a range of temperature conditions. A working hypothesis was that strains collected from different latitudes may differ in temperature dependency of the eclosion rhythm.

The aim of the present study was a comparative investigation of effects of temperature level on the daily pattern of adult eclosion in *T. embryophagum* (Hartig) and *T. principium* Sugonjaev et Sorokina. *T. embryophagum* is dominant in temperate regions (central Russia, Belarus, Ukraine, Western Europe), whereas *T. principium* occurs mainly in the areas with continental climate (Southern Kazakhstan, Uzbekistan, Turkmenistan) (Sorokina, 2001).

Material and methods

Two *Trichogramma* strains were used in experiments: *T. embryophagum* (Hartig), parthenogenetic strain, originated from Moscow region and *T. principium* Sugonjaev et Sorokina from Chimkent Region, Kazakhstan. The parasitoids were reared at collection sites from natural host (lepidopteran eggs) and since then have been maintained for 10 years in laboratory on eggs of the grain moth *Sitotroga cerealella* Oliv. (Gelechiidae).

For parasitization the host eggs glued on paper cards were placed for 24 h into a cage with newly emerged females of *Trichogramma*. Then the cards were put into glass tubes and transferred to experimental conditions (thermostatic chambers) under the same photoperiod (L : D = 12 : 12, light-on at 12 a.m.). Illumination was provided by fluorescent lights (50 lx). Eclosed adults were counted visually every two hours. Males and females were not recorded separately since the previous studies have demonstrated that the differences in eclosion rhythm between sexes are negligible (Reznik *et al.*, 1998).

The experiment was conducted under the same photoperiod (L : D = 12 : 12) but different constant temperature: 15, 20 or 25 °C. The entire development of parasitoids occurred under the same regimes, except the variant with 15 °C. In the latter experiment the parasitoids initially developed under 20 °C, to prevent induction of diapause, and were placed to 15 °C after the pupation (at 16 day of development). The records of eclosing adults started after 5 days of exposition under the low temperature. In all the regimes, eclosion was recorded during 48 h in 3-8 replicates.

Distribution of eclosion time was compared among treatments by Kolmogorov-Smirnov test.

Results

The daily patterns of adult eclosion under photoperiod L : D = 12 : 12 and different constant temperatures are shown at Fig. 1. In both species, eclosion during dark period was rather week under all temperature regimes, not more than 17% of adults emerged from host eggs by light-on (records at 12 h), most parasitoids eclosed within first 4 h of photophase (by 16 h). Pattern of eclosion depended on temperature: the lower was the temperature, the higher was a percentage of adults emerging at the middle of the photophase (16-20 h). Differences between the regimes were statistically significant in both species (Kolmogorov-Smirnov test, $p<0.001$). *T. embryophagum* changed the dynamics of eclosion under low temperature conditions to a greater extent than *T. principium*: at 15 °C the peak of eclosion shifted from 14 to 16 h, whereas in *T. principium* the peak occurred at 14 h in all the regimes. To the contrary, the latter species was more sensitive to high temperature conditions and started eclosion early under 25 °C, in comparison with *T. embryophagum.*

Discussion

The pattern of eclosion in studied species strongly depended on temperature level. The tendency was the same in both *T. embryophagum* and *T. principium*: under lower temperatures the percentage of adults eclosing during the dark period and within 2 h after light-on was lower. Correspondingly, more adults eclosed at the middle of photophase (Fig. 1).

Such a temperature dependency of circadian rhythms was repeatedly observed in many insects. As a rule, under low temperatures the level of day-time activity increases, while night-time activity decreases. For instance, peak of adult eclosion in fruit fly *Chimomyza costata*, sunflower moth *Homoesoma electellum* and midge *Chironomus thummi* shifted to the middle of photophase under low temperature conditions (Kureck, 1979; Riemann, 1991; Lankinen & Riihimaa, 1997). Many species of crickets are active primarily during night hours under higher temperatures, but at light period under lower temperatures (Loher & Wiedenmann, 1981; Ikeda & Tomioka, 1993). Recent studies suggest that such temperature effects may be based on molecular mechanisms universal for many insects (Sidote *et al.*, 1998; Majercak *et al.*, 1999). This feature apparently allows individuals to adjust the timing of activity to seasonal changes of temperature factor so that during cold periods insects use light hours (the warmest part of daily cycle) most effectively and reduce activity during the night (Loher & Wiedenmann, 1981). Noteworthy also is an importance of air humidity for minute insects. At low temperature conditions it may be risky for adults to eclose during the night because high saturated humidity can prevent normal spreading and hardening of wings (Riihimaa, 1996; Lankinen & Riihimaa, 1997). Thus, the inhibition of emergence of *Trichogramma* from host eggs at night and shortly after light-on (the coldest part of daily cycle in nature) and, correspondingly, intensified eclosion during the light period, observed in the experiments with 15 °C seem to have an adaptive significance.

The second significant finding was the interspecies differences in effect of temperature level on the daily pattern of eclosion. *T. embryophagum* changed the rhythm under low temperature conditions to a greater extent than *T. principium.* The latter species, in turn, responded to highest temperature level (25 °C) by strongly intensified eclosion in dark period, in contrast to *T. embryophagum* (Fig. 1).

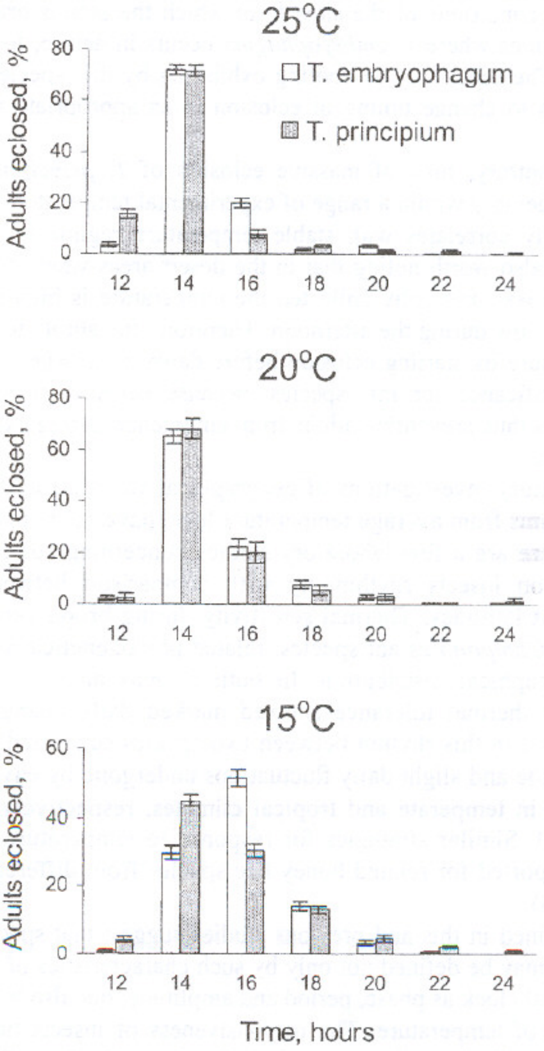

Fig. 1. Daily dynamics of eclosion in *T. embryophagum* and *T. principium* under different constant temperatures (photoperiod L : D = 12 : 12). Records were made only during photophase. At first record (12 h after light-off) all adults eclosing within dark period were counted. The error bars indicate confidence intervals

Such a species-specific temperature dependency apparently correlated with climatic conditions of the sites from which the strains originated. In temperate regions where *T. embryophagum* occurs in nature, frosts are not uncommon. The sensitivity to cooling exhibiting by this species probably allows insects to change timing of eclosion in an appropriate way during cold periods.

To the contrary, time of massive eclosion of *T. principium* did not change considerably within a range of experimental temperatures. This feature apparently correlates with stable temperature regime in continental climate. It is also worth noting that in the desert areas where the strain of *T. principium* was originally collected the temperature is high and air humidity is very low during the afternoon. Therefore, the ability to respond to high temperature by starting eclosion before dawn, in darkness, may be of adaptive significance for this species, because eclosion is restricted to morning hours thus preventing adults from emergence during extremely hot and dry period.

No laboratory investigations of geographical variation in dependency of daily rhythms from average temperature level have come to our notice. However, there are a few laboratory studies concerning some effects of temperature on insects rhythmicity with comparison between species from different latitudes. Thermal sensitivity during brood care was analysed in two *Camponotus* ant species, related phylogenetically but disparate in geographical distribution. In both *C. mus* and *C. rufipes*, the threshold for thermal tolerance showed marked daily changes. Differences observed in this rhythm between two species suggested an adaptation to the wide and slight daily fluctuations undergone by environmental temperatures in temperate and tropical climates, respectively (Roces & Nunez, 1995). Similar strategies for response to temperature influences have been reported for related honey bee species from different latitudes (Nunez, 1979).

Data obtained in this and previous studies suggest that specificity of a daily rhythm may be defined not only by such characteristics of an endogenous biological clock as phase, period and amplitude, but also by sensitivity to influences of temperature. The responsiveness of insects time-keeping mechanisms to this factor is apparently defined genetically as far there are evidences that temperature exerts its influence on circadian oscillators through certain molecular substrates (specific proteins) (Sidote *et al.*, 1998; Yoshii *et al.*, 2002). Thus, the characteristic can be submitted to natural selection.

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References

Corbet, P.S. 1966. Dial patterns of mosquito activity in a high arctic locality. *Canad. Entomologist* 98: 1238-1252.

Corrigan, J.E, Laing, J.E. & J.S. Zubricky. 1995. Effects of parasitoid to host ratio and time of day of parasitism on development and emergence of *Trichogramma minutum* (Hymenoptera: Trichogrammatidae) parasitizing eggs of *Ephestia kuehniella* (Lepidoptera: Pyralidae). *Ann. entomol. Soc. Am.* 88: 773-780.

Dahiya, A.S., Tshernyshev, W.B. & V.M. Afonina. 1993. Diurnal rhythm of emergence from pupae in parasitic wasp. *J. Interdiscipl. Cycle Res.* 24: 162-170.

Edery, I. 2000. Circadian rhythms in a nutshell. *Physiol. Genomics* 3: 59-74.

Ikeda, M. & K. Tomioka. 1993. Temperature dependency of the circadian locomotor rhythm in the cricket *Gryllus bimaculatus*. *Zool. Sci.* 10: 597-604.

Kureck, A. 1979. Two circadian eclosion times in *Chironomus thummi*, alternately selected with different temperatures. *Oecologia* 40: 311-323.

Lankinen, P. 1993. North-south differences in circadian eclosion rhythm in European populations of Drosophila subobscura. *Heredity* 71: 210-218.

Lankinen, P. & A. Riihimaa. 1997. Effects of temperature on weak circadian eclosion rhythmicity in Chymomyza costata (Diptera: Drosophilidae). *J. Insect Physiol.* 43: 251-260.

Loher, W. & G. Wiedenmann. 1981. Temperature-dependent changes in circadian patters of cricket premating behaviour. *Physiol. Entomol.* 6: 35-43.

Majercak, J., Sidote, D., Hardin, P.E. & I. Edery. 1999. How a circadian clock adapts to seasonal decreases in temperature and day length. *Neuron* 24: 219-230.

Muller, K. 1973. Circadian rhythms of locomotor activity in aquatic organisms in the subarctic summer. *Aquilo Ser. Zool.* 14: 1-18.

Nunez, J.A. 1979. Comparative study of thermoregulation between European and Africanized Apis mellifera in Brasil. *J. Apic. Res.* 18: 116-121.

Pittendrigh, C.S., Kyner, W.T. & T. Takamura. 1991. The amplitude of circadian oscillations: temperature dependence, latitudinal clines, and the photoperiodic time measurement. *J. Biol. Rhythms* 6: 299-313.

Pompanon, F., Fouillet, P. & M. Bouletreau. 1995. Emergence rhythms and protandry in relation to daily patterns of locomotor activity in *Trichogramma* species. *Evol. Ecol.* 9: 467-477.

Reznik, S.Ya., Zinovjeva, K.B., Umarova, V.A. & V.A. Zaslavski. 1998. Dependence of eclosion rhythm on photoperiod and thermoperiod in species of the genus *Trichogramma* Westw. (Hymenoptera, Trichogrammatidae). *Entomol. Rev.* 78: 13-19.

Riemann, J.G. 1991. Effect of thermoperiod and photoperiod on the eclosion rhythm of the sunflower moth (Lepidoptera: Pyralidae). *Environ. Entomol.* 20: 1322-1326.

Riihimaa, A. 1996. *Genetic variation in diapause, cold-hardiness and circadian rhythm in Chymomyza costata*. Oulu. 52 pp.

Roces, F. & J.A. Nunez. 1995. Thermal sensitivity during brood care in workers of two *Camponotus* ant species: Circadian variation and its ecological correlates. *J. Insect Physiol.* 41: 659-669.

Saunders, D.S. 1982. *Insect clocks.* Pergamon Press. Oxford. 409 pp.

Sidote, D., Majercak, J., Parikh, V. & I. Edery. 1998. Differential effects of light and heat on the *Drosophila* circadian clock proteins PER and TIM. *Molec. Cell. Biol.* 18: 2004-2013.

Sorokina, A.P. 2001. *Estimation of Trichogramma species perspective for plant protection.* St.Petersburg, Russian Institute of Plant Protection. 44 pp. (In Russian).

Tavares, J. & J. Voegele. 1991. Influence of the photoperiod on the egg laying rhythm and emergence of three *Trichogramma* species (Hymenoptera, Trichogrammatidae). *Redia* 74: 309-314.

Tshernyshev, W.B. 1984. *Sutochnye ritmy aktivnosti nasekomykh [Diurnal rhythms of activity in insects].* Moscow State Univ. 216 pp. (In Russian).

Zaslavski, V.A., Zinovjeva, K.B., Umarova, T.Ya. & S.Ya. Reznik. 1999. Interaction of circadian rhythm synchronised by photoperiod and by thermoperiod with direct influence of light and temperature as factors determining rhythm of adult eclosion in two species of *Trichogramma* (Hymenoptera, Trichogrammatidae). *Entomol.* Rev. 79: 1-10.

Yoshii, T., Sakamoto, M. & K. Tomioka. 2002. A temperature-dependent timing mechanism is involved in the circadian system that drives locomotor rhythms in the fruit fly *Drosophila melanogaster. Zool. Sci.* 19: 841-850.