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Circadian Rhythm of Light Sensitivity in the Beetle *Coccinella septempunctata* (Coleoptera, Coccinellidae): Effects of Illumination and Temperature

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Abstract—Beetles *Coccinella septempunctata* L. are active only during daylight. It is demonstrated that sensitivity to light, the photopreferendum, and locomotor activity are regulated by endogenous circadian oscillators. The percentage of beetles that became active during the first 10 min after light-on was determined. Sensitivity to light (100, 1000 or 7000 lx) reached the maximum in the daytime (period of activity) and the minimum at night (resting period) irrespective of temperature (17 or 26°C). Illumination and temperature serve as modifying factors for the pattern and amplitude of the circadian rhythm of light sensitivity.

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In insects, excitability of the sensory system, especially of its visual section, is affected by the circadian modulation. This is manifested in significant daily changes in the amplitude of electroretinogram of the response of the compound eye to stimulation by the standard light impulse (Koehler and Fleissner, 1978; Fleissner, 1982; Yamazaki et al., 1989) or in the intensity of behavioral response to illumination (Zotov, 1983; Chernyshev, 1984). It is assumed that the circadian rhythm of light sensitivity in insects is determined by a complex of retinomotor phenomena (Sakura et al., 2003) and is regulated by the same endogenous oscillators that control the circadian rhythm of activity (Wills et al., 1985; Chang, Lee, 2001). It is, however, unclear, whether the rhythm of light sensitivity is purely endogenous or its parameters are also determined by exogenous environmental factors. It is known that illumination and temperature modify the amplitude and pattern of the rhythm of activity. We decided to reveal, whether the illumination and temperature correspondingly modify the rhythm of sensitivity to light as well.

Coccinella septempunctata L. were used in the experiment; these beetles are characterized by the circadian rhythm of light sensitivity with the maximum expressed during the daylight hours (Zotov, 1983). The rhythm was studied at different values of constant illumination and constant temperature in the experiment. The photopreferendum was also observed at different hours of the day.

MATERIALS AND METHODS

Beetles *C. septempunctata*, collected in different regions of Moscow Province, were used in our experiments. The studies were performed in the laboratory at constant temperature (20–21°C) and in darkness (degree of illumination <1 lx).

Photopreferendum was studied with the use of alternative chambers. Each chamber was made of a Petri dish 10 cm in diameter. Each half of the upper and lower covers of the dish was patched with black paper. A blind was fixed between the dark and illuminated halves of the chamber; this blind allowed free passage of beetles from one half to the other, but isolated the darkened half from light. Ten beetles were placed simultaneously in each chamber. For one day, beetles freshly collected in nature were maintained in alternative chambers in total darkness; after that, the chambers were illuminated from above with a table lamp (illumination at the bottom of each chamber constituting 450 lx). A total of six one-day-long observations were performed. Five Petri dishes (a total of 50 beetles) were used on each day of experiment. Visual observations were made after each hour, when the number of beetles in the illuminated half of the chamber was recorded.

The number of specimens that became active during the first 10 min after the beginning of illumination demonstrated the degree of light sensitivity. Before the exposure to light, beetles stayed for a day in a thermo-

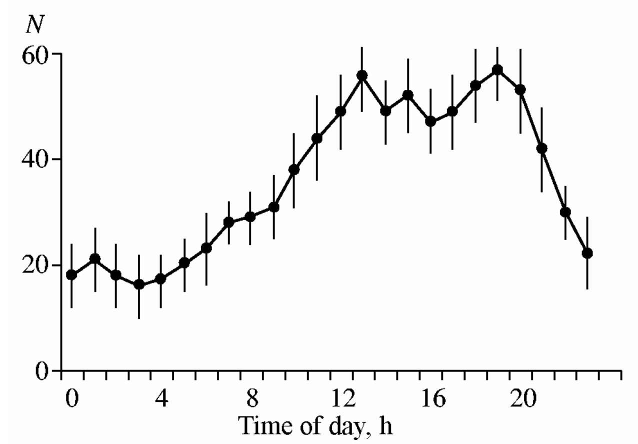

Fig. 1. Daily changes of photopreferendum in beetles *Coccinella septempunctata* at 20°C. (N) fraction of beetles that stayed in illuminated (450 lx) half of the alternative chamber in the given time of the day (average value, %).

stat at 20°C in Petri dishes (10 beetles in each dish). The experiment includes two variants and three replications in each variant.

In the first variant, reaction of beetles to light with the intensity constituting 100,1000 or 7000 lx was studied. During the whole day, each four h two Petri dishes with beetles were replaced from the thermostat to a table; a lamp with a reflector providing the necessary degree of illumination was switched on. In order to maintain the constant surface temperature (20°C), a flat microbiological bottle with a 10% sulphate of copper solution was used as a heat filter.

In the second variant, the influence of temperature on the light reaction of beetles was examined. For this purpose, Petri dishes with beetles were previously maintained in darkness in thermostats. In one thermostat, the temperature was 17°C; in the other one, 26°C. After one day, with a two-hour interval, two dishes from each thermostat were placed into two other thermostats with similar temperatures. Inside these thermostats, light (about 1000 lx) was switched on and the behavior of beetles was observed through a glass door of the thermostat.

In both variants, percentage of active beetles was recorded during the first 10 min after the beginning of illumination; the data were summarized and the average percentage with the confidence interval (at $P = 0.95$) was calculated.

New beetles were used in each variant. During experiments, beetles were fed on sugar syrup (a cotton plug saturated with sugar solution was placed in each Petri dish).

RESULTS AND DISCUSSION

In the laboratory, when the beetles were isolated from external light-dark and temperature cycles, they demonstrated the circadian rhythm of the photopreferendum (Fig. 1). In the morning, the beetles actively responded to light, trying to fly out of chambers. In the afternoon, they quietly crawled and fed in the illuminated half of the dish; in the evening (after 8 p.m.),

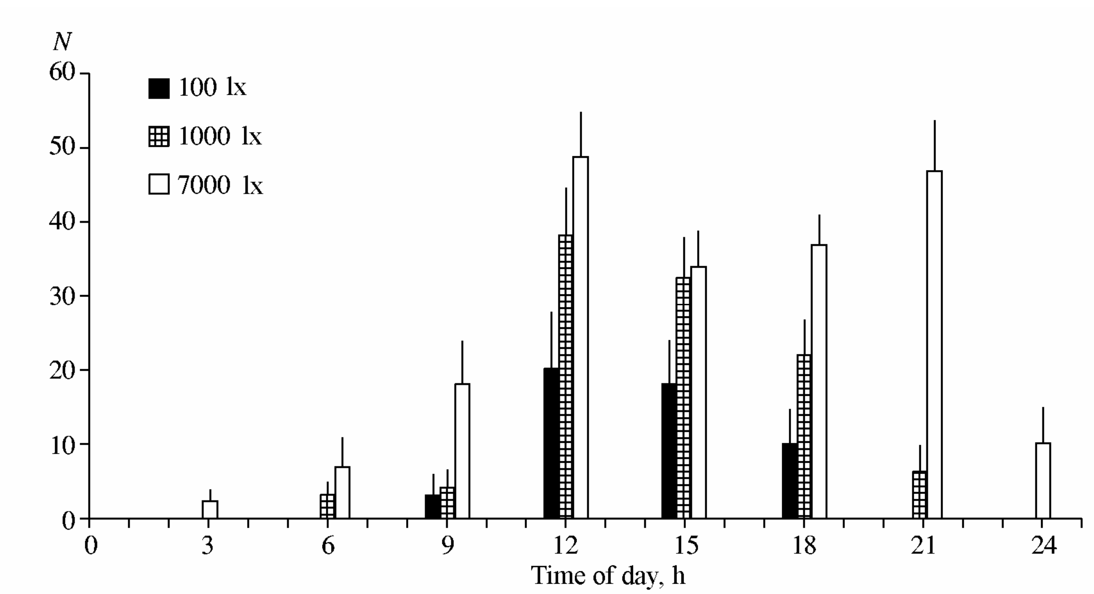

Fig. 2. Daily changes of sensitivity in the beetles *C. septempunctata* to light of different intensity at 20°C. (*N*) here and in Fig. 3, fraction of beetles that became active during the first 10 min after switching the light on at the given time of the day (average value, %).

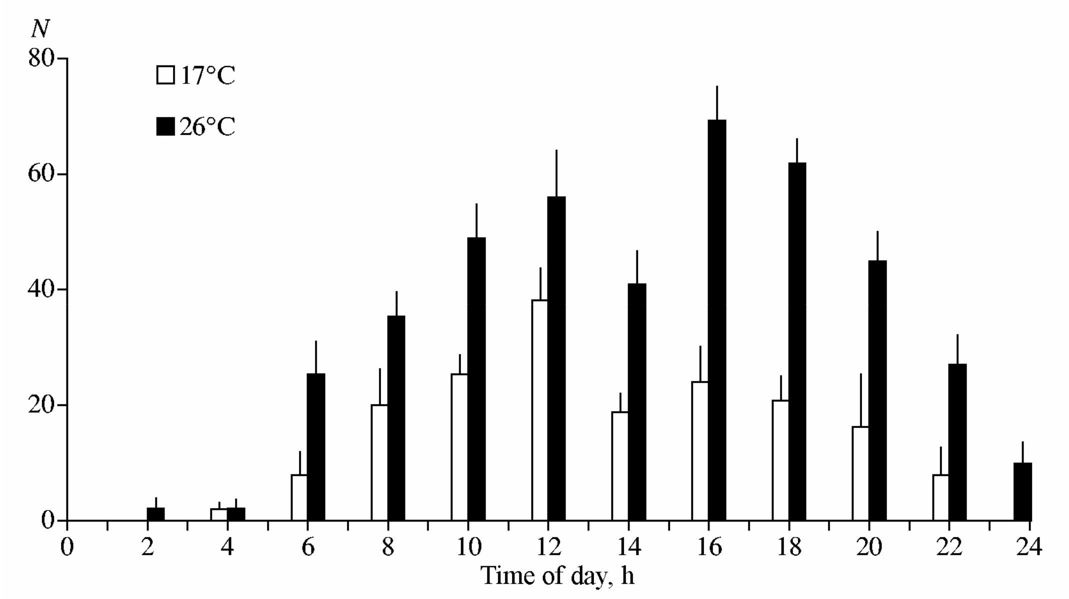

Fig. 3. Daily changes in sensitivity of beetles *C. septempunctata* to light (1000 lx) at 17 and 26°C.

they began walking into the darkness. A certain number of beetles stayed in the illuminated area also during the night; as a rule, they were very active. In the opinion of Chernyshev (1984), the circadian rhythm of photopreferendum is most characteristic of insects that change their habitats during the day, e.g., they are active in the open air but rest in shelters. Ladybeetles *C. septempunctata* live on exposed plants and don't use shelters for rest. Nevertheless, vertical migrations on plants during the day are characteristic of ladybird larvae and beetles (Kaufman, 1981; Banks, 1957). We assume that the rhythm of the photopreferendum is associated with vertical migrations.

The circadian rhythm of light sensitivity was registered in ladybirds at all the values of illumination and temperature examined (Figs. 2 and 3). The minimal number of specimens that became active during the first 10 min after the light was switched on was noted in the first hours of the resting period and the maximal number, in the daytime hours of the period of activity. Illumination and temperature significantly modified the amplitude and pattern of the rhythm. The highest values of the amplitude of the rhythm and the appearance of an additional evening maximum were observed at high values of illumination (7000 lx) and temperature (26°C).

Thus, on the one hand, the results of our studies confirmed the assumption on the existence of the single circadian regulation of activity and light sensitivity rhythms. On the other hand, they allow explaining the role of the circadian rhythms in the adaptive strategy of *C. septempunctata*. In nature, beetles are active only during the lighttime of the day. At night, they are always passive. Without changing their habitat during the day, beetles are constantly influenced by such a stable synchronizing factor, as the 24-hour-long light-dark cycle. The modifying effects of illumination and temperature allow beetles rapidly adapt to specific environmental conditions and be active during each favorable moment. Apparently, circadian organization of the behavior similar to that revealed in *C. septempunctata* seems to be also characteristic of insects dwelling in temperate and northern latitudes with unstable weather.

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