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Rhythmicity in life events of an aphidophagous ladybird beetle, *Cheilomenes sexmaculata*

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Abstract: The present laboratory experiments have been carried out to investigate rhythms in various life events, viz. mating, oviposition, hatching, moulting, pupation and eclosion, of an aphidophagous ladybird beetle, *Cheilomenes sexmaculata* under laboratory conditions. Incidences of mating and eclosion occurred mostly (66% and 73% respectively) in photophase. While oviposition, hatching, moulting and pupation occurred during scotophase with the peaks of the former two distributed between time intervals 12:00–15:00 h and 21:00–00:00 h respectively. Confinement of life events that are associated with vulnerable stages in scotophase might be an adaptation to prevent their exposure to natural enemies.

Key words: *Cheilomenes sexmaculata*, Coccinellidae, eclosion, hatching, mating, moulting, oviposition, pupation, rhythmicity

1 Introduction

Rhythms in organisms have evolved as adaptive strategies for successful survival with their phases changing in response to important environmental and temporal cues, such as daily light and temperature cycles. The rhythms may be free running with a period of about 24 h under constant conditions and are regulated by endogenous clocks or oscillators. Many internal clocks forming a complex circadian system provide synchronization of life events of an organism with environmental variables (Plautz et al. 1997; Giebultowicz 1999; Edery 2000; Myers 2003).

Rhythmicity in life-events, viz. mating, oviposition, hatching, moulting, pupation, emergence, foraging, and flight activity is well known in insects (Saunders 1982; Schotzko and O'Keeffe 1986, 1990; Zinov'eva et al. 1996; Jimenez-Perez et al. 2002; LiJuan et al. 2002). Those pupating above ground are likely to be more sensitive to daily changes in light than temperature and hence more synchronized to the former. Amongst such insects, predatory ladybird beetles (Coleoptera: Coccinellidae) are of pragmatic value being potential biocontrol agents of several insect and mite pests of agricultural and horticultural crop plants. Although ample literature exists on the ecology and behaviour of coccinellids (Hodek and Honěk 1996; Dixon 2000), the rhythmicity of life events has been meagerly studied. A few previous studies reveal prominent diurnal periodicity in foraging and feeding patterns of ladybird beetles (Hull et al. 1977; Mack and Smilowitz 1978; Miura and Nishimura 1980; Nakamuta 1987). Some recent attempts have also been made to provide information on the biorhythms of predaceous ladybirds (Mishra and Omkar 2004; Omkar et al. 2004a).

More such studies elucidating the spatial and temporal occurrence of life events and identifying the underlying patterns will enhance understanding of basic ecology along with providing cues that may facilitate mass multiplication and utilization of ladybird beetles in biocontrol of target insect pests. Thus, the present investigation has been carried out to study and analyse rhythmicity in various life events, viz. mating, oviposition, hatching, moulting, pupation and emergence, of a ladybird beetle, *Cheilomenes sexmaculata* (Fabricius) under laboratory conditions. *Cheilomenes sexmaculata* is a native predator of aphids in Oriental and Palaearctic regions and found abundantly almost throughout the year in areas around Lucknow, India (Hussein 1991; Omkar and Bind 1993; Omkar and Pervez 2004). Its high reproductive and predatory response on many aphid species (Omkar and Bind 2004; Omkar et al. 2004b) and better performance in competitive environment (Agarwala and Yasuda 2000; Omkar et al. 2005) has been the criterion for its selection as an experimental model.

2 Materials and Methods

2.1 Stock culture

Adults of *C. sexmaculata* were collected from agricultural fields adjoining the city of Lucknow (26°50′N, 80°54′E), India and brought in the laboratory to establish stock culture in Environmental Test Chamber (ETC) (at 25 ± 2°C, 65 ± 5% RH; 10L: 14D; white light) along with prey *Aphis craccivora* Koch infested on *Dolichos lablab* Linnaeus. They were paired and allowed to mate in transparent Petri dishes (9.0 × 1.5 cm). Eggs were collected daily and hatched instars reared till pupation in glass beakers (11.0 × 9.0 cm) on daily replenishment of *ad libitum* prey. Beakers containing pupae were inspected every 4 h and emerging new adults were sexed and isolated in Petri dishes (space and prey as above) for further use in experiments.

2.2 Experimental setup

The experimental ladybird beetles were kept in beakers in ETC provided with the above prey. The lights for maintaining the desired photophase were kept switched on between 00:00 and12:00 h (photophase) and switched off between 12:00 and 00:00 h (scotophase) with experimental conditions similar to those for stock culture. For recording data in scotophase the ladybird beetles were exposed to brief duration of red light (under ~3.8 V red light bulb having light intensity ~190–200 lux; measured by an EP628 digital lux meter by Eurisem technics (Eurisem technics, Leicester, Leicestershire, UK)).

2.3 Mating rhythm

For the study of mating rhythms over a 24-h period, 10-day-old ladybird beetle pair ($n = 10$) was selected and placed in a Petri dish under suitable abiotic and biotic conditions (as above). Each pair was observed at an interval of 3 h for the incidence of mating and its occurrence recorded. The recordings were made for five consecutive days.

2.4 Ovipositional rhythm

To record the ovipositional rhythm an unmated mating pair after being subjected to single mating was observed after every 3 h from 10th day of oviposition for two consecutive days. The observations were taken in 10 replicates.

2.5 Hatching rhythm

One hundred eggs were selected from the above experiment and hatching was recorded at an interval of 3 h. The experiment was replicated 10 times, thus hatching in a total of 1000 eggs was recorded.

2.6 Moulting, pupation and eclosion rhythms

Ten first instars of *C. sexmaculata* were placed in a beaker (6.5 × 9.5 cm) with prey and conditions as above and observed every 2 h through their inter developmental period for the incidence of moulting, pupation and eclosion. The experiment was replicated 10 times, thus in a total of 100 individuals.

2.7 Data analysis

The data on number of incidences of various life-events, viz. mating, oviposition, hatching, moulting, pupation and eclosion, at different time intervals were subjected to two-way ANOVA taking time interval as row factor and days as a column factor followed by *post hoc* test for comparison of means. The percent data on total incidences of life-events in the photophase and scotophase over different days were subjected to chi square analysis. All statistical analyses were performed on PC using computer software MINITAB (Minitab Inc., State Coltege, Pennsylvania, USA).

3 Results

3.1 Mating rhythm

In a diel cycle, mating occurred mostly throughout photophase (66%; $\chi^2 = 20.48$; $P < 0.001$; fig. 1) and early scotophase, and was relatively reduced during late scotophase as lower percentages (1% and 3%) of mating were recorded between 18:00–21:00 and 21:00–00:00 h respectively (fig. 2). The differences in means of mating incidences at 3-h interval were statistically significant ($F = 10.13$; $P < 0.001$; d.f. = 7, 399). However, the rhythm was not found to change significantly between days ($F = 0.63$; $P > 0.05$; d.f. = 4, 399). The interaction between days and time intervals was statistically significant ($F = 1.77$; $P < 0.05$; d.f. = 28, 399).

3.2 Ovipositional rhythm

In the diel cycle, peak of oviposition (62%) was attained during early scotophase (12:00–15:00 h). Relatively lower percentages of eggs (1–15%) were laid during rest of the day and none during 0600–0900 h

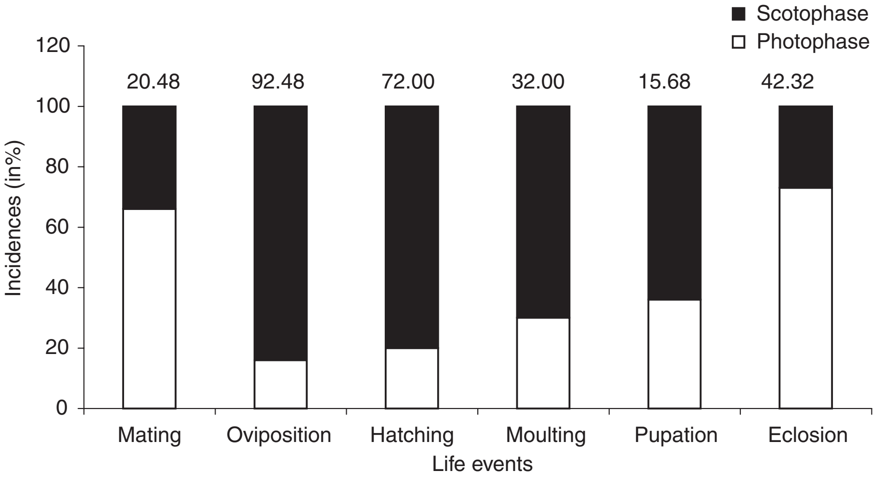

Fig. 1. *Incidence of various life events in photophase and scotophase; chi square test values, given above the bars significant at P < 0.001 level*

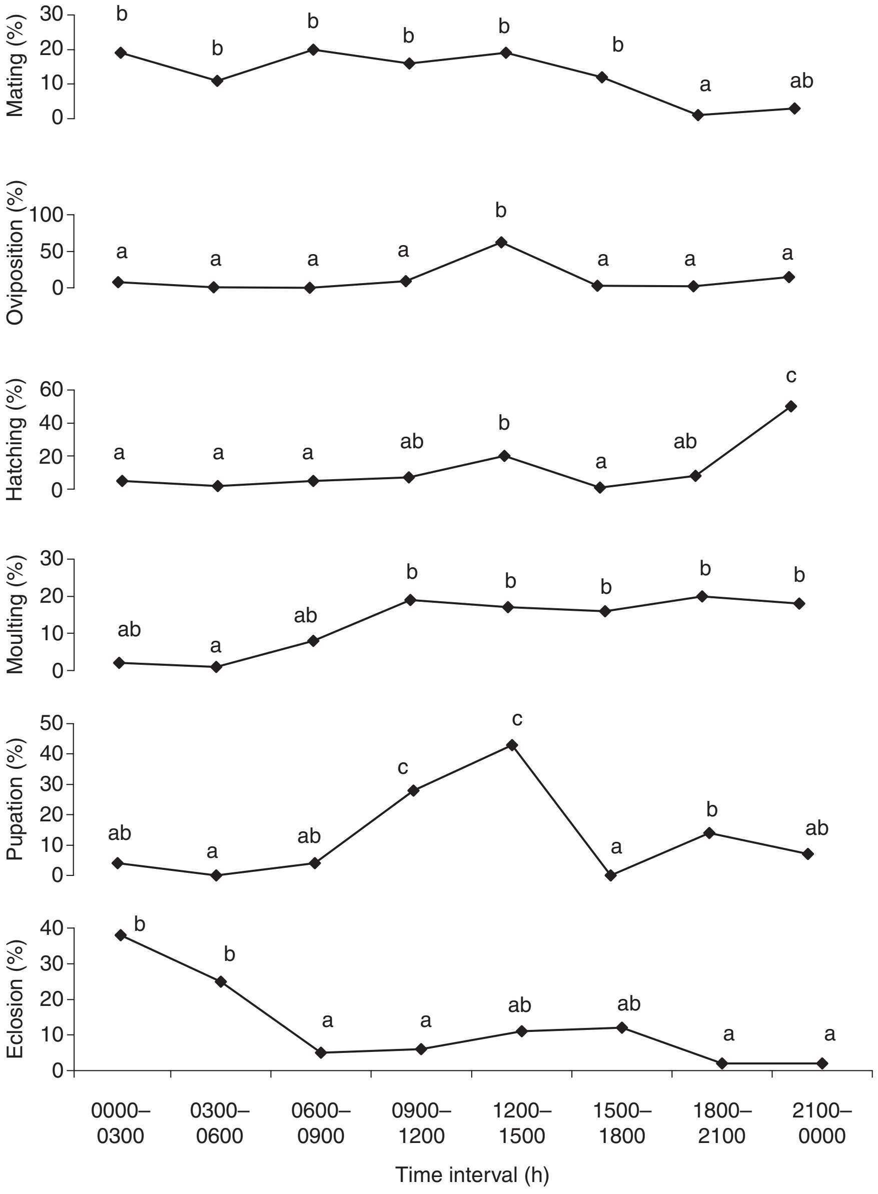

Fig. 2. *Diel rhythms of various life events; different letters denote data significant at $P < 0.001$*

(fig. 2). Overall oviposition was significantly higher (82%; $\chi^2 = 92.48$; $P < 0.001$; fig. 1) in the scotophase. The differences in means of oviposition at 3-h interval were statistically significant ($F = 5.93$; $P < 0.001$; d.f. = 7, 159). However, the rhythm did not change ($F = 0.20$; $P > 0.05$; d.f. = 1, 159) in between the days. The interaction between days and time intervals was also not significant statistically ($F = 0.18$; $P > 0.05$; d.f. = 7, 159).

3.3 Hatching rhythm

In a diel cycle, significantly high incidences ($F = 13.61$; $P < 0.01$; fig. 2) of hatching were observed during early scotophase (12:00–15:00 h) and a peak (50%) during late scotophase (21:00–00:00 h). Significant lower hatching incidences were observed during 15:00–18:00 h, major part of the photophase (00:00–09:00 h) and slightly increased levels during 18:00–21:00 h and late photophase (09:00–12:00 h). Overall, significantly more hatching incidences (80%; $\chi^2 = 72.00$; $P < 0.001$; fig. 1) were observed during scotophase. Differences in means of incidences of hatching within 24 h were statistically significant ($F = 13.61$; $P < 0.001$; d.f. = 7, 159). Differences in means of incidences of hatching in between different days ($F = 31.07$; $P < 0.001$; d.f. = 1, 159) and interaction between time interval and days ($F = 9.54$; $P < 0.001$; d.f. = 7, 159) were also statistically significant.

3.4 Moulting rhythm

In a diel cycle, relatively high levels (16–20%) of moulting were observed during late photophase and throughout scotophase (09:00–00:00 h). Levels of moulting were significantly decreased (1–8%) during rest of the photophase (00:00–09:00 h; $F = 2.98$; $P < 0.01$; fig. 2). Overall, moulting incidences were significantly higher (70%; $\chi^2 = 32.00$; $P < 0.001$; fig. 1) during scotophase, which apparently increased during the later half of the scotophase. Differences in means of moulting incidences at 3-h intervals were statistically significant ($F = 2.99$; $P < 0.01$; d.f. = 7, 239). Differences in means of incidences of moulting in between different days ($F = 1.58$; $P > 0.05$; d.f. = 2, 239) were not statistically significant, however, interaction between time interval and days was statistically significant ($F = 3.15$; $P < 0.001$; d.f. = 14, 239).

3.5 Pupation rhythm

Relatively higher incidences (28% and 43%) of pupation were observed during late photophase (09:00–12:00 h) and early scotophase (12:00–15:00 h) respectively. Significantly less incidences (4%, 4% and 7%, 14%) of pupation were recorded during early photophase (00:00–03:00 h), between 06:00–09:00 h and later half of the scotophase (18:00–00:00 h) respectively, while none during 03:00–06:00 h and 15:00–18:00 h. Most of the pupations were concentrated (64%; $\chi^2 = 15.68$; $P < 0.001$; fig. 1) in the scotophase. Differences in means of pupation over 24 h ($F = 11.19$; $P < 0.001$; d.f. = 7, 239), in between different days ($F = 15.14$; $P < 0.001$; d.f. = 2, 239) and interaction between the time interval and different days ($F = 5.89$; $P < 0.001$; d.f. = 14, 239) were statistically significant.

3.6 Eclosion rhythm

Higher incidences (38% and 25%) of eclosion were observed in the first half of the photophase (00:00–03:00 and 03:00–06:00 h respectively); however, relatively lesser incidences (2–12%) of eclosion were observed in the later half of the photophase (06:00–12:00 h). Similar trend was followed in the scotophase with slightly increased levels during its earlier half (12:00–18:00 h) and decrease in the later half (18:00–00:00 h) (fig. 2). Overall incidences of eclosion were significantly higher (73%; $\chi^2 = 42.32$; $P < 0.001$; fig. 1) in the photophase. The differences in means of eclosion within a day ($F = 9.18$; $P < 0.001$; d.f. = 7, 159), in between different days ($F = 10.54$; $P = 0.001$; d.f. = 1, 159) and interaction between time interval and days ($F = 4.07$; $P < 0.001$; d.f. = 7, 159) were statistically significant.

4 Discussion

Our results reveal prominent diel rhythmicity in various life events, viz. mating, oviposition, hatching, moulting, pupation and eclosion, in the ladybird, *C. sexmaculata* under laboratory conditions. Maximum incidences of mating were recorded in the photophase in contrast with those found in another ladybird beetle, *Propylea dissecta* (Mulsant), where they were mostly concentrated in the scotophase (Mishra and Omkar 2004).

Significantly high oviposition was observed during scotophase as also reported in other ladybird beetles, viz. *Coccinella septempunctata* Linnaeus, *C. transversalis* and *P. dissecta* (Omkar et al. 2004a). We speculate that absence of egg laying during afternoon hours and lesser egg laying during rest of the photophase might be a strategy to avoid exposure of eggs to high temperatures at more vulnerable stages of development; eggs normally hatch within 2 days at 25°C. Egg-laying by insects at certain times of the day is well documented. In many insects the ultimate cause of such periodicity, whether environmentally controlled or internally driven by biological clocks, are largely unknown (Saunders 1982).

Hatching, moulting and pupation occurred mainly during scotophase in this ladybird beetle having the peak of the former in the late scotophase (21:00–00:00 h), while in *P. dissecta* they were constricted in the same phase with the peaks distributed between 01:00 and 03:00 h (Mishra and Omkar 2004). In a pyralid, *Diatrea grandiosella* Dyar, moulting occurred almost in the same period (Takeda 1983). This appears to be a survival strategy because newly hatched instars are relatively more vulnerable to predation.

Adults of *C. sexmaculata* eclosed mostly during early photophase (i.e. 00:00–03:00 h). Similarly, in *Bombyx mori* emergence occurs early in the morning (Anonymous 1987). Peak emergence in the photophase have also been recorded for southern pine beetle, *Dendroctonus frontalis* Zimmerman, albeit during its later part (Kinn 1978), while in *P. dissecta* it was recorded in the scotophase with a peak between 01:00 and 03:00 h (Mishra and Omkar 2004). In other insects as well, e.g. lepidopterans *Heliothis armigera* (Huebner), *Opisina arenosella* Walker, aphid *Aphis gossypii* Glover and African rice gall midge, *Orseolia oryzivora* Harris and Gagne, prominent eclosion was found to occur during scotophase (Tripathi and Singh 1989; Babu 1990; Omoloye and Odebiyi 2001; XiangDong et al. 2003). A recent review explained that the sequence of behaviours necessary for eclosion is activated by hormones that relay developmental readiness. The circadian clock controlling the timing of behaviours, such as, the rest activity rhythm of adult insects also controls eclosion timing (Myers 2003).

It is apparent from the results that life events in *C. sexmaculata* are distinctly distributed in a diel cycle. Mating and eclosion rhythms occur in photophase suggesting the probable role of light in controlling these activities, as visual cues and increased temperature during daytime might aid mate search and elytral hardening, respectively. Oviposition, hatching, moulting and pupation occurred in synchrony with the scotophase when foraging activity of conspecifics and other predators was low, which might be an ecological adaptation to prevent the exposure of most vulnerable stages to natural enemies that might attack during photophase and also to the higher temperature during the daytime. This enhances the chances of survival since moulting, newly moulted instars and pupae are vulnerable stages for cannibalism and intraguild predation (Dixon 2000). Rhythmicity of behavioural activities and life events also reduces direct competition between species sharing the same resources and the synchronization of sexual activities in a population increases the efficiency of genetic isolation of sibling species during evolutionary divergence (Saunders 1982). However, in ladybird beetles further experimentation is needed to explore the rhythms over different seasons, prey and its synchronization with natural cycles of light and temperature in order to explore the origin of rhythmicity and understand the ecological significance.

Thus, it can be concluded that in *C. sexmaculata*: (i) various life events are rhythmic in principle at least under laboratory conditions; (ii) mating and eclosion occurs mostly in photophase, while oviposition, hatching, moulting and pupation in scotophase and (iii) confinement of those events that are associated with vulnerable stages mostly in scotophase might be a survival strategy to prevent their exposure to natural enemies.

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References

Agarwala BK, Yasuda H, 2000. Competitive ability of ladybird predators of aphids: a review of *Cheilomenes sexmaculata* (Fabr.) (Coleoptera: Coccinellidae) with a worldwide checklist of preys. J. Aphidol. **14,** 1–20.

Anonymous, 1987. FAO manuals on sericulture. Central silk board, Bangalore.

Babu PBS, 1990. Eclosion rhythm and its entrainment by photoperiodism in *Opsina arenosella* Walker (Lepidoptera: Xyloryctinae). Uttar Pradesh J. Zool. **10,** 115–121.

Dixon AFG, 2000. Insect predator-prey dynamics, ladybird beetles and biological control. Cambridge University Press, Cambridge, UK.

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

Giebultowicz JM, 1999. Insect circadian clocks: is it all in their head? J. Insect Physiol. **45,** 791–800.

Hodek I, Honěk A, 1996. Ecology of Coccinellidae. Kluwer Academic Publishers, Dordrecht/Boston/London.

Hull LA, Asquith D, Mowery PD, 1977. The mite searching ability of *Stethorus punctum* within an apple orchard. Environ. Entomol. **6,** 85–90.

Hussein MY, 1991. *Menochilus sexmaculatus* Fabr. (Coleoptera: Coccinellidae) its biology, prey requirement and artificial diets. J. Plant Prot. Tropics **8,** 153–160.

Jimenez-Perez A, Wang Q, Markwick NP, 2002. Adult activity patterns of *Cnephasia jactatana* Walker (Lepidoptera: Torticidae). N. Z. Plant Prot. **55,** 374–379.

Kinn DN, 1978. Diel emergence patterns of the southern pine beetle (D*endroctonus frontalis* Zimm.). J. Georgia Entomol. Soc. **13,** 80–85.

LiJuan S, Huaguo D, WeiXian Y, YongQing L, 2002. The adult emergence rhythm and mating rhythm of rice host population and water-oats host population of the rice stem borer, *Chilo suppressalis*. Entomol. Knowl. **39,** 421–423.

Mack TP, Smilowitz Z, 1978. Diurnal, seasonal, and relative abundance of *Myzus persicae* (Sulzer) predators. J. N. Y. Entomol. Soc. **86,** 305.

Mishra G, Omkar, 2004. Diel rhythmicity in certain life events of a ladybird, *Propylea dissecta* (Mulsant). Biol. Rhythm Res. **35,** 269–276.

Miura T, Nishimura S, 1980. The larval period and predaceous activity of an aphidophagous coccinellid, *Harmonia axyridis* Pallas. Bull. Fac. Agric. Shimane Univ. **14,** 144–148.

Myers EM, 2003. The circadian control of eclosion. Chronobiol. Int. **20,** 775–794.

Nakamuta K, 1987. Diel rhythmicity of prey-search activity and its predominance over starvation in the ladybeetle, *Coccinella septempunctata brucki* Mulsant (Coleoptera: Coccinellidae): releasing stimuli and decision of giving-up time. Jpn. J. Appl. Entomol. Zool. **29,** 55–60.

Omkar, Bind RB, 1993. Records of aphid-natural enemies of Uttar Pradesh. II. The Coccinellids. J. Adv. Zool. **14,** 96–99.

Omkar, Bind RB, 2004. Prey quality dependent growth, development and reproduction of a biocontrol agent, *Cheilomenes sexmaculata* (Fabricius) (Coleoptera: Coccinellidae). Biocontrol Sci. Technol. **14,** 665–673.

Omkar, Pervez A, 2004. Predaceous Coccinellids in India: predator-prey catalogue. Orient. Insects **38,** 27–61.

Omkar, Mishra G, Srivastava S, Gupta AK, 2004a. Ovipositional rhythmicity in ladybirds (Coleoptera: Coccinellidae): a laboratory study. Biol. Rhythm Res. **35,** 277–287.

Omkar, Singh SK, Pervez A, Mishra G, 2004b. Age-specific fecundity and natality life-table of an aphidophagous ladybird, *Cheilomenes sexmaculata*. Biol. Mem. **30,** 20–25.

Omkar, Pervez A, Mishra G, Srivastava S, Singh SK, Gupta AK, 2005. Intrinsic advantages of a ladybird, *Cheilomenes sexmaculata* over the relatively bigger two co-occurring *Coccinella* species. Insect Sci. **12,** 179–184.

Omoloye AA, Odebiyi JA, 2001. Endogenously entrained emergence and oviposition rhythm in the African rice gall midge, *Orseolia oryzivora* H. and G. (Dipt., Cecidomyiidae). J. Appl. Entomol. **125,** 105–107.

Plautz JD, Kaneko M, Hall JC, Kay SA, 1997. Independent photoreceptive circadian clocks throughout *Drosophila*. Science **278,** 1632–1635.

Saunders DS, 1982. Insect clocks, 2nd edn. Pergamon Press, Oxford.

Schotzko DJ, O'Keeffe LE, 1986. Ovipositional rhythms and egg melanisation rate of *Sitona lineatus* (L.) (Coleoptera: Curculionidae). Environ. Entomol. **15,** 601–606.

Schotzko DJ, O'Keeffe LE, 1990. Ovipositional rhythms of *Thyanta pallidovirens* (Hemiptera: Pentatomidae). Environ. Entomol. **19,** 630–634.

Takeda M, 1983. Ontogeny of the circadian system governing ecdysial rhythms in a holometabolous insect, *Diatraea grandiosella* (Pyralidae). Physiol. Entomol. **19,** 55–63.

Tripathi SR, Singh R, 1989. Calling and mating behaviour in *Heliothis armigera* (Hubner) (Lepidoptera: Noctuidae). Ann. Entomol. **7,** 21–25.

XiangDong L, Zhang XX, Zhai BP, 2003. Flight activity rhythm of the cotton aphid, *Aphis gossypii* Glover in Nanjing and its flight capacity. Acta Entomol. Sin. **46,** 489–493.

Zinov'eva KB, Reznic SY, Zaslavskii VA, Umarova TY, 1996. Effect of photoperiod and thermoperiod on the eclosion rhythm of adults in *Alysia manducator* (Hymenoptera: Braconidae) and *Trichogramma evanescens* (Hymenoptera: Trichogrammatidae). Zool. Zh. **75,** 1496–1504.

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