Photoperiod and Temperature Effects on the Adult Eclosion and Mating Rhythms in *Pseudopidorus fasciata* (Lepidoptera: Zygaenidae)

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**Photoperiod and Temperature Effects on the Adult Eclosion and
Mating Rhythms in *Pseudopidorus fasciata* (Lepidoptera: Zygaenidae)**

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24 **ABSTRACT**

25 Daily distributions of eclosion and mating activities of *Pseudopidorus fasciata* Walker
26 (Lepidoptera: Zygaenidae) were recorded under natural and various laboratory conditions.
27 Eclosion of this insect exhibited circadian gating in constant darkness (DD) but not in
28 constant light (LL) at 28 °C. Under natural conditions, the majority of adults emerged in
29 mid-morning with an eclosion peak around 10:00 a.m. The eclosion distribution was
30 significantly affected by ambient temperature but not by photoperiod under laboratory
31 conditions. Eclosion was more spread out at 22 °C than at higher temperatures, and peak
32 eclosion times were advanced at higher temperatures up to 30 °C. Under natural and
33 laboratory diurnal cycles, adults of *P. fasciata* preferred to mate at dusk, within a few hours
34 before the start of the scotophase. Photoperiod and ambient temperature interacted in
35 regulating the mating distribution in *P. fasciata*. Mating rhythmicity disappeared under
36 DD and LL, under which the insect either mated arrhythmically (DD) or barely mated (LL).
37 Overall, eclosion rhythm in this insect was predominantly regulated by temperature rather
38 than photoperiod, whereas photoperiod appeared to be more influential than temperature in
39 rhythmic gate of mating patterns.

40 **KEY WORDS**

41 *Pseudopidorus fasciata*; eclosion rhythm; mating rhythm; photoperiod; temperature

42 **Introduction**

43 Many insect activities, such as flight, feeding, oviposition, eclosion and mating, are often
44 repeated either by individuals or within a population in circadian rhythmic patterns
45 (Saunders 2002). The distribution of each event can be modulated by light, temperature,

46 or both (Pittendrigh 1954, Saunders 1976, Smith 1985, Lankinen and Riihimaa 1997).
47 The eclosion distribution has been widely studied in many insect species, mainly from
48 Diptera and Lepidoptera, such as *Drosophila* spp. (Bünning 1935, Kalmus 1935,
49 Pittendrigh 1954, Brett 1955, Palaksha and Shakunthala 2014), the onion fly, *Delia*
50 *antiqua* (Watari 2002, Watari 2005, Watari and Tanaka 2010); the southwestern corn
51 borer *Diatraea grandiosella* (Takeda 1983), the fall webworm *Hyphantria cunea* (Morris
52 and Takeda 1994), the corn earworm *Heliothis zea* (Callahan 1958), and the Indian meal
53 moth *Plodia interpunctella* (Kikukawa et al. 2012), etc. If an insect population is
54 synchronized to emerge into adults within the same time window each day, its eclosion is
55 considered to be gated, or restricted by its internal circadian clock (Myers 2003).
56 Likewise, mating behaviors of insects are governed by circadian clock genes (Sakai and
57 Ishida 2001). Mating mechanisms in insects, like that in many other animals, are critical
58 to breeding and enlarging their populations for better survival of the species. Studies on
59 mating pattern in insects, along with eclosion distribution, can help our understanding of
60 their reproductive strategies, and perhaps better improve timing of pest control options.

61 The zygaenid moth *Pseudopidorus fasciata* Walker (Lepidoptera: Zygaenidae) is a
62 significant defoliator of Chinese sweetleaf, *Symplocos chinensis* (Lour.) Druce
63 (Symplocaceae). There are one to three generations per year in Jiangxi Province.
64 Diapause occurs in the fourth instar in winter, and individuals aestivate as prepupae in the
65 summer (Shen and Xue 1991, Xue and Kallenborn 1998). It has been shown that winter
66 diapause induction and termination in this moth are under photoperiodic control (Wei et
67 al. 2001, Li et al. 2003, Hua et al. 2005a, Hua et al. 2005b), and summer aestivation
68 could serve as a “bet hedging” mechanism against changing environments (Wu et al.

2006). Here we show how exogenous factors, like photoperiod and temperature, impact the daily cycles of population eclosion and mating of *P. fasciata*.

Materials and Methods

The population of *P. fasciata* used in this study was collected in the suburbs of Nanchang (28° 46' N, 116° 50' E). The longest period of daylight in a year is approximately 14 h 56 min (including twilight), and the shortest is 11 h 9 min, in Jiangxi Province, Peoples' Republic of China. To observe the eclosion and mating distributions under natural conditions, overwintered (OW) full-grown larvae were collected in the field in late April, first generation (G1) larvae in early June and second generation (G2) larvae in early September. These larvae were allowed to form cocoons on foliage of Chinese sweetleaf, *S. chinensis* in round plastic boxes (diam. 15 cm, height 6 cm). The cocoons along with attached foliage were then transferred to nested cages (30 cm × 30 cm × 35 cm) and allowed to emerge into adults. All the boxes and cages were placed outdoors under natural photoperiod and temperature. The average temperature during the pupal stage was 22.1°C for OW, 30 °C for G1, and 26.3 °C for G2.

To observe the impact of photoperiod and temperature on the eclosion and mating rhythms, newly hatched larvae from G1 were reared as described above at 25 °C, LD 14:10 (light 14 h: dark 10 h) until they formed cocoons. Newly formed cocoons were then transferred to various photoperiod (LD 11:13, LD 13:11 or LD 15:9) and temperature (22, 25, 28 or 30 °C) combinations to monitor eclosion and mating activities. Also, constant darkness and constant light at 28 °C were used to investigate the inherent eclosion and mating distribution patterns excluding the interference from photoperiod. Insects were kept under these conditions from cocoon formation to adult eclosion. The

average time required for pupal development was 16 days at 22 °C, 14 days at 25 °C, and 13 days at 28 °C and 30 °C. Prepupae that did not emerge within 20 days were assumed to be in diapause and were not used in the experiments.

Emerged adults were counted hourly and transferred to nested cages to mate. The number of mating couples was recorded every half an hour and removed. Eclosion and mating activities in darkness were observed under dim red light (wavelength > 600 nm). Laboratory experiments were performed in illuminated incubators (LRH-250-GS, Guangdong Medical Appliances Plant, Guangdong, P. R. China) equipped with four fluorescent 30 W tubes. Light intensity applied was 500 - 700 lx and variation of temperatures was $\pm 1^{\circ}\text{C}$. The scotophase was controlled manually by covering the cage with a black opaque hood.

Rhythmicity in the temporal pattern of eclosion and mating was evaluated by Rayleigh's test and by cosinor analysis (Refinetti et al. 2007). Pairwise comparison of temporal patterns was conducted by the Kolmogorov-Smirnov test for two samples (Siegel 1956). Because multiple tests were conducted, a significance level of 0.01 was set for each comparison, in an attempt to avoid an increase in the probability of family-wise Type I error without greatly reducing statistical power.

Results

1. Eclosion distribution

Eclosion of *P. fasciata* exhibited daily rhythmicity in constant darkness (Rayleigh test, $\chi^2(2) = 17.328$, $P = 0.0002$) but not in constant light ($\chi^2(2) = 0.531$, $P = 0.767$) at 28 °C

(Fig. 1). Under a light-dark cycle, eclosion events were clustered in the mid-morning both under natural conditions and in the laboratory (Figs. 2 and 3).

Under natural conditions, adult eclosion was recorded for three generations within a year (Fig. 2). All three generations showed robust daily rhythmicity, as determined by Rayleigh and cosinor tests ($P < 0.0001$). Eclosion of the overwintered population (OW) was spread out over the daytime and peaked at 10:00 (acrophase at 11:00), whereas eclosion of G1 and G2 was more concentrated in the early hours of the day with peaks at 8:00 and 9:30, respectively. Kolmogorov-Smirnov tests indicated significantly different temporal distributions for OW-G1, OW-G2, and G1-G2 ($P < 0.001$). It is noteworthy that OW and G1 were exposed to similar photoperiods but OW was exposed to a lower temperature than G1 (22.5 °C for OW; 30.2 °C for G1 on average).

To investigate if temperature or photoperiod plays a more important role than the other on the eclosion daily distribution in *P. fasciata*, three photoperiods, LD 11:13, LD 13:11, LD 15:9, and four constant temperatures, 22, 25, 28, 30 °C, were applied. It was found that the eclosion distribution was dispersed through the photophase at 22 °C, but more condensed at other temperatures (Fig. 3). Under each photoperiod, the temporal distribution of eclosion changed significantly with ambient temperature (Kolmogorov-Smirnov test, $P < 0.01$), with an apparent advance of the peak time at higher temperatures. Although small differences in the temporal distribution of eclosion between the three photoperiods were observed under each temperature, the general temporal pattern was not affected by changes in photoperiod (Kolmogorov-Smirnov test, $P > 0.05$).

2. Mating distribution

Pseudopidorus fasciata barely mated under constant light conditions (data not shown). A few adults mated in constant darkness at 28 °C, and the events occurred at no particular time of the day (Rayleigh test, $\chi^2(2) = 5.830$, $P > 0.05$), but mating was clustered in a few hours before dusk under natural conditions (Fig. 4). Both generations tested under natural conditions showed robust daily rhythmicity, as determined by Rayleigh and cosinor tests ($P < 0.0001$). The exact temporal distribution of mating differed between the two generations (Kolmogorov-Smirnov test, $P < 0.001$). Peak mating and acrophase were about an hour earlier in G2 (18:00) than in G1 (19:00).

Mating distribution in *P. fasciata* was further studied in the laboratory under various combinations of photoperiod and temperature (Fig. 5). Three constant temperatures (22, 25, and 28 °C) and three photoperiods (LD11:13, LD13:11 and LD15:9) were used. Similar to the temporal pattern observed under natural conditions, mating was concentrated on the few hours before lights-off in the laboratory. As expected because of the later time of lights-off in the longer photoperiods, peak times were later under longer photoperiods, but variations in photoperiod had otherwise modest though significant effects on the temporal distribution of mating (Kolmogorov-Smirnov tests, $P < 0.001$). Variations in temperature also had a modest effect on the temporal distribution of mating, with significant differences due to temperature under LD 11:13 and LD 13:11 (Kolmogorov-Smirnov tests, $P < 0.001$) but not under LD 15:9 (Kolmogorov-Smirnov tests, $P > 0.05$).

Discussion

Effects of photoperiod and temperature on the eclosion distribution in *P. fasciata*

Adults of *P. fasciata* emerged from cocoons mostly in the mid-morning except under constant light or darkness. Similarly, the giant silk moth, *Hyalophora cecropia*, was found to emerge 1-9 h after dawn (Truman and Riddiford 1970). Many insects emerge into adults in the morning, and one possible explanation is that the relatively humid, cool air favors expansion of wings in the newly emerged adults (Pittendrigh 1954). For example, the kauri moth, *Agathiphaga vitiensis*, requires relative humidity greater than 80 percent to eclose (Robinson and Tuck 1976). In contrast, eclosion activities of the Chinese oak silk moth, *Antheraea pernyi*, peak in late afternoon (Truman and Riddiford 1970), and the southwestern corn borer *D. grandiosella* (Takeda 1983) and the fall webworm *H. cunea* (Morris and Takeda 1994) emerge close to dusk, and the corn earworm *H. zea* emerges mainly in the evening (Callahan 1958).

The eclosion of *P. fasciata* was gated to a narrow time window under natural conditions and at various laboratory temperatures, except at 22 °C. Compared with 22 °C, eclosion events at higher temperatures had fewer and earlier eclosion peaks and increased amplitude (Fig. 3). Significant differences were also detected among the four temperatures. The temporal distribution of eclosion changed significantly with ambient temperatures, with an apparent advance of the peak time at higher temperatures. The impact of temperature on eclosion distribution was further demonstrated under natural conditions. At the relatively low temperature of 22.5 °C, OW of *P. fasciata* showed a more dispersed eclosion distribution and a later eclosion peak than G1 (30.2 °C) when they experienced similar average day lengths (Fig. 2). These results indicate that temperature had a significant impact on the eclosion distribution in *P. fasciata*.

179 In addition, we observed that the average time required for *P. fasciata* pupal
180 development was affected by temperature (16 days at 22 °C; 14 days at 25 °C; 13 days at
181 28 °C and 30 °C). Thus, it is likely that high temperatures accelerated the development
182 rate of pupae. It is also possible that the natural increase in temperature in the middle of
183 the day could have interfered with the wing expansion of newly emerged adults, as
184 verified in the onion fly *D. antiqua* by Tanaka and Watari (2009).

185 Under natural conditions, the eclosion distribution in OW and G1 of *P. fasciata* were
186 significantly different from each other (Fig. 2). Conditions differed not only in
187 temperature, but also in photoperiod. Although the average photoperiod was 14 h 30
188 min for both OW and G1, the photoperiod was increasing daily for OW (May) while
189 decreasing daily for G1 (July). In addition, the eclosion distribution in OW differed
190 significantly from G2, when temperature was close (22.5 °C for OW; 23.3 °C for G2) but
191 photoperiod was distinct (14 h 30 min for OW; 13 h 05 min for G2) (Fig. 2). Thus, we
192 assume ~~changing~~ photoperiod might also have an impact on the eclosion distribution in *P.* change of
193 *fasciata*. However, such a hypothesis was rejected in the tests under laboratory diurnal
194 conditions. Although small differences in the temporal distribution of eclosion between
195 the three photoperiods were observed under each temperature, the general temporal
196 pattern was not affected by ~~the~~ changes in photoperiod (Fig. 3). This is different from
197 the findings of Kikukawa et al. (2012), in which photoperiod acted as an effective
198 zeitgeber in the adult eclosion of the Indian meal moth *P. interpunctella*.

199 Because ambient temperature did not cycle daily in the laboratory, and because
200 photoperiod did not seem to play a crucial role in the timing of eclosion, the “gate” of
201 eclosion in the mid- to late morning in the field must be due to other environmental or

endogenous factors. When we kept pupae under constant darkness at 28°C, eclosion still oscillated daily (Fig. 1), even though the temporal distribution was much wider. Thus, as has been shown in many other insect species (Myers 2003), eclosion in *P. fasciata* is possibly gated by an endogenous circadian clock.

Effects of photoperiod and temperature on the mating distribution in *P. fasciata*

Adults of *P. fasciata* initiated mating at dusk, or within a few hours before lights-off. Typically, mating behavior of insects occurs at a preferred light intensity at dusk or after dusk. For example, the coconut moth, *Atheloca subrufella*, mated between 19:00 and 23:00 (Bento et al. 2006), and the legume pod borer, *Maruca vitrata*, mainly mated between 23:00 and 24:00 for 3-day-old moths (Lu et al. 2007).

Saunders (2002) stated that mating activity could be suppressed by either (or both) high light intensity and darkness. We verified this in our study. *Pseudopidorus fasciata* rarely mated in darkness or continuous light at the intensity of 500 - 700 lx (Fig. 4). Under natural conditions, the moths preferred to mate at dusk with decreasing light intensity (Fig. 4). However, under artificial light-dark cycles with constant light intensity, the moths still initiated mating before lights-off, and the mating peak was delayed as the onset of scotophase was delayed under different photoperiods (Fig. 5). Variations in temperature also had a modest but significant effect on the temporal distribution of mating, with significant differences due to temperature under LD 11:13 and LD 13:11 but not under LD 15:9 (Fig. 5). This was probably because the short night (LD15:9) had a more determinant effect than temperatures had on the mating events, although

temperatures might have played an important role in gating the rhythmicity when combined with longer nights (LD 11:13 and 13:11).

Sakai and Ishida (2001) indicated that mating distribution might be subject to the circadian control of either pheromone release or male copulation, or both. We observed that during dusk, wild male moths of *P. fasciata* could often be attracted by the female moths in outdoor cages, and the males were extremely active when the mating peak approached. We suggest that sexual pheromone release and locomotor activities, as pre-mating signals, might be controlled in a circadian rhythmic pattern in *P. fasciata*. This needs to be verified. In our study, mating did not exhibit daily rhythmicity in constant darkness (Fig. 4), under which there were relatively fewer mating events than that under diurnal cycles. In particular, *P. fasciata* barely mated under constant light conditions. Thus, mating is likely inhibited in both constant light and darkness in this insect.

To conclude, *P. fasciata* displayed clear rhythmicity on adult eclosion and mating events under circadian light-dark cycles. Eclosion distribution pattern was predominantly regulated by temperature rather than photoperiod, whereas photoperiod appeared to have a more influential role than temperature had in mating activities. Eclosion of this insect exhibited circadian gating in constant darkness but not in constant light, and mating rhythmicity was lost under both constant light and darkness. The underlying mechanism of how ambient conditions affect the rhythmicity regulation in this insect merits further evaluation.

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327

328

Figure Legends

Fig. 1. Adult eclosion rhythm in *Pseudopidorus fasciata* observed under constant light (LL) and constant darkness (DD) at 28 °C. Φ_E represents the median eclosion time; N = total number of emerged adults.

Fig. 2. Adult eclosion rhythm in *Pseudopidorus fasciata* observed under natural conditions for three generations, with an average temperature of 22.5 °C, and day-length (including the twilight) of 14 h 30 min for the overwintered generation (OW); 30.2 °C, 14 h 30 min for the first generation (G1); 23.3 °C, 13 h 05 min for its second generation (G2). Φ_E represents the median eclosion time. (■ = night time; □ = day time for each hour; within each plot, N = total number of emerged adults)

Fig. 3. Eclosion rhythms in *Pseudopidorus fasciata* observed at different temperature / photoperiod conditions. Φ_E represents the median eclosion time. (■ = night time; □ = day time for each hour; within each plot, N = total number of emerged adults)

Fig. 4. Mating rhythms in *Pseudopidorus fasciata* observed under constant darkness (DD) at 28 °C, or under natural conditions for two generations, with an average temperature of 29.9 °C and day-length of 14 h 30 min for the first generation (G1); 23.3 °C and 13 h 05 min for its second generation (G2). Φ_M represents the median mating time. (■ = night time; □ = day time for each hour; within each plot, N = total number of mating pairs observed)

Fig. 5. Mating rhythm in *Pseudopidorus fasciata* observed at different temperature / photoperiod conditions. Φ_M represents the median mating time. (■ = night time; □ = day time for each hour; within each plot, N = total number of mating pairs observed)