Circadian rhythm of adult emergence in the tiger-fly *Coenosia attenuata* (Diptera: Muscidae)

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Resumo

Coenosia attenuata Stein, vulgarmente conhecida como mosca-tigre, foi detectada em Portugal em 2001, na região Oeste, a predar adultos de mosquinha branca e de larvas mineiras. Os indivíduos desta espécie apresentam particular importância como agentes de luta biológica, uma vez que as larvas de mosca-tigre predam diversos organismos no solo e os adultos são predadores de importantes pragas agrícolas, na parte aérea das culturas. No decurso da criação de mosca-tigre em laboratório, registou-se o período do dia em que ocorreram emergências dos adultos. Foram testadas duas modalidades: numa, o *puparium* foi colocado em caixa sem substrato de criação, portanto directamente exposto à luz; na outra, o *puparium* foi colocado em caixa com substrato. Verificou-se que as emergências ocorreram maioritariamente nas primeiras seis horas depois de as luzes da câmara de criação se acenderem. O pico de emergências ocorreu significativamente mais cedo na ausência de substrato e foi mais prolongado no caso dos machos. Os resultados obtidos indicam que a emergência dos adultos da mosca-tigre, apesar de esta espécie pupar, naturalmente, enterrada no solo, foi influenciada pelo ciclo luz-escuridão.

Palavras- chave: fotoperíodo, padrão circadiano, "Zeitgeber", relógio biológico

Abstract

Coenosia attenuata Stein, commonly known as tiger-fly, was detected in Portugal for the first time in 2001 in the Oeste region, feeding on adults of whitefly and leafminer. Individuals of this species can play an important role as biological control agents, since the larvae of tiger-fly prey soil-dwelling organisms, and adults are predators of important agricultural pests, above the soil. During tiger-fly rearing in laboratory, the time of the day when adult emergences occurred was registered. Two modalities were performed: without substrate, and so, with the *puparium* directly exposed to the light; and with substrate, then, the *puparium* was not directly exposed to light. Tiger-fly adult emergences occurred mainly in the first six hours after the rearing chamber lights were turned on, being the peak of emergences significantly earlier in the absence of substrate, and the period when most of the emergences occurred longer in males. In spite of pupating naturally in the soil, results indicate that tiger-fly adult emergence from the *puparium* responded to the light-night cycles.

Key words: photoperiod, circadian pattern, Zeitgeber, biological clock

Introduction

Day-night cycles have been reported as influencing deeply physiological and behavioural patterns in organisms (Sharma, 2003; Yerushalmi & Green, 2009), in particular in insects (Saunders et al., 2002; Danks, 2005), with an

adaptive significance (Emerson et al., 2008; Yerushalmi & Green, 2009). Eclosion and ecdysis (for example, emergence from pupae) and activity-rest patterns are among the best-known examples of circadian rhythms in insects (Bertossa et al., 2010). Correct coordination and timing of several behaviours at ecdysis (such as muscle movements) are essential for insect survival. These ecdysis behaviours differ among insect species. In most cases, emergence from pupal integument is gated by the endogenous circadian system to a restricted period of the day (Myers, 2003). In *Drosophila pseudoobscura* Frolova & Astaurov, *D. melanogaster* (Meigen), *D. victoria* Fallén, *D. gangottri* Muniyappa & Reddy and *D. jambulina* Parshad & Paika (Diptera: Drosophilidae) emergence from *puparium* occurs in the hours immediately following the onset of light: individuals that reach maturity outside this gate will emerge at the following gate, usually 24h later (Pittendrigh, 1954; Pittendrigh & Shopik, 1970; Kouser & Shakuntala, 2012). In the silkmoth *Antheraea pernyi* Guérin-Méneville (Lepidoptera: Saturniidae) the emergence of the adults occurs in late afternoon, while for *Hyalophora cecropia* (L.) (belonging to the same family) it occurs in the morning (Truman & Riddiford, 1970); however, for several other silkmoth species the egg-hatching gate anticipated lights-on in the morning (Sauman & Reppert, 1998).

The factors influencing the emergence of adults from pupae are strongly associated to where pupae are located (above or beneath the soil) and the factors to which insects are more sensitive (temperature, light) are influenced by their habitat. For example, emergence patterns in insects emerging from the soil will probably be more sensitive to daily changes in temperature (thermoperiod) than in light (photoperiod) (Myers, 2003). Soil temperature has a daily cyclic variation: it increases with solar radiation during the day and it decreases during night with outward radiation. *Mamestra brassicae* (L.) (Lepidoptera: Noctuidae), which pupates in the soil, proved to have daily rhythms of emergence temperature regulated, with the timing of emergence varying with the amplitude of the temperature (Tanaka et al., 2013). In *Heliothis virescens* (Fab.), another noctuid species, adult emergence can be regulated either by thermoperiod or photoperiod (Roush & Schneider, 1985). In *Delia antiqua* (Meigen) (Diptera: Anthomyiidae)

(Tanaka & Watari, 2003) and in *Sarcophaga crassipalpis* Macquart (Diptera: Sarcophagidae) (Miyazaki et al., 2011) the emergence from soil is also thermoperiod regulated. Furthermore, *D. antiqua* and *S. crassipalpis* as well as *M. brassicae* are able to compensate for the depth dependent phase temperature delay and amplitude of the temperature cycle (Tanaka & Watari, 2003; Miyazaki et al., 2011; Tanaka et al., 2013).

The tiger-fly, *Coenosia attenuata* Stein (Fig. 1), a Paleotropical species (Hennig, 1964; Kühne, 2000), was detected in Portugal for the first time in 2001 (Prieto et al., 2005); it is nowadays distributed worldwide in several agricultural systems. Being a predator of important agricultural pests in both larval and adult stages (Kühne, 1998; Moreschi & Colombo, 1999), it can play an important role as a biocontrol agent. Tiger-fly adults prey on flying insects on the aerial part of the plants; females lay their eggs in the soil interstices and larvae are predators of soil-dwelling organisms. Adults sometimes kill their prey without feeding on it (Martinez & Cocquempot, 2000), and it has been reported that this species has little impact on natural enemies (Téllez & Tapia, 2006; Garcia, 2011; Martins et al., 2012).
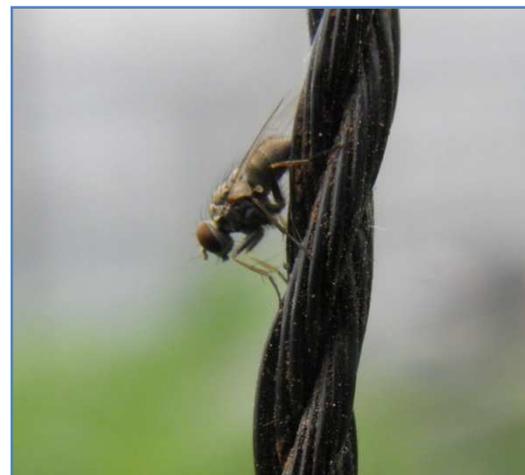

Figure 1. *Coenosia attenuata* female adult standing on a tomato holding twine. © E. Figueiredo.

This tiger-fly is adapted to high temperatures (Gilioli et al., 2005) which is the case of Mediterranean greenhouses in summer. It is therefore important to take this predator into account in conservation strategies. For laboratory predation assays, it was necessary to determine the age of tiger-fly adults reared in laboratory,

and so the time of the day when adults emerged in the laboratory rearing units was registered.

Material and Methods

Tiger-fly rearing

Coenosia attenuata larvae were reared in a substrate constituted by soil rich in organic material, mixed with coconut fibres, and oak flakes previously inoculated with *Pleurotus ostreatus* (Jacq. ex Fr.) P. Kummer (Basidiomycota: Pleurotaceae). For the larvae of tiger-fly, *Bradysia difformis* (Diptera: Sciaridae) larvae were used as prey. For tiger-fly adults, adults of this fungus gnat species were used as prey, and also adults of *Drosophila melanogaster* were used as a supplement. Rearing took place in an acclimatized breeding room, at 25ºC, 60% mean RH and photoperiod 12h L: 12h D (Martins et al., 2012).

Tiger-fly adult emergence

Puparia of the tiger-fly were removed from the rearing substrate with a paintbrush and placed individually in closed transparent cylindrical boxes (4.0 cm ø; 2.2 cm high).
Two modalities were performed (Fig. 2): M1- without substrate - consisted in placing a *puparium* directly exposed to the light on a filter paper disk (humidified periodically) at the base of the box; M2 – with substrate - consisted in placing one *puparium* buried under 1cm of rearing substrate at the base of a box.

Figure 2. Emergence modalities tested:
a) M1 – without substrate. © J. Martins;
b) M2 – with substrate. © J. Martins;
c) tray with M1 plastic boxes in the acclimatized chamber. © J. Martins.

The boxes were maintained in an acclimatized chamber at 25.0±0.1°C, 80± 5% RH and photoperiod 14h L: 10h D, with a 30min light intensity ramp (from 10% to full intensity) to simulate dawn and dusk. To register the period when adult emergence occurred, seven daily observations were performed: at the beginning of the day (when the lights of the rearing chamber turned on) and 1h, 2h, 3h, 6h, 9h and 11h hours after this event.

The emergence of 162 tiger-fly adults (95 females, 67 males) in the modality without substrate (M1) and 166

individuals (112 females, 54 males) in the other modality (M2) was registered. Data were analysed using contingency-tables (cross-tabs) with the statistical software IBM SPSS v. 20.

Results and Discussion

Tiger-fly adult emergences occurred mainly in the first six hours after lights were turned on in the chamber (Table 1). This pattern corresponds to literature references in which many dipteran insects emerge from the *puparium* in the early morning (Denlinger & Ždárek, 1994),

responding to light or temperature fluctuations or to both (e.g. Pittendrigh, 1954; Pittendrigh & Shopik, 1970; Saunders, 1976; Roberts et al., 1983; Lankinen & Riihimaa, 1997, Tanaka & Watari, 2003). The adult emerged at the beginning of the morning has an adaptive advantage: with the cooler air in the morning and the higher relative humidity wing expansion is facilitated; at noon and particularly in the afternoon, lower humidity but especially higher temperatures would difficult the correct wing expansion (Pittendrigh, 1954; Tanaka & Watari, 2009).

Table 1. Adult emergences of the tiger-fly *Coenosia attenuata* during seven observation periods along the day, in each experimental modality (M1 = without substrate; M2 = with substrate) (n = number of observed emergences).

Observation (time after lights on)	M1						M2					
	Male		Female		Total		Male		Female		Total	
	n	Mean (%)	n	Mean (%)	n	Mean (%)	n	Mean (%)	n	Mean (%)	n	Mean (%)
0h*	3	4.5	7	7.4	10	6.2	5	9.3	11	9.8	16	9.6
1h	21	31.3	26	27.4	47	29.0	6	11.1	14	12.5	20	12.0
2h	11	16.4	32	33.7	43	26.5	9	16.7	31	27.7	40	24.1
3h	17	25.4	18	19.0	35	21.6	12	22.2	27	24.1	39	23.5
6h	15	22.4	9	9.5	24	14.8	16	29.6	26	23.2	42	25.3
9h	0	0.0	3	3.2	3	1.8	6	11.1	3	2.7	9	5.4
11h	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

*Observation at the time lights were turned on recorded emergences corresponding to the period 11h to 24h (i.e. emergences with up to 13 hours) (photoperiod 14h L: 10 h D).

There were significant differences between the modalities regarding the total of emergences (chi-square = 12.401; n = 328; p = 0.03), females (chi-square = 12.916; n = 207; p = 0.024), and males (chi-square = 23.631; n = 121; p <0.001), being the peak of emergences earlier in the absence of substrate. The most evident difference between the two modalities was that in M1 pupae were directly exposed to the light, in opposition to M2, where pupae were buried. The air temperature of the climatic chamber was relatively constant to eliminate the potential effect of temperature changes, except when lights turned on or off; at that time, a temperature adjustment period of 10-20 min long with a 0.5°C maximum amplitude occurred. Results suggest an influence of dark/light changes

in tiger-fly emergence of the adults at fairly constant temperatures.

The effect of light in adults' emergence was also observed when comparing males and females in M1. Significant differences were detected between females and males in M1 (chi-square = 16.248; n = 162; p =0.006), in opposition to M2 (chi-square = 8.842; n = 166; p =0.116); in the absence of substrate (direct exposition to light), females responded more promptly than males, anticipating the emergence peak period and shortening the period when most emergences occurred.

For insects which pupate in the soil, daily temperature cycle is a more probable cue for the adults' emergence than light cycle, since light does not penetrate into the soil

(Tanaka & Watari, 2003; Watari, 2005). However, for *Heliothis virescens*, which also pupates in the soil, adult emergence was found to be regulated by soil temperature cycles whenphotoperiodic cycles were absent or regulated by photoperiodic cycles in the absence of soil temperature cycles ; in the absence of either of these cycles, *H. virescens* adults emerged in an approximately 24.6h cycle (Roush & Schneider, 1985). Watari & Tanaka (2010) also found that at a 12h periodic fluctuating temperature from 24.5 to 25.5ºC, the emergence rhythm of *Delia antiqua* from *puparium* was completely regulated by the light-dark cycles. Our results also indicate that the tiger-fly, in spite of pupating in the soil, reacted to the light/dark cycles for the regulation of adult emergence from the *puparium*, at constant temperature.

It is important to note that it is not known if the emergences from pupa and from *puparium* occur always at the same period. There are some species, particularly endoparasitoids, whose emergence from pupa and from their hosts can be regulated by different factors and occur with a time delay. In the case of the egg parasitoids *Trichogramma embryophagum* Htg. (Hymenoptera: Trichogrammatidae) and the males of *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae), the emergence from the host is a circadian behaviour, but not the previous emergence from their own pupal integument (Bertossa et al., 2010; Reznik et al., 2008).

Conclusion

Results suggest that the tiger-fly, in spite of pupating in the soil, reacted to the photoperiod for the regulation of adult emergence from the *puparium*. Furthermore, females responded more promptly than males to the light stimulus.

Acknowledgements

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