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Photoperiodic control of diapause in *Pseudopidorus fasciata* (Lepidoptera: Zygaenidae) based on a qualitative time measurement

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Abstract

In the zygaenid moth, *Pseudopidorus fasciata*, both larval diapause induction and termination are under photoperiodic control. In this study, we investigated whether photoperiodic time measurement (with a 24-h light–dark cycle) in this moth is qualitative or quantitative. Photoperiodic response curves, at 22, 25, and 28 °C indicated that the incidence of diapause depended on whether the scotophases exceeded the critical night length (CNL) or not. All scotophases longer than the CNL-induced diapause; all scotophases shorter than the CNL-inhibited diapause. The CNL was 10.5 h at 25 and 28 °C, and 10 h at 22 °C. By transferring from various short photoperiods (LD 8:16, LD 9:15, LD 10:14, LD 11:13, LD 12:12, and LD 13:11) to a long photoperiod (LD 16:8) at different times, the number of light–dark cycles required for 50% diapause induction at 25 °C was 7.14 at LD 8:16, 7.2 at LD 9:15, 7.19 at LD 10:14, 7.16 at LD 11:13, and 7.13 at LD 12:12, without showing a significant difference between the treatments. Only at LD 13:11 (near the CNL), the number of light–dark cycles was significantly increased to 7.64. The intensity of diapause induced under different short photoperiods (LD 8:16, LD 9:15, LD 10:14, LD 11:13, and LD 12:12) at 25 °C was not significantly different with an average diapause duration of 36 days. The duration of diapause induced under LD 13:11 was significantly reduced to 32 days. All results indicate that the night-lengths are measured as either "long" or "short" compared with some critical value and suggest that photoperiodic time measurement for diapause induction in this moth is based on a qualitative principle.

Keywords: *Pseudopidorus fasciata*; Diapause; Photoperiod; Temperature; Qualitative time measurement

1. Introduction

Diapause is under photoperiodic control in most insects (Tauber et al., 1986). In studies of the effects of photoperiod on the induction of diapause, different groups of experimental insects are usually exposed to a series of day-lengths (all with a 24-h light–dark cycle) and to continuous darkness (DD) and continuous light (LL). The diapause response is most frequently measured in terms of the percentage incidence of diapause in the experimental population. All photoperiodic responses have a feature in common, involving the apparent 'measurement' of day-length (or night-length) (Saunders, 2002). The most important feature of the response is the so-called critical day length (CDL) or critical night length (CNL) that separates the long photophases resulting in non-diapause development from the short photophases that ultimately lead to winter diapause. First proposed over 20 years ago, the concept of CDL is based on the assumption that the response threshold is measurable in terms of absolute day-length, i.e., that the insect is not responding to daily increments of seasonal changes (increases or decreases) (Beck, 1980). In other words, the photoperiodic clock merely distinguishes long from short days (or more commonly, short from long nights) by a qualitative or all-or-nothing mechanism. In recent years, however, a number of papers have discussed the possibility of

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quantitative time measurement, i.e., a "clock" mechanism that distinguishes between actual day- or night-lengths, rather than merely discriminating "long" from "short" (Kimura, 1982 for *Drosophila testacea*; Zaslavski and Fomenko, 1983 for *Megoura viciae*; Numata, 1985 for *Riptortus clavatus*; Hori, 1987 for *Palomena angulosa*; Kimura, 1990 for *Drosophila auraria*; Hardie, 1990 for *Megoura viciae*; Spieth and Sauer, 1991 for *Pieris brassicae*). Three experimental methods are usually used to test whether photoperiodic time measurement in insects is based on a qualitative or quantitative principle. The first method is to study the incidence of diapause in different short photophases at different temperatures. The second method is to assess the number of days required to induce 50% diapause at different short photophases. The third method is to test the intensity (or duration) of diapause at different short photophases. If results for all three experimental approaches show no significant differences, it is generally considered that time measurement is qualitative. If all results show significant differences, quantitative time measurement is indicated.

The zygaenid moth *Pseudopidorus fasciata* (Felder and Felder, 1862) enters winter diapause as a fourth instar larva when exposed to short days with a CNL of 10.5 h (Xue and Kallenborn, 1998). Both diapause induction and termination in this moth have been systematically investigated; all results indicate that the larvae are highly sensitive to photoperiod and both diapause induction and termination depend on whether the night- lengths exceed the CNL or not, i.e., 10.5 h for diapause induction; 10 h for diapause termination (Wei et al., 2001; Li et al., 2003). Thus, this moth is an excellent organism to examine whether photoperiodic time measurement is based on a qualitative or quantitative principle by using the three experimental methods mentioned above.

2. Materials and methods

2.1. Experimental material and rearing conditions

The population of *P. fasciata* used in this study was collected in the suburbs of Nanchang (28 °46′N, 116 °50′E), Jiangxi Province, PR China. Full-grown larvae prior to cocooning were collected in the field. The larvae were allowed to form cocoons and to emerge under natural conditions. Eggs were collected daily. Newly hatched larvae were transferred to round plastic boxes ($\pi \times 7.5 \times 7.5 \times 6$ cm) with fresh foliage of Chinese sweet-leaf, *Symplocos chinensis* (a deciduous shrub) and then were placed for different treatments.

All experiments were performed in illuminated incubators (LRH-250-GS) equipped with four fluorescent 30 W tubes controlled by an automatic time switch. Light intensity at the level of larvae was 500–700 lx and variation of temperatures was ± 1 °C.

2.2. Experimental design

2.2.1. Effect of temperature on the photoperiodic responses

Larvae were reared at 22, 25, and 28 °C combined with various photoperiods (including DD and LL) until diapause was induced. The number of larvae used in each test photoperiod was at least 50. Diapause larvae of *P. fasciata* were easy to identify by their small size and brown body color, and by the observation that they had ceased feeding (Xue and Kallenborn, 1998).

2.2.2. Effect of the number of photoperiodic cycles on diapause incidence in six short light–dark regimes

Newly hatched larvae were transferred to diapause-inducing photoperiods (LD 8:16 h, LD 9:15 h, LD 10:14 h, LD 11:13 h, LD 12:12 h, and LD 13:11 h) at 25 °C, and were exposed to these photoperiods for different lengths of time (2, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 14 days), and were then transferred to a diapause-averting photoperiod of LD 16:8 h. Eighteen days after the beginning of the experiment, the percentage of diapause incidence was determined for each treatment. Experiments in which larvae were exposed to between 2 and 14 light–dark cycles were carried out 3 times. The number of larvae used in each test photoperiod was at least 50.

Logistic regression analysis was used to compare effects of the number of light–dark cycles on diapause incidence. The number of light–dark cycles required for 50% diapause induction was estimated by fitting the data according to the model of Haanstra et al. (1985), for which the STATA package was used.

$$Y = c/[1 + \exp(b(X - a))],$$

where $Y =$ (100 - percentage diapause induction),

X is the natural logarithm of number of light–dark cycles experienced,

a the logarithm of number of cycles required for 50% diapause induction,

b the slope parameter, and

c the maximum diapause induction.

Treatments were compared by one-way analysis of variance (ANOVA) to determine whether differences between them were significant.

2.2.3. Effect of photoperiod of the induction regime on diapause duration

Newly hatched larvae were transferred to various diapause-inducing regimes (LD 8:16 h, LD 9:15 h, LD 10:14 h, LD 11:13 h, LD 12:12 h, and LD 13:11 h) at 25 °C and kept there for 18 days. Under this condition all individuals enter a larval diapause. Then all