

Effect of photoperiod on the duration of summer and winter diapause in the cabbage butterfly, *Pieris melete* (Lepidoptera: Pieridae)

HAI-JUN XIAO, DONG YANG and FANG-SEN XUE*

Institute of Entomology, Jiangxi Agricultural University, Nanchang 330045, People's Republic of China

Key words. Lepidoptera, Pieridae, *Pieris melete*, photoperiod, pupal diapause, diapause termination, aestivation, hibernation

Abstract. Effect of photoperiod on the duration of summer and winter diapause was investigated in the cabbage butterfly, *Pieris melete*. By keeping naturally induced aestivating and hibernating pupae under various photoperiods, it was shown that diapause duration of aestivating pupae was significantly longer at long than at short daylengths, whereas diapause duration of hibernating pupae was significantly shorter at long than at short daylengths, suggesting both aestivating and hibernating pupae require opposite photoperiodic signals to promote diapause development. By transferring diapausing pupae, induced under various photoperiods, to 20°C with a naturally changing summer daylength, the diapause induced by short daylengths was easier to terminate than diapause induced by long daylengths. When naturally induced aestivating and hibernating pupae were kept under natural conditions, aestivating pupae had a long diapause (mean 155 days) and wide range of emergence (90 days), whereas hibernating pupae had a short diapause (mean 105 days) and a relatively synchronized emergence (lasted 30 days). Finally, the ecological significance of photoperiodic regulation of diapause duration is discussed.

INTRODUCTION

The cabbage butterfly, *Pieris melete* Ménétériés is a serious pest of crucifers in the mountain areas of the Jiangxi Province, PR China. It is a multivoltine species, which undergoes summer and winter diapause as a pupa (Xue et al., 1996). The seasonal life history of the cabbage butterfly comprises two different photoperiodically induced developmental arrests: aestivation at daylengths >13 h and hibernation <12 h. At intermediate daylengths (12 h to 13 h), the butterfly does not diapause (the proportion depends on temperature). The larval instars are sensitive to photoperiod (Xue et al., 1997). In the field, the spring generation of this butterfly appears between early March and early April. The larvae hatch from early April to early May and begin to pupate in late April. Almost all of the spring generation pupae enter summer diapause. During late August to early November, adult butterflies emerge from aestivating pupae. The butterflies, which emerge in late August develop without diapause and produce three generations in autumn; those emerging before mid-October produce two generations; those emerging after mid-October, produce only one generation. Thus, there are two to four generations per year. All individuals that pupate after early November enter winter diapause.

Whether aestivating or hibernating pupae maintain or terminate diapause depends directly on temperature. High temperatures delayed and relatively low temperatures enhanced diapause development (Xue et al., 1997). However, it is unknown whether photoperiod also influences diapause duration in this butterfly. A number of experiments have shown that the duration of diapause can be affected by photoperiod during diapause (Sugiki &

Masaki, 1972 for *Spilarctia imparilis*; Tauber & Tauber, 1976 for *Chrysopa downesi*; Gomi & Takeda, 1992 for *Elcysma westwoodii*; Spieth, 2002 for *Pieris brassicae*) or by pre-diapause photoperiod (see Danks, 1987, pp. 136–137; Nechols et al., 1987 for *Chrysopa ocellata*; Beck, 1989 for *Ostrinia nubilalis*; Kimura, 1990 for *Drosophila auraria*; Košťál & Hodek, 1997 for *Cymbalophora pudica*; Nakamura & Numata, 2000 for *Riptortus clavatus*). In the present study, we examined (1) diapause duration of naturally aestivating and hibernating pupae when kept under natural conditions and different photoperiods, and (2) duration of diapausing pupae induced under different photoperiods when kept under identical conditions.

MATERIAL AND METHODS

To obtain naturally hibernating or aestivating pupae, full-grown larvae of *Pieris melete* were collected from the vegetable gardens in the suburbs of Nanchang (28°46'N, 115°50'E; at an altitude of between 120–200 m a.s.l.), Jiangxi Province, in late April and mid-November 2003, respectively. They were transferred to wooden insectaries (30 × 30 × 35 cm) to pupate under natural conditions and examined daily for pupation. The pupae, which developed in the first three days, were used in this study. These pupae were divided into two groups. Those in the first group were kept in wooden insectaries under natural conditions until the adults emerged. Those in the second group were transferred to incubators and kept under a photoperiod of 10L : 14D, 12L : 12D or 14L : 10D at 18, 20 or 22°C until the adults emerged. These experiments of the second group were done in illuminated incubators (LRH-250-GS) equipped with four fluorescent 30 W tubes controlled by a time switch. Light intensity at the level of the pupae was 500–700 lx and the variation in temperature was ± 1°C.

* Corresponding author; e-mail: fangsen@nc.jx.cn

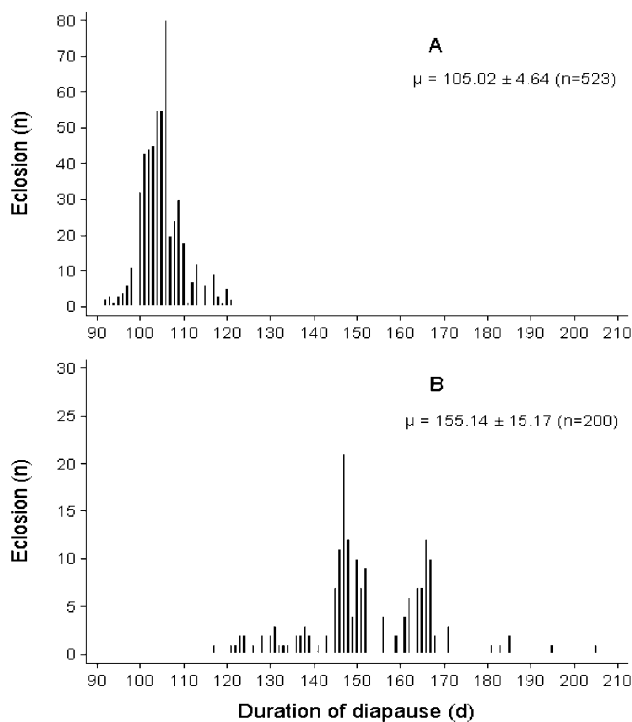


Fig 1. Duration of diapause of hibernating (A) and aestivating pupae (B) kept under natural conditions. During the period they were in diapause, most of the aestivating pupae experienced long days for about 5 months when daylengths were > 13 h and temperatures were high (a mean of 25.3°C), whereas most of the hibernating pupae experienced short days for about 3 months when daylengths were < 12 h 30 min and temperatures were low (a mean of 9.7°C).

To determine whether pre-diapause photoperiod influences diapause intensity, newly hatched larvae (from eggs produced by the spring generation in an outdoor screened insectary) were transferred to round plastic boxes (7.5 × 6 cm) containing fresh radish leaves (*Raphanus sativus*) and then reared under one of six photoperiodic regimes ranging from 8L : 16D to 16L : 8D at 18°C until they pupated (under these conditions diapause was induced in over 80% of the pupae). Each box contained about 25 larvae. Each treatment comprised 3 boxes. The boxes were cleaned and supplied with fresh leaves daily. On the tenth day after pupation, diapausing pupae were transferred to an incubator equipped with 3 side glass windows and kept at 20°C to terminate diapause. In this incubator the diapausing pupae were exposed to a naturally changing summer daylength.

The data presented in Fig. 1B is normally distributed (Skewness/Kurtosis test, $P = 0.9217$). It is important to note that the mortality of aestivating pupae was very high this year (about 80%) because of high summer and autumn temperatures, especially on 25–30 September (i.e., the 153–160 day of Fig. 1B), the daily mean temperature exceeded 25°C and the highest temperature was 30°C. Thus, a lot of adults were unable to emerge successfully from pupae on these three days.

Statistical analyses were conducted using STATA Version 8.0. All data were tested using one-way analysis of variance (ANOVA). For post hoc comparisons the Bonferroni test was used (Zolman, 1993).

TABLE 1. Diapause duration of aestivating pupae of *Pieris melete* kept at 18, 20 or 22°C and under different photoperiods.

Temperature	Photoperiod	Number observed	Duration of diapause (d)* $\bar{x} \pm SD$
18°C	10L : 14D	176	92.93 ± 13.96 B
	12L : 12D	122	84.96 ± 14.81 C
	14L : 10D	172	98.44 ± 13.16 A
20°C	10L : 14D	190	87.42 ± 12.48 B
	12L : 12D	179	83.30 ± 13.48 C
	14L : 10D	177	91.20 ± 14.03 A
22°C	10L : 14D	132	95.59 ± 11.77 B
	12L : 12D	114	94.71 ± 10.84 B
	14L : 10D	110	102.75 ± 12.03 A

* Values followed by different letters are significantly different based on a Bonferroni test and one-way analysis of variance (ANOVA) of results obtained at 18°C ($F = 33.56$, d.f. = 2,467, $P (= 0.0000) < 0.01$), 20°C ($F = 15.64$, d.f. = 2,543, $P (= 0.0000) < 0.01$) and 22°C ($F = 16.43$, d.f. = 2,353, $P (= 0.0000) < 0.01$).

RESULTS

Diapause duration of aestivating or hibernating pupae under different photoperiods

At all of the temperatures used, the duration of diapause of the aestivating pupae differed significantly under different photoperiods ($F = 33.56$, d.f. = 2,467, $P (= 0.0000) < 0.01$ at 18°C; $F = 15.64$, d.f. = 2,543, $P (= 0.0000) < 0.01$ at 20°C; $F = 16.43$, d.f. = 2,353, $P (= 0.0000) < 0.01$ at 22°C) (Table 1). The duration of diapause was shortest when the aestivating pupae were kept at a photoperiod of 12L : 12D, followed by 10L : 14D and then at 14L : 10D.

At all of the temperatures used, the duration of diapause of hibernating pupae also depended on the photoperiod ($F = 48.87$, d.f. = 2,329, $P (= 0.0000) < 0.01$ at 18°C; $F = 21.56$, d.f. = 2,323, $P (= 0.0000) < 0.01$ at 20°C; $F = 10.16$, d.f. = 2,330, $P (= 0.0000) < 0.01$ at 22°C) (Table 2). However, the duration of diapause was the opposite of that recorded for aestivating pupae. It was shortest when the hibernating pupae were kept at a photoperiod of 14L : 10D, followed by exposure to 12L : 12D, and 10L : 14D.

Effect of pre-diapause photoperiod on diapause intensity

The duration of diapause was significantly influenced by the pre-diapause photoperiod ($F = 125.371$, d.f. = 5,351, $P (= 0.0000) < 0.01$) (Table 3). At 20°C the duration of diapause induced by photoperiods of 12L : 12D, 10L : 14D or 8L : 16D were significantly shorter than those of 13L : 11D, 14L : 10D or 16L : 8D. However, there were no significant differences in the intensity of diapause induced by short photoperiods of 8L : 16D, 10L : 14D and 12L : 12D, whereas differences induced by the long photoperiods of 13L : 11D, 14L : 10D and 16L : 8D were significantly different.

As all diapausing pupae terminated their diapause before 19 August, they experienced photoperiods that exceeded 13 h 50 min.

TABLE 2. Diapause duration of hibernating pupae of *Pieris melete* kept at 18, 20 or 22°C and under different photoperiods.

Temperature	Photoperiod	Number observed	Duration of diapause (d)* $\bar{x} \pm SD$
18°C	10L : 14D	109	102.26 \pm 15.68 A
	12L : 12D	112	92.99 \pm 15.37 B
	14L : 10D	111	82.20 \pm 14.10 C
20°C	10L : 14D	105	98.22 \pm 16.57 A
	12L : 12D	108	92.43 \pm 16.69 B
	14L : 10D	113	84.11 \pm 14.66 C
22°C	10L : 14D	112	100.51 \pm 16.57 A
	12L : 12D	110	93.21 \pm 16.43 B
	14L : 10D	111	91.06 \pm 16.22 B

* Values followed by different letters are significantly different based on a Bonferroni test and one-way analysis of variance (ANOVA) of results obtained at 18°C ($F = 48.87$, d.f. = 2,329, $P (= 0.0000) < 0.01$), 20°C ($F = 21.56$, d.f. = 2,323, $P (= 0.0000) < 0.01$) and 22°C ($F = 10.16$, d.f. = 2,330, $P (= 0.0000) < 0.01$).

Diapause duration of aestivating and hibernating pupae kept under natural conditions

When the aestivating pupae of the spring generation were kept under summer conditions, diapause lasted for 120–210 days and adult emergence extended over about 90 days, whereas when hibernating pupae of the autumn generation were kept under winter conditions, diapause lasted for 90–125 days and adult emergence was relatively synchronized within one month (Fig. 1).

The difference in the diapause duration of aestivating and hibernating pupae may be due to the naturally changing temperature and photoperiod experienced by larvae and pupae. For the aestivating pupae, diapause was induced by gradually increasing daylength in April (from 13 h 20 min to 13 h 58 min, including twilight) and relatively low temperature (a mean of 19.2°C). Most of these pupae experienced long days for a long period (> 13 h for about 5 months) and high temperatures (a mean of 25.3°C) during their diapause. For the hibernating pupae, diapause was induced by gradually decreasing daylength in November (from 11 h 50 min to 11 h 22 min) and a mean temperature of 15.1°C. While in diapause most of the hibernating pupae experienced short days for a relatively short period (< 12 h 30 min for about 3 months) and low temperatures (a mean of 9.7°C) during their diapause. These results show that photoperiod and temperature have an important influence on the maintenance and termination of both summer and winter diapause.

DISCUSSION

In some insects, the duration of diapause is clearly affected by photoperiod during diapause. The pupae of *S. imparilis*, reared at a photoperiod of 12L : 12D as larvae required a longer period for adult emergence than those reared at 16L : 8D (Sugiki & Masaki, 1972). In *C. downesi*, the rate of diapause development of diapausing adults transferred from the field (on 22 December) to one of five photoperiodic regimes ranging from 12L : 12D to

TABLE 3. Duration of pupal diapause in *Pieris melete* kept at 20°C and natural changing daylengths. Diapause was induced at six different photoperiods.

Diapause-inducing photoperiod	Number observed	Duration of diapause (d)* $\bar{x} \pm SD$
8L : 16D	60	68.07 \pm 11.54 D
10L : 14D	44	69.66 \pm 10.12 D
12L : 12D	60	65.93 \pm 9.31 D
13L : 11D	47	94.45 \pm 12.80 A
14L : 10D	63	102.87 \pm 10.23 B
16L : 8D	69	80.58 \pm 10.12 C

* Values followed by different letters are significantly different based on a Bonferroni test and one-way analysis of variance (ANOVA). $F = 125.371$, d.f. = 5,351, $P (= 0.0000) < 0.01$.

16L : 8D increased with increase in photoperiod (Tauber & Tauber, 1976). In the tailed zygænid moth, *E. westwoodii*, the duration of summer diapause of aestivating prepupae kept under one of five photoperiodic regimes ranging from 12L : 12D to 16L : 8D gradually increased with increase in photoperiod (Gomi & Takeda, 1992). In the case of *P. melete*, however, the diapause duration of aestivating pupae was significantly shorter at short daylengths of 12 and 10 h than that at a long daylength of 14 h, whereas diapause duration of hibernating pupae was significantly shorter at a long daylength of 14 h than at short daylengths of 10 and 12 h (Table 1). This result suggests that short daylengths favour the development of aestivating pupae and long daylengths the development of hibernating pupae. This may imply that aestivation and hibernation are physiologically different, as they are induced by opposite photoperiodic signals. A very similar result is recorded for the Iberian population of *P. brassicae* (Spieth, 2002). When the hibernating and aestivating pupae were kept at either 15L : 9D or 10L : 14D, the hibernating pupae required longer to terminate diapause than aestivating pupae at a short photoperiod (10L : 14D); and aestivating pupae required more days than hibernating pupae to terminate diapause at a long photoperiod (15L : 9D).

That the pre-diapause photoperiod affects diapause intensity has been reported for a number of insects. In the fruit flies, *D. auraria*, *D. subauraria* and *D. triauraria*, the photoperiods with longer scotophases induce more intense diapause than those with shorter scotophases (Kimura, 1983, 1990). In the green lacewing, *C. ocellata* and the European corn borer, *O. nubilalis*, a 12 h scotophase evokes a greater intensity of diapause than either longer or shorter scotophases (Nechols et al., 1987; Beck, 1989). In the Mediterranean tiger moth *C. pudica*, short photophases (11 or 12 h) induce a long prepupal diapause (mean 88 days) whereas long photophases (14, 16 h) induce a short diapause (mean 52 days) (Košťál & Hodek, 1997). In the bean bug, *R. clavatus*, diapausing adults kept at a photoperiod of 13L : 11D as nymphs start to oviposit earlier than those kept at shorter photoperiods (Nakamura & Numata, 2000). In the present study on *P. melete*, short photophases ≤ 12 h induced a short diapause

(65–69 days) at 20°C, whereas long photophases ≥ 13 h induced a long diapause (80–102 days) (Table 3). This difference in the duration of diapause induced by short and long photoperiods might be due to the long summer photoperiods (> 13 h 50 min) experienced by diapausing pupae. That is, summer conditions can shorten diapause induced by short daylengths and lengthen diapause induced by long daylengths.

Under natural conditions, aestivating pupae had a long diapause (mean 155 days) and emerged over a period of 90 days, whereas hibernating pupae had a short diapause (mean 105 days) and a relatively synchronized emergence (about 30 days). This is also reported for the regulation of diapause in the leaf-mining fly, *Pegomyia bicolor*. In the field, the duration of diapause of aestivating pupae (> 175 days) is significantly longer than that of hibernating pupae (< 116 days) (Xue et al., 2001).

As mentioned above, in addition to the effect of temperature, photoperiod could also affect the duration of diapause of naturally aestivating and hibernating pupae of *P. melete* by increasing or decreasing the initial intensity of diapause and diapause development. In nature, diapause in summer is induced by a gradually increasing daylength (from 13 h 20 min to 13 h 58 min) during the development of the larvae, which may strengthen the initial intensity of diapause. This diapause was maintained by daylengths > 13 h before 17 September (for about 5 months), which may reduce the rate of diapause development in summer. In fact, 70% of the adults emerged from aestivating pupae after 17 September (Fig. 1B). This delay in development is important ecologically as it prevents adult emerging in summer and synchronizes adult emergence with the occurrence of lower temperatures and an abundance host-plants in autumn. In fact, high temperatures are unsuitable for the reproduction of the cabbage butterfly and the growth of its host-plants. In nature, winter diapause was induced by a gradually decreasing daylength (from 11 h 50 min to 11 h 22 min) in November, which may weaken the initial intensity of diapause. After that diapause was maintained by short daylengths of < 12 h before 9 February (for about 2.5 months), which may accelerate the termination of winter diapause. Therefore, adults that emerged from winter pupae have a much shorter emergence period in spring (one month). This increase in winter development ensures the spring generation of butterflies synchronizes its reproduction and off-spring development with the availability of food plants, as almost all cruciferous vegetables complete their develop-

ment in spring and are harvested within two months, i.e., before mid-May.

ACKNOWLEDGEMENTS. We thank H.R. Spieth for his critical reading of the manuscript and very helpful comments and also thank two anonymous referees for their valuable comments. The research was supported by a Grant from National Natural Science Foundation of P.R. China (30460074).

REFERENCES

- BECK S.D. 1989: Factors influencing the intensity of larval diapause in *Ostrinia nubilalis*. *J. Insect Physiol.* **35**: 75–79.
- DANKS H.V. 1987: *Insect Dormancy: An Ecological Perspective*. Biological Survey of Canada, Ottawa, 439 pp.
- GOMI T. & TAKEDA M. 1992: A quantitative photoperiodic response terminates summer diapause in the tailed zygaenid moth, *Elcysma westwoodii*. *J. Insect Physiol.* **38**: 665–670.
- KIMURA M.T. 1983: Geographic variation and genetic aspects of reproductive diapause in *Drosophila triauraria* and *D. quad-raria*. *Physiol. Entomol.* **8**: 181–186.
- KIMURA M.T. 1990: Quantitative response to photoperiod during reproductive diapause in the *Drosophila auraria* species-complex. *J. Insect Physiol.* **36**: 147–152.
- KOŠTÁL V. & HODEK I. 1997: Photoperiodism and control of summer diapause in the Mediterranean tiger moth *Cymbalophora pudica*. *J. Insect Physiol.* **41**: 767–777.
- NAKAMURA K. & NUMATA H. 2000: Photoperiodic control of the intensity of diapause and diapause development in the bean bug, *Riptortus clavatus* (Heteroptera: Alydidae). *Eur. J. Entomol.* **97**: 19–23.
- NECHOLS J.R., TAUBER M.J. & TAUBER C.A. 1987: Geographical variability in ecophysiological traits controlling dormancy in *Chrysopa oculata* (Neuroptera: Chrysopidae). *J. Insect Physiol.* **33**: 627–633.
- SPIETH H.R. 2002: Estivation and hibernation of *Pieris brassicae* (L.) in southern Spain: synchronization of two complex behavioral patterns. *Popul. Ecol.* **44**: 273–280.
- SUGIKI T. & MASAKI S. 1972: Photoperiodic control of larval and pupal development in *Spilarctia imparilis* Butler (Lepidoptera: Arctiidae). *Kontyu* **40**: 269–278.
- TAUBER M.J. & TAUBER C.A. 1976: Environmental control of univoltinism and its evolution in an insect species. *Can. J. Zool.* **54**: 260–266.
- XUE F.S., ZHU X.F. & WEI H.Y. 1996: Biology of *Pieris melete* Ménétriés. *Jiangxi Plant Prot.* **19**: 1–4 [in Chinese].
- XUE F.S., KALLENBORN H.G. & WEI H.Y. 1997: Summer and winter diapause in pupae of the cabbage butterfly, *Pieris melete* Ménétriés. *J. Insect Physiol.* **43**: 701–707.
- XUE F.S., ZHU X.F. & SHAO Z.Y. 2001: Control summer and winter diapause in the leaf-mine fly *Pegomyia bicolor* Wiedemann (Dipt., Anthomyiidae). *J. Appl. Entomol.* **125**: 181–187.
- ZOLMAN J.F. 1993: *Biostatistics: Experimental Design and Statistical Inference*. Oxford University Press, Oxford, 344 pp.

Received January 19, 2006; revised and accepted February 13, 2006