Influence of photoperiod on the development of diapause in larvae and its cost for individuals of a univoltine population of Dendrolimus punctatus (Lepidoptera: Lasiocampidae)

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Abstract. Larvae of Dendrolimus punctatus overwinter in diapause. In the Yangtze River Region, this species is multivoltine at altitudes below 400 m and univoltine above 700 m a.s.l. The photoperiodic response of the univoltine population, investigated at five day lengths (11.5, 12.5, 13.5, 14.5 and 15.5 h) at 27 ± 1°C, indicates that D. punctatus is a long-day species. Diapause was induced in 95–100% of the individuals by three short photophases (11.5–13.5 h), while diapause incidence decreased steeply with increase in day length down to 10% at 15.5 h. The critical daylength (CDL = day length resulting in a 50% incidence of diapause) is 14.6 h. When induced by critical or longer photophases (14.5 and 15.5 h), diapause terminated spontaneously, without any change in photoperiod or temperature. Diapause induced at shorter photophases was more intense and not terminated without activation by diapause averting conditions. Larvae in diapause lived for up to 190 days when kept under a 11.5 h photophase during which they remained sensitive to the photoperiodic signal. In transfer experiments, diapause was terminated after an increase in photoperiod, even if it was within the range of diapause inducing photophases: from 11.5 h to 13.5 h. This study revealed that the univoltine hill dwelling population of D. punctatus undergoes a facultative diapause. Although potentially multivoltine they are univoltine because of the low temperatures at that altitude.

INTRODUCTION

Photoperiod is the most important cue initiating diapause in insects (Danilevsky et al., 1970; Tauber et al., 1986; Bradshaw & Holzapfel, 2007) and there are two major types of photoperiodic response, a long-day response in which diapause is induced by short photoperiods and a short-day response in which diapause is induced by long photoperiods (Beck, 1980; Tauber et al., 1986). The photoperiodic response of many insects varies geographically and is genetically determined (Danks, 1987; Bradshaw & Holzapfel, 2007), which is expressed in terms of variation in the critical photoperiod (day length for a 50% diapause response). Insect diapause is not a simple arrest of morphological development (Tauber et al., 1986; Danks, 1987; Saunders et al., 2002), but a dynamic process of diapause development (Tauber et al., 1986; Danks, 1987; Hodek & Hodková, 1988; Hodek, 1996, 2002; Denlinger, 2002; Koštál, 2006; Ragain et al., 2009), consisting of several successive phases, such as, maintenance, termination etc. (Tauber et al., 1986; Koštál, 2006). In some insects, responsiveness to photoperiod may diminish gradually during diapause development, however, this sensitivity may persist throughout diapause and is an important diapause-maintaining factor in some insects. In these latter insects a specific photoperiodic condition is required before diapause terminates (Tauber et al., 1986). However, our understanding of how diapause ends (termination) is still very incomplete (Koštál, 2006) and the details of the processes that occur during diapause development poorly known (Hodek, 2002). Therefore, further research on the photoperiodic termination of diapause in insects is needed.

The pine caterpillar Dendrolimus punctatus is a major pest of pine forest consisting of Pinus massoniana. Outbreaks of this species are frequently recorded and often destroy the agro-forestry systems in China (Chen, 1990). It has a broad distribution ranging from 20°N to 34°N (Hou, 1987) and a variable life history. For instance, the number of generations per year decreases with increase in latitude, from four or five generations in southern China, decreasing to two generations in northern China (Hou, 1987). In the region of the Yangtze River (around latitude 30°N), two or three generations are produced per year at low altitudes (below 400 m) (Hou, 1987), but only one at high altitudes (above 700 m) (Chen et al., 1992). D. punctatus enters larval diapause mostly during the third or fourth instar (Hou, 1987; Li & Gia, 1991; Huang et al., 2005), mainly in response to short day lengths (Li & Gia, 1991; Huang et al., 2005). In the region of the Yangtze River, the multivoltine populations enter diapause mostly
in late summer or early autumn (Hou, 1987) and have a CDL of about 13.5 h (Li & Gia, 1991; Huang et al., 2005). However, the univoltine populations enter diapause earlier (e.g. in July, Chen et al., 1992) and thus we assume their CDL is “longer”. But there are no studies on the photoperiodic response of individuals from univoltine populations and whether they have a “longer” CDL is unknown. Moreover, previous studies on this moth were mainly on photoperiodic induction (Li & Gia, 1991; Li et al., 1994, 1995; Huang et al., 2005) or coldhardiness of larvae in diapause (Han et al., 2005, 2008; Zeng et al., 2008), but not on the photoperiodic regulation of diapause development, including diapause intensity and post-diapause development. The present study analyzes the photoperiodic response of individuals from a univoltine population of *D. punctatus* in terms of the above diapause parameters. In addition, we also compared some life history traits of non-diapause and diapause generations.

**MATERIALS AND METHODS**

**Insect culture**

The cocoons of *D. punctatus* were collected in a forest of masson pine, *Pinus massoniana*, at altitudes between 700 and 800 m in Suining County (26.35°N, 110.09°E), Hunan Province, China in June 2007. This species completes only one generation per year and the early larval instars mostly occur in July (Chen et al., 1992), which is the hottest month at these altitudes with a mean air temperature of 27 ± 1°C. The cocoons collected were transferred to insect cages (60 × 60 × 60 cm) and the adults that were each transferred and kept individually in a glass tube (dia. 8.5 cm, length 20 cm) sealed with a ball of cotton and the hatching larvae reared at 27 ± 1°C under different photoperiods. Fresh needles of masson pine, *Pinus massoniana*, were also provided.

The photoperiodic experiments were conducted in illuminated chambers (LRH-250-GS) equipped with eight fluorescent 30W tubes (light intensity 800–1000 lux) controlled by electric timers. In special cases the scotophase was also controlled manually by enclosing the rearing tubes in opaque hoods.

**Diapause induction and identification**

To induce diapause the newly-laid eggs were kept at 27 ± 1°C under one of five photoperiods: 11.5L : 12.5D, 12.5L : 11.5D, 13.5L : 10.5D, 14.5L : 9.5D or 15.5L : 8.5D. In the experiments, newly-laid eggs from mated females were transferred to insect cages (60 × 60 × 60 cm) and the adults that were each transferred and kept individually in a glass tube (dia. 8.5 cm, length 20 cm) sealed with a ball of cotton and the hatching larvae reared at 27 ± 1°C under different photoperiods.

Data were recorded daily when fresh needles of masson pine were provided.

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Furthermore, we weighed three-day old non-diapause or diapause pupae, using an electronic balance (sensitivity: 0.1 mg, Sartorius, R200 D.A.G., Göttingen, Germany), and determined the life-span of the adults. In each experimental regime, 30 to 218 eggs were used, and all experiments were terminated when the last adult died.

### Statistical analysis

One-way analysis of variance (ANOVA) was used to analyze the data with *P* < 0.05, and Tukey’s Honest Significant Difference test for multiple comparisons. Statistical analyses were conducted using SPSS 13.0 software.

### RESULTS

**Photoperiodic response of the univoltine population of *D. punctatus* reared at 27 ± 1°C**

The photoperiodic response of the univoltine population reared under five different day lengths, 11.5 h, 12.5 h, 13.5 h, 14.5 h and 15.5 h were recorded (Fig. 1). The response curve was of the long-day type. From the curve, the CDL was estimated as 14.6 h. Diapause incidence of nearly 100% was induced by three short day lengths, 11.5, 12.5 and 13.5 h; then the incidence steeply declined with increase in day length. The lowest incidence (10%) was recorded at the longest day-length (15.5 h) used (Fig. 1).

**Influence of photoperiod on the intensity, maintenance and termination of diapause in *D. punctatus***

The 60 day-old larvae that had been in diapause were kept under one of five diapause inducing photophases (11.5 h, 12.5 h, 13.5 h, 14.5 h or 15.5 h) or manually transferred to a longer photophase, 13.5 h or 15.5 h (see Fig. 2 for details). Fig. 2 shows that the intensity of dia-
pause was relatively low when induced by the near-critical photophases 14.5 h and 15.5 h as development of the larvae ceased for less than a month and then started again (Fig. 2) and the larvae pupated. However, the intensity of diapause was much greater when the larvae were reared and kept at the three short day lengths 11.5 h, 12.5 h and 13.5 h. These larvae eventually died if kept in these diapause inducing conditions (Fig. 2), with those kept in the 13.5 h photophase treatment dying first (within 110d) and those in the 12.5 h and 11.5 h photophase treatments surviving longer, up to 170 d and 190 d, respectively (Fig. 2).

The results of the four photophase-transfer treatments presented in Fig. 2 (11.5 h → 13.5 h, 11.5 h → 15.5 h, 12.5 h → 13.5 h, 12.5 h → 15.5 h, 13.5 h → 13.5 h, 13.5 h → 15.5 h, arrows indicate the transfer when 60 days-old). DP – diapause; PD – post-diapause development.
12.5 h → 15.5 h and 13.5 h → 15.5 h, but not 12.5 h → 13.5 h) show that larvae in diapause can respond to an increase in day length by awaking from diapause and resuming growth. We calculated the time from the day on which the photoperiod was increased to the day on which the post-diapause larvae showed signs of a slight darkening in the colour of the body and of actively feeding on needles and expressed this in terms of days in Table 1. This revealed that diapause was terminated after different periods of time in these four transfer treatments (F₃, ₉₉ = 44.937, P < 0.001). The shortest period (22.4 d) was recorded in the 13.5 h → 15.5 h photophase treatment and longer periods of 37 d and 38 d, respectively, in the 11.5 h → 13.5 h and 11.5 h → 15.5 h treatments, i.e. a more intense diapause was induced by the shorter 11.5 h day length (Fig. 2).

The time taken by larvae after awaking from diapause to complete their development and pupate is given in Table 1. It was significantly different in the above four transfers between photophases (F₃, ₄₉ = 10.787, P < 0.001). The longest was recorded in 11.5 h → 13.5 h treatment, with the larvae requiring 92.5 d to complete their development and pupate when reared under the relatively short 13.5 h photophase.

**Comparisons of pupal weights and adult life-spans of individuals of D. punctatus, which as larvae did not or did enter diapause**

The cost of diapause was measured in terms of the pupal weight and adult life-span of individuals that as larvae did not enter diapause (NP) and those that did (DP) and the results are presented in Fig. 3. The pupal weights of the NP and DP individuals reared under 14.5 h and 15.5 h photophases did not differ significantly (respectively F₁, ₂₄ = 0.074, P = 0.788 and F₁, ₉₉ = 0.151, P = 0.699). However, the adult life-span of the DP individuals was significantly shorter in the 15.5 h treatment (F₁, ₅₃ = 9.025, P = 0.004) (see Fig. 3).

**DISCUSSION**

Response of individuals from multivoltine and univoltine populations of D. punctatus to photoperiod

Our results confirm earlier findings that the pine caterpillar D. punctatus is a long-day animal (Hou, 1987; Li & Gia, 1991; Huang et al., 2005). Beck (1980) says that long-day insects only enter diapause when days are short and consistently reproduce, grow and develop under the long-day conditions prevailing in late spring and early summer (Tauber et al., 1986). As for D. punctatus, it usually defoliates forests of masson pine from May to July in the Yangtze River region (Hou, 1987; Chen, 1990). The natural day-length at that time is 14.0 h or above, longer than either the CDL value of 13.3 h cited by Huang et al. (2005) or that of 13.5 h by Li & Gia (1991), which are the minimum day lengths required for fast growth and reproduction of this pest. However, the CDL varies with latitude in many species, mostly greater in the north than in the south (Danks, 1987). In Daphnia pulex, for example, its CDL increases from 13 h at 45°N to 22 h at 71°N (Stross, 1971). D. punctatus also has a broad distribution, from 20° to 34°N latitude (Hou, 1987), and Gia (1993) indicates that its CDLs increase from 13.0 h at 26.0°N to 13.5 h at 30.0°N, with an increase of about one hour for...
every 8° latitude. A similar increase of one hour per 5.4° latitude is reported for *Wyeomyia smithii* (Bradshaw, 1976; Bradshaw & Holzapfel, 2007) and one hour per 7° latitude for *Phyllonorycter ringoniella* (Ujiye, 1985). As the CDL increases toward the north, an insect can complete fewer generations per annum in the north than the south, e.g., in *D. punctatus* it declines from 5 to 2 (Hou, 1987).

Furthermore, there is also an increase in CDL with increase in altitude (Danks, 1987; Bradshaw & Holzapfel, 2007), which is confirmed by our results. In the region of the Yangtze River, the CDL is relatively “long”, 14.6 h, in the univoltine population of *D. punctatus* that occurs at an altitude of 700 m or above (Chen et al., 1992), compared to the 13.3 h or 13.5 h CDLs of multivoltine populations that occur at altitudes below 400 m, and increases about one hour per 400 m increase in altitude in this region. The growing season for all organisms is shorter at high than at low altitudes and they are more likely to enter winter diapause earlier at high altitudes (Hodkinson, 2005). This study provides support for the day length triggering diapause being significantly longer for high than low altitude populations of the same species. Field observations indicate that highland populations of *D. punctatus* mostly enter diapause early in mid-July (Chen et al., 1992) when the day length is 14.5 h or so and diapause is triggered one month or earlier than in lowland populations, where they mostly enter diapause in late August or September. In summer, the temperature decreases rapidly after July in the high regions of Suiing, e.g. in 2007 the average air temperature decreased from 27.6°C in July down to 26.8°C in August and to 22.0°C in September. At these temperatures, the larvae enter winter diapause sooner, which synchronizes their life cycle with the highland season; this represents an important adaptive benefit for this species.

**Diapause intensity, maintenance and termination under constant photoperiod or a change of photoperiod in *D. punctatus***

Diapause intensity is measured in terms of the duration of the developmental arrest (diapause) at a given moment and under given environmental conditions (Vinogradova, 1974). Results of this study indicate that diapause intensity in *D. punctatus* depends on the length of the inducing photoperiod, whereas in other insects the response is all or none (Danks, 1987), such as in the blow fly *Lucilia caesar* (Ring, 1968) and butterfly *Pieris brassicae* (Tyshchenko & Goryshin, 1979). A relatively weak diapause (low intensity) was induced by near-critical photoperiods (photophase 14.5 h and 15.5 h) and terminated spontaneously (Fig. 2), as occurs in many other insects and mites kept under constant photoperiod or temperature conditions in a laboratory (Danks, 1987; Koštál, 2006). Larvae in intense diapause, induced by short photoperiods (photophases 11.5 h, 12.5 h and 13.5 h), did not resume development and eventually died (Fig. 2) if not exposed to long day conditions. This also occurs in *Diprion pini*, as the eonymphs in diapause will not pupate and eventually die if not exposed to long days (Eichhorn, 1977). We also found that larvae in deep diapause kept under very short day conditions (e.g. at 11.5 h) survived for longer than those kept at 13.5 h. This indicates that larvae in deep diapause may have a lower metabolic rate, which enables them to survive longer.

Photoperiod is one of two major factors that maintain diapause (Tauber et al., 1986). However, in our study only three short photophases (11.5 h, 12.5 h and 13.5 h) maintained diapause in *D. punctatus* (see Fig. 2). Many species gradually become less sensitive to photoperiod during diapause development, e.g. the Odonate *Lestes eurinus* (Lutz, 1968), linden bug *Pyrrhocoris apterus* (Hodek, 1971) and flesh fly *Sarcophaga bullata* (Denlinger, 1972). *D. punctatus*, however, remained sensitive to photoperiod throughout diapause. In the transfer experiments, for instance, diapause was terminated when they were transferred from a short (11.5 h) inducing photophase to a “longer” photophase (e.g. 13.5 h or 15.5 h). Here, the “short” photophase of 13.5 h (a diapause-inducing and maintaining photophase) terminated diapause evidently due to the 2 h lengthening of the photophase (e.g. 11.5 h → 13.5 h in Fig. 2). This indicates that the thresholds photoperiods critical for diapause induction and termination usually differ (Danks, 1987). For instance, in the bug *Pyrrhocoris apterus*, the critical photoperiod for the end of diapause is one hour longer than that for induction (Saunders, 1983). And in the facewing *Meleona signoretti*, the difference is about two hours (Tauber et al., 1986). In addition, a change in the length of the short photoperiods, which induce diapause, can terminate diapause development in this moth, as in some other insects (see Danks, 1987, pp. 143–144). For example, eonymphs of the sawfly *Neodiprion pini* will emerge if transferred from short days to somewhat longer days, which normally induce diapause (Eichhorn, 1977).

In *Pectinophora gossypiella* daily photophases of 14–16 h terminate diapause more rapidly than 8- or 12-h photophases (Watson et al., 1973). In addition, the time taken to terminate diapause may be also dependent on the diapause-inducing conditions. For instance, in *D. punctatus*, it took longer to terminate diapause induced by the “short” short-photophase of 11.5 h than that induced by the “long” short-photophase of 13.5 h (see Table 1). This again suggests that deep diapause takes longer to terminate. However, it is worth mentioning that post-diapause development is more dependent on current photoperiods than on the diapause inducing photoperiods (see Table 1).

In addition to the effect of photoperiod diapause can be terminated by high temperature. For instance, diapause in *Telogryllus commodus* persists for long periods (60–80 days) at 20°C, but is terminated very quickly after a brief exposure to high temperatures (e.g. 30°C) (Masaki et al., 1979).

In insects, the individuals that diapause require greater energy reserves for maintenance than those that do not. This indicates there is a cost associated with diapause that might affect other life-history traits. For example, in *Acanthoscelides pallidipennis*, the fecundity and body size of adults that spent some time in diapause during
their development are less than those that did not diapause (Sakaiyko & Ishihara, 2012). Our results also indicate there is a cost as those individuals of *D. punctatus* that entered diapause had a shorter adult life-span than those that did not (see Fig. 3). A similar situation occurs in *Leptinotarsa decemlineata* (Jansson et al., 1989) and *Busseola fusca* (Gebre-Amlaka, 1989).

In conclusion, our results reveal that a single environmental factor, photoperiod, can greatly influence the expression of diapause development. Larval diapause was relatively deep when induced by short photoperiods and can be maintained by these inducing photoperiods. However, a relatively weak diapause was induced by near-critical or long photoperiods and is terminated spontaneously. Changes in photoperiod accelerated diapause development and decreased the intensity gradually or rapidly. This supports the possibility that there are “multiple pathways of diapause development in every individual (Hodek, 2002).”

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