

## Mixed life cycles in the transitional zone between voltinisms in the fall webworm, *Hyphantria cunea*

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**Abstract.** The fall webworm, *Hyphantria cunea* Drury, was introduced from North America into Japan at half a century ago. At present, the populations north of 36° N are bivoltine and those south of 36° N are trivoltine. In the present study, the life cycle in the transitional zone between voltinisms was clarified in Tsukuba (36.1° N). When fourth instar larvae of the second generation were collected in the field and maintained in the laboratory, the incidence of pupal diapause increased when the sampling date was delayed from 16 August to 5 September. The fact that some developed without diapause indicates the occurrence of a trivoltine life cycle in this locality, where a bivoltine life cycle is predominant. The critical photoperiod for diapause induction at 20 and 25 °C was 14 h 13 min and 14 h 10 min, respectively. The photoperiodic response may explain the rapid increase in diapause incidence in late summer. In laboratory-reared diapause pupae, the time and period of chilling (5 °C) greatly influenced the time required for adult emergence at the final incubation temperature of 25 °C. However, diapause pupae chilled for a sufficiently long period developed to adulthood rapidly, irrespective of the conditions before chilling. Therefore, the difference in timing of entering diapause between pupae of the second and third generations would not result in their temporal reproductive isolation in the following spring. It is concluded that the mixed voltinism in Tsukuba is not a result of a mixture of genetically distinct bivoltine and trivoltine populations, but a result of the phenotypic plasticity in a genetically rather homogeneous population.

**Key words.** Diapause; *Hyphantria cunea*; photoperiodic response; transitional zone; voltinism.

Introduced insects are suitable for studying the evolution of life histories<sup>1</sup>. The fall webworm, *Hyphantria cunea*, was introduced from North America into Japan, and first found in Tokyo (35.7° N) in 1945<sup>2</sup>. Since then, the range of its distribution has expanded to 32° N in the south and to 40° N in the north<sup>3</sup>. In many insects, the induction of diapause is controlled by photoperiod and temperature<sup>4</sup>. *H. cunea* overwinters as a pupa in a state of diapause which is primarily induced by larval photoperiod<sup>5</sup>. A bivoltine life cycle, i.e. two generations per year, prevailed throughout its distribution until the early 1970s. Adults of the overwintering generation emerged in the late spring and those of the first generation in midsummer<sup>2</sup>. However, the cycle shifted to a trivoltine one, i.e. three generations per year, in the southwestern areas of Japan. The boundary between the two types of life cycle occurs around 36° N<sup>3,6,7</sup>. Elucidation of the life cycle in the transitional zone between bivoltine and trivoltine life cycles in this introduced insect may contribute to an understanding of the evolution of life histories.

Morris and Takeda<sup>8</sup> reported that in the transitional zone between voltinisms, larvae of the first generation occurred in late May, those of a second generation in

early August, and those of a third generation in mid September, when the cumulative heat units above 10 °C, from the spring, reached about 1700 degree-days. This value is 300 degree-days smaller than that in a southwestern city (34.7° N) where a trivoltine life cycle is known to occur<sup>6</sup>, although the first two generations appear at nearly the same heat-unit point in both localities. It is thus possible that in the transitional zone only some of the individuals in the second generation, which appear relatively early in the season, develop without diapause and produce a third generation. In this study, this hypothesis was tested by determining the incidence of diapause in the second generation and the photoperiodic response for the induction of diapause in this population.

If some individuals of *H. cunea* enter diapause in the second generation, and the others in the third, the former would experience relatively high temperatures for a longer period as diapause pupae than the latter, before winter comes. The false melon beetle, *Atrachya menetriesi*, overwinters in the egg stage, and the intensity of diapause varies depending on the temperature during the early phase of the egg stage<sup>9</sup>. The intensity of diapause can be estimated as the time required for termination of diapause under given conditions, e.g. low temperature<sup>4</sup>. If a difference in temperature conditions before winter influences the intensity of diapause in *H. cunea*, it may bring about a difference in the timing of

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adult emergence in the following spring, and consequently reproductive isolation. To examine this possibility, the effects of the pre-chilling and chilling of diapause pupae on adult emergence were also investigated.

## Materials and methods

**Rearing of insects.** Middle instar larvae of *Hyphantria cunea* Drury were collected from *Liquidambar styraciflua* L. in Tsukuba, Japan, in June 1993. The larvae were reared until adult emergence on an artificial diet of 'Insecta LF' (Nihon Nosan Kogyo), in transparent plastic cups (500 ml) with continuous light at  $25 \pm 1$  °C. The diet and cups were changed every 2–5 days depending on the larval instar. Adults and egg-batches were held at 16L-8D (16 h light-8 h dark) and  $20 \pm 1$  °C. Hatchlings were incubated under various experimental conditions.

**Incidence of diapause in the second generation.** As the stage sensitive to photoperiod occurs in the first three of the seven instars in this species<sup>10</sup>, fourth instar larvae were collected from *L. styraciflua* between 16 August and 5 September every 5 days in 1993. The larvae taken from different nest-webs were reared separately on the artificial diet in continuous light at 25 °C, and the incidence of diapause was determined for each nest-web.

**Photoperiodic response.** Insects were kept in light-proof wooden cabinets (40 cm × 70 cm × 55 cm height) kept in constant temperature rooms or in incubators (45 cm × 60 cm × 45 cm height). Each cabinet or incubator was equipped with a 10W or a 6W fluorescent lamp fixed on the ceiling to control the photoperiod. The photoperiodic response controlling diapause induction was investigated by rearing insects from hatching to adult emergence with photoperiods varying from 13.75L-10.25D to 14.75L-9.25D, at 20 and 25 °C. Pupae producing adults within 50 days (20 °C) or 40 days (25 °C) after pupation were regarded as non-diapause individuals, and the remaining living pupae as diapause individuals. The critical photoperiod was calculated as the day-length inducing diapause in 50% of the individuals at each temperature.

**Effects of the pre-chilling and chilling periods on adult emergence.** Diapause pupae obtained under a regimen of 12L-12D and 25 °C were kept under the same conditions for 5, 30 or 60 days and chilled at 5 °C for 60, 70, 80, 90 or 100 days to break diapause. Before and after chilling, all pupae were exposed to a series of decreasing and increasing temperatures, i.e. 10, 15 and 20 °C for 5 days each, for acclimation. They were finally incubated at 25 °C and 12L-12D, and the time required for adult emergence at this final incubation temperature was recorded.

Table. The incidence of diapause in the second generation of *Hyphantria cunea* in Tsukuba in 1993.

Date of collection*	Nest-webs	Incidence of diapause (%)	N
16 August	A	43.72	119
	B	36.51	189
	C	21.40	271
	Total	36.96 <sup>a</sup>	579
21 August	A	97.94	97
	B	74.02	127
	C	66.67	162
	Total	76.94 <sup>b</sup>	386
26 August	A	92.35	170
	B	89.94	159
	C	51.79	199
	Total	76.33 <sup>b</sup>	528
31 August	A	100	103
	B	89.86	148
	Total	94.02 <sup>c</sup>	251
5 September	A	98.18 <sup>c</sup>	110

\*Larvae were collected at the fourth instar. Values followed by the same letters are not significantly different by  $\chi^2$ -test ( $p > 0.05$ ).

## Results

Nest-webs containing fourth instar larvae were collected in the field and maintained in the laboratory (constant light, 25 °C) to determine the incidence of diapause in the second generation in Tsukuba (table). The total incidence of diapause increased as the date of sampling became later. In the nest-webs sampled on 16th August, it was less than 50%, and significantly lower than that of the following samples ( $\chi^2$ -test;  $p < 0.05$ ). The incidence of diapause in the nest-webs sampled on 21st and 26th August varied between 52 and 98%. However, the total incidence of diapause was not significantly different between the two samples ( $p > 0.05$ ). The average incidence of diapause in the last two samples was significantly higher than that of the other samples ( $p < 0.05$ ).

The photoperiodic response curves for diapause induction were obtained at 20 and 25 °C (fig. 1). They were similar to each other. The incidence of diapause varied from 100 to 0% over a range of about 45 min. The critical photoperiod was 14 h 13 min at 20 °C and 14 h 10 min at 25 °C.

The effects of timing and period of chilling on adult emergence were investigated by first incubating diapause pupae at 25 °C for 5, 30 or 60 days and then exposing them to 5 °C for various periods (fig. 2). All chilled pupae were finally incubated at 25 °C for adult emergence. The timing of chilling greatly influenced the subsequent development of diapause pupae, and the earlier the time of chilling, the longer it took the pupae to emerge as adults upon transfer to 25 °C. Irrespective of the time of transfer to the chilling conditions, the time required for adult emergence at the final tempera-

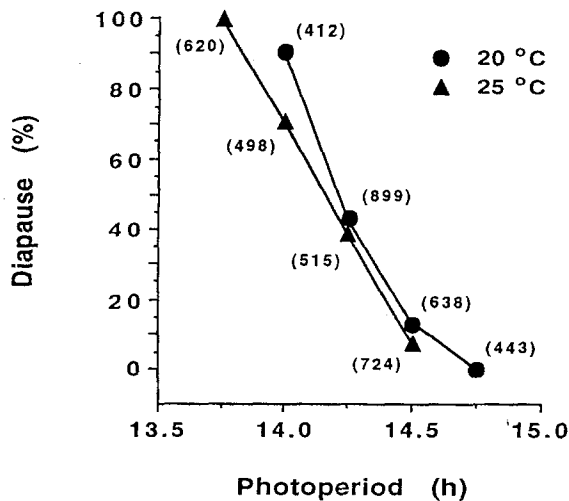


Figure 1. Photoperiodic response curves for induction of pupal diapause in the Tsukuba population of *H. cunea* at two temperatures. Sample sizes are in parentheses. Circles and triangles show results at 20 and 25 °C.

ture of 25 °C rapidly decreased as the chilling period increased. The differences caused by the pre-chilling conditions became smaller as the chilling period increased, although they were still significant even after chilling of 100 days (Kruskal-Wallis test;  $p < 0.05$ ).

**Discussion**

The present results showed that some of the individuals in the second generation of *H. cunea* developed without diapause, and the remaining individuals, which appeared relatively late in the season, entered a pupal diapause in Tsukuba (table). Thus, this species is partially trivoltine in this locality. The incidence of diapause increased markedly between 16 and 26 August, during which natural day-lengths including 1 h twilight<sup>11</sup> decrease from 14 h 19 min to 14 h 10 min. The critical photoperiod for diapause induction of this population is 14 h 10–13 min (fig. 1). Therefore, the seasonal change in the incidence of diapause may be explained by the photoperiodic response. The critical photoperiod determined two decades after the introduction into Japan was 14 h 35 min at 25 °C both in the Yokohama (35.3° N) and the Oomagari (39.5° N) populations<sup>4</sup>. In the present study, the Tsukuba population was found to have a critical photoperiod of 14 h 10 min, 25 min shorter than that in the above populations. This difference may mean that the critical photoperiod was shifted as a result of local adaptation in Tsukuba.

An alternative hypothesis is that this population is a mixture of genetically distinct bivoltine and trivoltine individuals. The time required for adult emergence after chilling tended to decrease as the chilling period increased (fig. 2), as found in many insects including *H. cunea* from Canada<sup>12–14</sup>. It was also influenced greatly

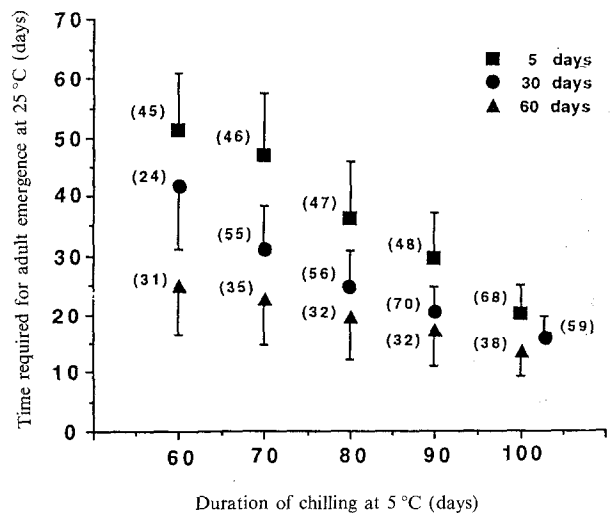


Figure 2. Time required for adult emergence at the final incubation temperature of 25 °C in diapause pupae treated with various lengths of pre-chilling (25 °C) and chilling conditions (5 °C). Pupae kept at 25 °C for 5, 30 and 60 days were incubated at 20, 15 and 10 °C for 5 days each before chilling at 5 °C for various lengths. After chilling, they were exposed to 10, 15 and 20 °C for 5 days each and incubated at 25 °C for adult emergence. Sample sizes are in parentheses. Squares, circles and triangles show the pre-chilling period for 5, 30 and 60 days after pupation.

by the timing of chilling. The differences caused by the timing of chilling became smaller as the chilling period increased. Although statistically significant even after chilling for 100 days, these differences were within six days. In temperate species of insects, a temperature range between 0 and 12 °C is the most suitable for diapause development<sup>15</sup>. In Tsukuba, such temperatures (<10 °C) occur during the winter months (Monthly Report of Meterology in Ibaraki Prefecture, 1993). Therefore, the difference in the time to enter diapause would not result in temporal isolation of mating between individuals having overwintered as second and third generations, respectively.

In *Dianemobius mikado* (formerly *Pteronemobius fascipes*), there is a difference in photoperiodic response controlling nymphal development between uni- and bivoltine populations<sup>16,17</sup>. This difference in photoperiodic response leads to a divergence in life cycles in the transitional zone from the univoltine to the bivoltine areas. In *H. cunea*, however, there is no evidence for the photoperiodic control of larval development<sup>3,7</sup>.

In *H. cunea*, the photoperiodically-controlled diapause may function as a buffer mechanism to decrease annual variance of fitness. In a warm year, insects would develop fast and experience relatively long daylengths in the second generation. They would thus undergo non-diapause development and can produce a third generation. Conversely, in a cool year, the proportion of individuals to enter diapause would be high in the second generation because they grow slowly and thus encounter short day-lengths.

Taylor<sup>18</sup> pointed out that if insects enter diapause too early in the season, reproductive potential would be reduced, and if they enter it too late, they may not develop to the appropriate stage for overwintering. In *H. cunea*, production of a third generation would be favorable for an increase in population size but involve a risk of failing to develop to the diapause stage by the arrival of winter. The risk would be reduced if a third generation starts early in the season. Thus the timing to enter diapause is important, and the critical photoperiod would be adjusted through natural selection so that diapause is induced with the optimal timing to maximize fitness of the population in each locality.

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