There was a significant difference in male response between the two populations. All values in the dose-response relationship were lower in the Thai population (Fig. 2), though the patterns were very similar. The lower response in the Thai population may be related to adaptation to the tropical environment, that is, the optimal temperature for male response may increase. However, male response is easily affected by environmental factors such as rearing conditions, and usually shows higher variability than is seen in female pheromone production. Actually, the level of male response in Fig. 3 was not so low in the percentages in similar condition (6:4 and 4:6). Therefore, it is difficult to discuss the differentiation of the male response system between two populations from these results alone. In order to solve this problem, a comparison of male response at various temperature conditions, especially at higher temperatures, is necessary.

The present results did not show a clear differentiation of characteristics in the pheromone system of *P. operculella* between the Japanese and Thai populations, though some possibilities of differentiation in male response still remain.

ACKNOWLEDGEMENTS

The authors thank Dr. A. YAMAMOTO for supplying the standard chemicals of the *P. operculella* sex pheromone.

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Slow Pupal Development Induced by Larval Temperature in the Fall Webworm, *Hyphantria cunea* DRURY (Lepidoptera: Arctiidae): Implication for Inter-Population Variation in Incidence¹

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(Received 15 January 1997)

(Accepted 7 April 1997)

Key words: *Hyphantria cunea*, pupal development, introduced insect, temperature, voltinism

The fall webworm, *Hyphantria cunea* DRURY, was

distributed exclusively in North American countries until 1940 (WARREN and TADIC, 1970). Its distribution expanded to central Europe and eastern Asia in the 1940's (UMEYA and ITÔ, 1977). In Japan, it was first found in Tokyo (35.7°N) in 1945, and then its range expanded to 32°N in the south and to 40°N in the north (GOMI and TAKEDA, 1996). It was bivoltine until the early 1970's throughout its distribution in Japan (MASAKI, 1975). However, a trivoltine life cycle was found in the south-western areas (UEZUMI, 1976; GOMI and TAKEDA, 1990) whereas a bivoltine life cycle has been maintained in the northern areas, and the transitional zone between the two areas occurs around 36°N (GOMI and TAKEDA, 1991, 1996).

This species overwinters as a pupa in diapause which is determined by photoperiod during the first half of the larval stage (MASAKI et al., 1968; MASAKI, 1977). Its photoperiodic response is a long-day type, and diapause is induced under short-day conditions (MASAKI et al., 1968). UMEYA and MASAKI (1969)

¹ Appl. Entomol. Zool. **32** (3): 517–521 (1997)

found that some pupae showed slow development even under long-day conditions where all individuals avert diapause, and that the incidence of such pupae increased with temperature. Although the physiological mechanism of slow pupal development is unknown, this phenomenon seems to be categorized neither as winter diapause nor as heat quiescence (UMEYA and MASAKI, 1969).

The pupal developmental time of this species does not differ significantly between trivoltine and bivoltine populations, although larval developmental time is shorter in the former than in the latter (GOMI and TAKEDA, 1991, 1996). This observation was made at 20°C, and the effect of high temperature on the occurrence of slow pupal development has not been examined. Slow pupal development may be related to a shift in the life cycle which occurred after the species was introduced into Japan. In the present study, the effect of temperature on the occurrence of slow pupal development was compared among five local populations of *H. cunea* from the bivoltine, trivoltine and transitional areas.

MATERIALS AND METHODS

Mid-instar larvae of *Hyphantria cunea* were collected on Honshu Island from Akita (39.6°N, 140.1°E) in September, 1991, Sendai (38.3°N, 140.9°E) in July, 1994, Tsukuba (36.1°N, 140.1°E) in June, 1993, Kobe (34.7°N, 135.2°E) in June, 1993 and on Kyushu Island from Kumamoto (32.8°N, 130.7°E) in June, 1994. Akita and Sendai are located in the bivoltine area, Kobe and Kumamoto in the trivoltine area and Tsukuba in the transitional area (GOMI, 1995, 1996 a; GOMI and TAKEDA, 1996). Larvae collected from these localities were reared at 20±1°C and a photoperiod of 16L-8D (16 h light-8 h dark) until they became adults and laid eggs. Hatchlings obtained were transferred to various experimental conditions, and reared on an artificial diet, "Insecta LF" (Nihon Nosan Kogyo), in transparent plastic cups (500 ml). The diet was changed every 2-5 days depending on the rearing temperature and larval age.

To examine the effect of larval and pupal temperature on pupal development, larvae of the Tsukuba population were reared at 20 or 25±1°C and 16L-8D, and pupae were transferred to 20, 25 or 30±1°C and 16L-8D on the day of pupation. The number of days required for adult emergence was recorded for all live pupae. As the temperature of 25°C during the larval and pupal stage effectively elicited slow pupal development, larvae of the other populations were

Fig. 1. Distribution of pupal period in the Tsukuba population of *H. cunea*. Panels A, B and C show results of individuals transferred to 20, 25 or 30°C and 16L-8D at pupation. Insets in each panel show probits of pupal period. Open and closed columns and squares show individuals reared at 20 and 25°C, respectively, and 16L-8D as larvae. Mean pupal developmental time (days) with SD (in parenthesis) and sample sizes (*N*) are given in each panel.

reared at 25°C and 16L-8D, and the duration of the pupal stage was determined under the same conditions. All experimental conditions were provided by constant temperature rooms equipped with two 40 W fluorescent lamps.

Variance of pupal development was analyzed using JMP 3.0.2 (SAS Institute, Cary, NC). Data of air temperatures for each sampling site were obtained from the National Astronomical Observatory (ed.) (1995).

Fig. 2. Distribution of pupal period at 25°C and 16L-8D in four populations of *H. cunea*. Insets in each panel show probits of pupal period. Mean pupal developmental time (days) with SD (in parenthesis) and sample size (N) are given in each panel.

RESULTS AND DISCUSSION

In the Tsukuba population, peaks of adult emergence occurred 19, 12 and 10 days after pupation at 20, 25 and 30°C, respectively (Fig. 1). The duration of pupal development was not influenced by larval temperature. However, for larvae reared at 20°C, the variance of pupal development was smaller and the mean pupal development significantly shorter than for those reared at 25°C (KOLMOGOROV-SMIRNOV test, $p < 0.001$, for Fig. 1A, B, $p < 0.005$, for Fig. 1C). A probit analysis indicated that adult emergence occurred simultaneously when reared at 20°C during the larval stage, although a small number of individuals kept at 30°C as pupae showed some developmental delay (Fig. 1C). More individuals showed slow pupal development when exposed to 25°C during the larval stage than when exposed to 20°C, and the incidence of such individuals does not appear to be influenced by pupal temperature. These results indicate that slow pupal development is mainly programmed

during the larval stage.

UMEYA and MASAKI (1969) found that the incidence of slow pupal development in larvae of the Hiratsuka (35°19'N, 139°28'E) population reared at 20°C tended to increase as pupal temperature rose from 20 to 30°C. This result was in contrast with the present findings and may suggest that pupae are less sensitive to high temperature in the Tsukuba population than in the Hiratsuka population.

Figure 2 compares the effect of temperature on pupal development in other populations. The variance of pupal development differed significantly among populations (LEVENE test, $p < 0.05$), except for that between the Akita and Sendai populations. The variance of the Tsukuba population was the largest among the populations examined (Figs. 1 and 2). The Kumamoto population showed relatively large variance in pupal development. A probit analysis indicated that the pattern of adult emergence was rather uniform except for the Kumamoto population in which some individuals showed slow pupal develop-

ment. The incidence of slow pupal development was considerably lower in the Kumamoto population than that in the Tsukuba population (Fig. 1B).

The difference in pupal response to high temperature among the populations may be related to the difference in their life cycles, although the significance of slow pupal development in the life cycle of the original populations in North America is unknown. The fact that almost none of the individuals underwent slow pupal development in the Akita, Sendai and Kobe populations is of interest. TAYLOR (1980) suggested that if insects enter diapause too early in the season, the reproductive potential would be reduced, and if too late, they may not develop to the overwintering stage. In localities where there are nearly sufficient available heat units to produce two or three generations per year, slow pupal development occurring in summer may cause the fall generation to fail to reach the diapause stage by the onset of winter. In *H. cunea*, the amounts of heat above 10°C available for completion of one generation were about 800 day-degrees (ITÔ et al., 1970). The amounts of heat above 10°C available in Akita, Sendai and Kobe per year are about 1,600, 1,700 and 2,500 day-degrees, respectively. These values represent nearly sufficient amounts of heat to complete two or three generations. Therefore, those individuals showing slow pupal development in the Akita, Sendai and Kobe populations might have been selected against. However, selection pressure against slow pupal development may be expected to decrease in the southernmost areas of the trivoltine populations where the amount of heat available for development is quite sufficient for completion of three generations per year. Kumamoto is located in an area where the amount of available heat above 10°C is about 2,650 day-degrees. A portion of individuals in the Kumamoto population showed slow pupal development in the present study. This result may support the above inference.

Tsukuba is located in the transitional zone between the bi- and trivoltine areas where thermal environments fluctuate yearly around the amount of heat required to produce three generations (GOMI, 1996 a). In this locality, production of a third generation entails the risk of failure to reach the diapause stage before the onset of winter. When the environment is variable and the onset of unfavorable conditions is unpredictable, a variable life cycle may be favored to disseminate the risk (PHILIPPI and SEGER, 1989). It is possible that the slow pupal development

of the Tsukuba population is associated with disseminating the risk. If slow development occurs in pupae of the first generation in Tsukuba, diapause would be induced in pupae of the next generation because they would be exposed to short-days (GOMI, 1996 a). In a cool year when the amount of heat available for development is slightly insufficient for three generations, individuals directly developing to adulthood may fail to reach the overwintering stage in the third generation, whereas pupae undergoing slow development would produce a second generation which could overwinter successfully. On the other hand, in a warm year with sufficient heat for completion of three generations, both individuals with and without slow pupal development would produce second and third generation individuals, respectively, which could overwinter successfully, although the former would suffer a reduction in fitness compared to the latter due to the decrease in the number of generations per year. Thus, slow pupal development may serve as a risk disseminating strategy and have been maintained in this population.

Various life-history traits including the photoperiodic response for diapause induction (GOMI, 1995; GOMI and TAKEDA, 1996) and other developmental characteristics varied with the shift in voltinism in *H. cunea* after its introduction to Japan (GOMI, 1996 a, b, c; GOMI and TAKEDA, 1990, 1991). In this context, the pupal response to high temperature in non-diapause generations might have been modified according to the local climatic conditions.

ACKNOWLEDGEMENTS

The author thanks Dr. S. TANAKA, National Institute of Sericultural and Entomological Science (NISES), TSUKUBA, for his critical reading of the manuscript and many useful suggestions, and Drs. K. KURATA, T. OKUDA, M. NAKAMURA and Ms. K. YASHIRO (NISES) for their kind support and encouragement. This research was supported in part by a grant from JSPS Fellowships.

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Comparison of Acetylcholinesterases from Adult Heads of the Flesh Fly, *Sarcophaga peregrina* (Diptera: Sarcophagidae) and from Its Cell Line, in Response to Several Inhibitors¹

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(Received 16 January 1997)

(Accepted 7 April 1997)

Key words: acetylcholinesterase, flesh fly, insect cell line, inhibitor

Acetylcholinesterase (AChE) from a flesh fly cell line was compared with that from adult heads in terms of sensitivity to several inhibitors. Among five inhibitors tested, both enzymes were inhibited most severely by eserine sulfate, and least by fenitrothion. The former AChE was more sensitive to the inhibitors than the latter.

We reported the presence of AChE activity in several insect cell lines (OHBAYASHI et al., 1997). Among the cell lines examined, the NIH-SaPe-4 cell line derived from embryonic tissues of the flesh fly, *Sarcophaga peregrina* (TAKAHASHI et al., 1980), showed high AChE activity, but could multiply in the presence of eserine, a potent inhibitor of AChE. This suggests that the cell line has AChE activity, but it is not essential for cell maintenance nor for multiplication. In the present study, with the purpose of exploitation of a new AChE inhibitor assay system, we examined whether the AChE from cultured cells showed similar responses to inhibitors including insecticides, as the AChE from intact insects.

MATERIALS AND METHODS

Enzyme sources and preparation of enzyme samples. NIH-SaPe-4 cells have been cultured in autoclaved MM medium (MITSUHASHI and MARAMOROSCH, 1964) free of serum (MM-SFA) at 25°C. For experiments, the cells were cultured at a density of 5×10^6 cells per ml for two days, and enzyme samples were prepared according to the method described in the previous paper (OHBAYASHI et al., 1997).

S. peregrina was a gift of Dr. H. KURAHASHI, National Institute of Health, Tokyo. Five adult heads were homogenized in a glass homogenizer with 1.0

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