A Mechanism for the Decrease in Developmental Period of a Trivoltine Population of *Hyphantria cunea* (Lepidoptera: Arctiidae)

Tadashi GOMI

Graduate School of Science and Technology, Kobe University, Nada, Kobe 657, Japan

(Received 3 August 1995; Accepted 19 January 1996)

The life cycle of the fall webworm, *Hyphantria cunea*, has differentiated from bivoltine to trivoltine since the early 1970's in the southwestern areas of Japan. Previous studies showed differentiation in developmental period as well: development was shorter in the trivoltine populations. In the present study, the precise mechanisms of this change were investigated by comparing developmental characteristics between bivoltine (Akita) and trivoltine (Urawa) populations. The theoretical lower threshold temperatures for larval and pupal development were not different between the two populations. The thermal constant for larval development was smaller in the trivoltine population than in the bivoltine population, but that for pupal development was almost identical for the two populations. Most individuals passed six larval instars, and some passed seven in both populations. The incidence of seven-instar larvae was significantly higher in the bivoltine population than in the trivoltine population. The larval period was significantly longer in the seven-instar type than in the six-instar type. Larvae of the six-instar type grew at similar rates in the two populations. Head width of last instar larvae was larger in the seven-instar type than in the six-instar type, but pupal weight was not statistically different between the two types. These results suggest that the reduction in developmental period in the trivoltine population can be attributed to a reduction in the thermal constant, especially that during the larval stage. A lower incidence of seven-instar larvae appears to be the major mechanism for reducing the thermal constant in the trivoltine population.

Key words: *Hyphantria cunea*, number of larval instars, thermal constant, threshold temperature for development, voltinism

INTRODUCTION

The fall webworm, *Hyphantria cunea*, was introduced from North America into Japan in 1945 (MASAKI, 1975). This species was first found in Tokyo (35.7°N) and has expanded its distribution from 32° to 40°N. The life cycle was bivoltine until the early 1970's throughout its distribution (MASAKI, 1975). Thereafter, the life cycle shifted to trivoltine in the southwestern areas of Japan, and the transitional zone between the voltinisms occurs around 36°N (GOMI, 1996; GOMI and TAKEDA, 1991, in press).

Two decades after colonization, ITÔ et al. (1968) showed that the theoretical lower threshold temperature for development of this species was 10–13°C for the egg, larval and pupal stages, and that the total thermal constant was 740 degree-days. The number of larval instars was predominantly seven in Japan (ITÔ and MIYASHITA, 1968), whereas it was six or a mixture of six and seven in North America and Europe (OLIVER, 1964; MORRIS and BENNETT, 1967; WARREN and TADIC, 1970).

Previous reports showed that the developmental period of trivoltine populations decreased in association with a shift in voltinism (GOMI and TAKEDA, 1990, 1991, in press).

This decrease might have been caused by changes in the lower threshold temperature for development and/or thermal constant. However, these developmental characteristics remained to be examined in the previous studies.

Changes in the number of larval instars may also influence the developmental time. MORRIS and FULTON (1970) pointed out that the developmental period does not differ significantly between larvae of the six- and seven-instar types. However, they compared a six-instar type of a Canadian population with a seven-instar type of a European population reported by JASIČ and MACKO (1961) (cited in MORRIS and FULTON (1970)). It is important to conduct such comparisons between different instar-types of the same geographic area.

In the present study, the following developmental characteristics between bivoltine and trivoltine populations of *H. cunea* were compared: 1) egg period; 2) lower threshold temperatures for development and thermal constants in the larval and pupal stages; 3) the number of larval instars; 4) duration of each instar; and 5) pupal weight. Further, the mechanisms by which developmental period was modified in association with the shift of voltinism were examined.

MATERIALS AND METHODS

Sources and rearing of insects. Mid-instar larvae of *Hyphantria cunea* DRURY were collected in Akita, Japan (39°43'N, 140°07'E) in September, 1990, and Urawa, Japan (35°40'N, 139°39'E) in June, 1991. The Akita population is bivoltine and the Urawa population is trivoltine (GOMI, 1992, 1995). Larvae were collected on *Platanus* sp. in Urawa and on *Prunus* sp. in Akita. All larvae were reared on an artificial diet, "Insecta LF" (Nihon Nosan Kogyo) in transparent plastic cups (500 ml) at 20°C. Adults of both populations were kept in cups at 20°C and 16L-8D (16 h light-8 h dark) to collect egg masses. Eggs were kept under the same conditions as the adult stage and hatchlings were reared under various experimental conditions.

Investigations of developmental characteristics. Developmental period of eggs was examined at 20 ± 1°C and 16L-8D. All eggs from the same egg mass hatched nearly synchronously and the egg period was determined as the number of days from oviposition to first hatching for each egg mass.

Thermal constants and lower threshold temperatures for larval and pupal development were estimated by rearing larvae at 18, 20, 23 and 25 ± 1°C, 16L-8D where all individuals avert diapause at the pupal stage. These values were calculated according to the method of DANKS (1987). The experimental conditions were provided by constant temperature rooms equipped with two 40 W fluorescent lamps at 20 and 25°C, and incubators (45 cm × 100 cm × 90 cm) equipped with a 20 W fluorescent lamp at 18 and 23°C.

As larval period is extended when larvae are destined to enter diapause in the pupal stage (WATANABE et al., 1970), the number of larval instars was investigated at 20°C and 16L-8D or 12L-12D where all individuals are destined to enter diapause (GOMI, 1992). MORRIS and FULTON (1970) found that there was no difference in head width between the six- and seven-instar types until the fourth larval instar. Therefore, larvae were reared in mass until the fourth ecdysis and individually, thereafter. In the mass rearing, larval instars were determined by measuring the width of head capsules of 80-120 individuals selected randomly in each sampling. Upon the fourth larval ecdysis, 72 and 60 larvae were randomly selected from the rearing mass of the Akita and Urawa populations, respectively, and transferred to small transparent plastic cups (80 ml) individually. The number and the

Table 1. Developmental period and rate in the Akita and Urawa populations of *H. cunea*

Population	Stage	Developmental period at 20°C, 16L-8D (days)	Regression equation* $Y = a + bX$	r	t_0 (°C)	K (degree-days)
Akita	Egg	15.25 ± 1.04 (64 ^{**}) ^a				
	Larva	42.04 ± 3.17 (69) ^a	$Y = -0.023 + 0.0023X$	0.983	10.09	436.68
	Pupa	20.57 ± 1.37 (69) ^a	$Y = -0.076 + 0.0063X$	0.957	12.10	160.00
Urawa	Egg	15.35 ± 1.26 (86 ^{**}) ^a				
	Larva	40.88 ± 2.04 (58) ^b	$Y = -0.025 + 0.0024X$	0.987	10.07	410.09
	Pupa	18.66 ± 1.41 (54) ^b	$Y = -0.078 + 0.0064X$	0.962	12.24	157.30

Developmental periods of each stage (mean ± SD) followed by the same letter were not significantly different (MANN-WHITNEY *U*-test; $p > 0.05$). Sample sizes are in parentheses.

* Developmental rate calculated from 4 temperature points. *Y*, developmental rate (1/days); *X*, temperature.

r , correlation coefficient; t_0 , lower threshold temperature for development ($-a/b$); K , thermal constant (1/°C).

** The number of egg batches examined.

interval of molts were recorded for each individual until pupation. Pupae were kept individually under the same conditions as in the larval stage and weighed 5 days after pupation.

RESULTS

There was no difference in egg period between the Akita and Urawa populations at 20°C and 16L-8D (MANN-WHITNEY *U*-test, $z = 0.698$, $p > 0.05$), while there was a significant difference in larval and pupal periods between the two populations ($z = 2.363$ for the larval stage and 6.149 for the pupal stage, $p < 0.05$, Table 1). The lower threshold temperatures for the larval and pupal development were similar in the two populations (Table 1). The thermal constant for larval development was larger in the Akita population than in the Urawa population, but that for pupal development was almost identical for the two.

The number of larval instars in both populations was a mixture of six and seven (Table 2). The incidence of seven larval instars was significantly higher in the Akita population (14.5%) than in the Urawa population (3.4%, $\chi^2 = 0.027$, $p < 0.05$). Similar results for the incidence of seven larval instars were observed at 12L-12D and 20°C where pupal diapause was induced in all individuals. In this case, the incidence of seven larval instars was 13.8% in the Akita population and 6.8% in the Urawa population, and was not significantly different from that at 16L-8D for either population ($\chi^2 = 0.973$ for the Akita population, $p > 0.05$; FISHER's exact probability test, $p = 0.74$ for the Urawa population).

As only a few seventh instar larvae occurred in the Urawa population (Table 2), the Akita population was used to compare the developmental characteristics between the six- and seven-instar types. The developmental period from hatching to the fourth ecdysis was significantly shorter in the seven-instar type than in the six-instar type (MANN-WHITNEY *U*-test, $z = 4.825$, $p < 0.05$, Table 2). However, the duration of larval development was significantly longer in the seven-instar type than in the six-instar type ($z = 5.054$, $p < 0.05$). The total larval period of the six- and seven-instar types was significantly longer in the Akita population than in the Urawa population (Table 1), but no significant difference in the larval period of the six-instar type was found between the two populations ($z = 1.136$,

Table 2. Developmental period and head width of each instar in the six- and seven-instar types of the Akita and the Urawa population of *H. cunea*

Population	Larval instar	Developmental period (days)		Head width (mm)	
		VI	VII	VI	VII
Akita	Hatch-4th	23.16 ± 0.77 (62) ^a	21.40 ± 0.52 (10) ^b		
	5th	6.42 ± 0.74 (62) ^a	6.30 ± 0.82 (10) ^a		
	6th	11.78 ± 1.20 (59) ^a	8.30 ± 1.57 (10) ^b	2.68 ± 0.11 (62) ^a	2.46 ± 0.24 (10)
	7th	—	11.90 ± 1.20 (10)	—	2.98 ± 0.11 (10) ^{b*}
	Total	41.08 ± 2.18 (59) ^{aA}	47.70 ± 2.00 (10) ^b		
Urawa	Hatch-4th	21.52 ± 0.75 (58)	21.50 ± 0.71 (2)		
	5th	6.67 ± 0.63 (58)	6.00 (2)		
	6th	12.55 ± 1.67 (56)	7.50 ± 0.71 (2)	2.48 ± 0.13 (58)	1.99 ± 0.09 (2)
	7th	—	10.50 ± 0.71 (2)	—	2.66 ± 0.05 (2)
	Total	40.71 ± 1.88 (56) ^{A**}	45.50 ± 0.71 (2)		

Values (mean ± SD) followed by the same letters are not significantly different between columns (MANN-WHITNEY *U*-test; $p > 0.05$).

Roman numerals show the ultimate number of larval instars (instar type). Sample sizes are in parentheses.

* Compared with the head width of the six instar larva in the six-instar type.

** Compared with the total developmental period of the six-instar type in the Akita population.

Table 3. Pupal weight in the Akita and Urawa populations of *H. cunea*

Population	Photoperiod	Instar type*	Male (mg)	Female (mg)
Akita	16L-8D	VI	120.94 ± 11.32 (29)	181.92 ± 21.77 (30) ^a
		VII	119.20 ± 1.97 (3)	185.86 ± 22.28 (7) ^a
	12L-12D	VI	158.12 ± 21.19 (36)	228.09 ± 15.98 (18)
		VII	165.12 ± 20.70 (5)	231.55 ± 13.31 (4)
Urawa	16L-8D	VI	123.68 ± 14.59 (31)	177.01 ± 18.07 (25)
		VII	—	177.40 ± 15.70 (2)
	12L-12D	VI	154.49 ± 12.04 (26)	199.51 ± 16.38 (15)
		VII	—	250.87 ± 17.07 (3)

Pupal weight (mean ± SD) followed by the same letter is not significantly different (MANN-WHITNEY *U*-test; $p > 0.05$). Sample sizes are in parentheses.

* Roman numerals show the ultimate number of larval instars.

$p > 0.05$, Table 2). The mean head width of the last instar larvae was significantly greater in the seven-instar type than in the six-instar type in the Akita population ($z = 4.747$, $p < 0.05$).

The pupal weight of the six-instar type did not differ significantly between the two populations (MANN-WHITNEY *U*-test, $z = 0.018$ for females at 16L-8D, $z = 0.964$ for males at 16L-8D, $z = 0.038$ for males at 12L-12D, $p > 0.05$), except for females at 12L-12D ($z = 27$, $p < 0.05$, Table 3). The weight of female pupae was not significantly different between the two instar types at 16L-8D in the Akita population ($z = 0.562$, $p > 0.05$). The pupal period at 16L-8D was 20.46 ± 1.26 (= mean ± SD) days in the six-instar type and 20.90 ± 1.45 days in the seven-instar type in the Akita population, and the difference was not significant ($z = 0.924$, $p > 0.05$).

DISCUSSION

The lower threshold temperatures for larval and pupal development were not different between the bivoltine and trivoltine populations (Table 1). However, the thermal constant for larval development was smaller in the trivoltine population than in the bivoltine population, while the two populations had similar thermal constants for pupal development. These results indicate that the decrease in developmental period in the trivoltine population is not due to a change in the lower threshold temperature but to a reduction in the thermal constant for larval development.

The larval period of the six-instar type was significantly shorter than that of the seven-instar type (Table 2). The incidence of seven-instar type was significantly lower in the Urawa population than in the Akita population. Therefore, an increase in the six-instar type appears to contribute to the reduction in developmental period in the trivoltine population (Table 1), and such a change may have contributed to the reduction of developmental period in other trivoltine populations (GOMI and TAKEDA, in press).

In some species, little inter-population difference has been observed in developmental period across a transition from univoltine to bivoltine areas (RITLAND and SCRIBER, 1985 for *Papilio glaucus*; PULLIN, 1986 for *Inachis io*; TAUBER et al., 1987 for *Chrysopa oculata*; BRADFORD and ROFF, 1995 for *Allonemobius socius*). BRADFORD and ROFF (1995) suggested that selection pressure may be weaker in developmental period than in diapause. However, in *Chilo suppressalis*, showing clinal variation of the critical photoperiod for diapause induction, a relatively large difference in larval period was found between univoltine and bivoltine populations (KISHINO, 1970). In *H. cunea*, differentiation in the photoperiodic response for diapause induction and its temperature dependency has been reported between bivoltine and trivoltine populations (GOMI, 1995; GOMI and TAKEDA, in press). Although the larval periods of the bivoltine and trivoltine populations were significantly different in the present study, the difference was rather small compared to *C. suppressalis* (Table 1). In *H. cunea*, the duration following the differentiation of voltinism from bivoltine to trivoltine may not be sufficient to cause a more apparent difference in developmental period between the bivoltine and trivoltine populations.

The number of larval instars in the Tokyo population was predominantly seven (ITÔ and MIYASHITA, 1968; ITÔ et al., 1968). In the present study, however, the number of larval instars was mainly six in both the Akita and Urawa populations (Table 2). Several authors cited in WARREN and TADIC (1970) indicated that the number of larval instars is affected by various environmental factors such as temperature, humidity and host plants. Food conditions might partially explain the difference in results between ITÔ et al. (1968) and the present study. The former study used leaves of *Platanus acerifolia* or *Cornus controversa*, whereas an artificial diet was used in the latter. The developmental period was significantly longer in individuals reared on leaves of *Pterocarya* sp. than in those on the artificial diet in a trivoltine population of this species (GOMI, unpublished data). The present study showed that the number of larval instars was not affected by photoperiod, i.e., it was independent of diapause induction.

In the course of the voltinism shift, individuals having a smaller thermal constant would be favored by natural selection in the trivoltine population because a shorter developmental period would reduce the risk of failure to complete three generations before winter. In general, a shorter developmental period would reduce fecundity because develop-

mental rate and body size are trade-offs, and fecundity is dependent on body size (ROFF, 1992). A positive correlation between fecundity and pupal weight was found in *H. cunea* (MORRIS and FULTON, 1970). In the present study, however, pupal weight was not statistically different between females of the two instar types of the Akita population at 16L-8D (Table 3), although there is a possibility that a significant difference was not detected between the two instar types because of the small sample size of the seven-instar type. This indicates that the decrease in developmental period does not lead to a reduction in fecundity of individuals of the six-instar type. On the other hand, head width at the last larval instar was significantly greater in the seven-instar type than in the six-instar type. Whether the six-instar type larvae decrease their developmental time at the expense of fecundity should be examined by direct observation on the relationship between these parameters.

ACKNOWLEDGEMENTS

I would like to thank Dr. M. TAKEDA, Kobe University, and Dr. S. TANAKA, National Institute of Sericultural and Entomological Science, for their critical reading of the manuscript and many useful suggestions. This research was supported in part by a grant from JSPS Fellowships.

REFERENCES

- BRADFORD, M. J. and A.D. ROFF (1995) Genetic and phenotypic sources of life history variation along a cline in voltinism in the cricket *Allonemobius socius*. *Oecologia* **103**: 319-326.
- DANKS, H.V. (1987) *Insect Dormancy: An Ecological Perspective*. Biological Survey of Canada, Ottawa, 439 pp.
- GOMI, T. (1992) The formation of geographic variation in the life cycle characteristics and the mechanism that enforced the voltinism change in the fall webworm, *Hyphantria cunea* DRURY (Lepidoptera: Arctiidae). Ph.D. dissertation, Kobe Univ. (in Japanese with English summary).
- GOMI, T. (1995) Effect of temperature on diapause response to photoperiod in *Hyphantria cunea* with special reference to local divergence. *Appl. Entomol. Zool.* **30**: 490-492.
- GOMI, T. (1996) Mixed life cycles in the transitional zone between voltinisms in the fall webworm, *Hyphantria cunea*. *Experientia* **52**: 273-276.
- GOMI, T. and M. TAKEDA (1990) The transition to a trivoltine life cycle and mechanisms that enforce the voltinism change in *Hyphantria cunea* DRURY (Lepidoptera: Arctiidae) in Kobe. *Appl. Entomol. Zool.* **25**: 483-489.
- GOMI, T. and M. TAKEDA (1991) Geographic variation in photoperiodic responses in an introduced insect, *Hyphantria cunea* DRURY (Lepidoptera: Arctiidae) in Japan. *Appl. Entomol. Zool.* **26**: 357-363.
- GOMI, T. and M. TAKEDA (1996) Changes in life-history traits in the fall webworm within half a century after introduction to Japan. *Funct. Ecol.* (in press).
- ITÔ, Y. and K. MIYASHITA (1968) Biology of *Hyphantria cunea* DRURY (Lepidoptera: Arctiidae) in Japan. V. Preliminary life tables and mortality data in urban areas. *Res. Popul. Ecol.* **10**: 177-209.
- ITÔ, Y., K. MIYASHITA and H. YAMADA (1968) Biology of *Hyphantria cunea* DRURY (Lepidoptera: Arctiidae) in Japan. VI. Effect of temperature on development of immature stages. *Appl. Entomol. Zool.* **3**: 163-175.
- KISHINO, K. (1970) Ecological studies on the local characteristics of seasonal development in the rice stem borer, *Chilo suppressalis* WALKER. II. Local characteristics of diapause and development. *Jpn. J. Appl. Entomol. Zool.* **14**: 1-11.
- MASAKI, S. (1975) Biology of *Hyphantria cunea* DRURY (Lepidoptera: Arctiidae) in Japan: A review. *Rev. Plant Protect. Res.* No. 8: 14-28.
- MORRIS, R.F. and C.W. BENNETT (1967) Seasonal population trends and extensive census methods for *Hyphantria cunea*. *Can. Ent.* **99**: 9-17.
- MORRIS, R.F. and W.C. FULTON (1970) Models for the development and survival of *Hyphantria cunea* in relation to temperature and humidity. *Mem. Entomol. Soc. Can.* No. 70: 1-60.
- OLIVER, A.D. (1964) Studies on the biological control of the fall webworm, *Hyphantria cunea* in Louisiana. *J. Econ. Ent.* **57**: 314-318.

- PULLIN, A.S. (1986) Effect of photoperiod on the life-cycle of the peacock butterfly *Inachis io*. *Entomol. Exp. Appl.* **41**: 237–242.
- RITLAND, D.B. and J.M. SCRIBER (1985) Larval development rates of three putative subspecies of tiger swallowtail butterflies, *Papilio glaucus*, and their hybrids in relation to temperature. *Oecologia* **65**: 185–193.
- ROFF, A.D. (1992) *The Evolution of Life Histories*. Chapman and Hall, New York, 535 pp.
- TAUBER, C.A., M.J. TAUBER and J.R. NECHOLS (1987) Thermal requirements for development in *Chrysopa oculata*: A geographically stable trait. *Ecology* **68**: 1479–1487.
- WARREN, L.O. and M. TADIC (1970) The fall webworm, *Hyphantria cunea* (DRURY). *Arkansas Agric. Exp. Stn. Bull.* No. 759: 1–106.
- WATANABE, N., K. UMEYA and S. MASAKI (1970) Biology of *Hyphantria cunea* DRURY (Lepidoptera: Arctiidae) in Japan. *Res. Bull. Pl. Prot. Jpn.* No. 8: 30–38.