

Geographic and annual variation of summer-diapause expression in the ladybird beetle, *Coccinella septempunctata* (Coleoptera: Coccinellidae), in Japan

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Abstract

Variation in the summer-diapause expression of the ladybird beetle, *Coccinella septempunctata*, among local populations and years was investigated by field observation and dissection of sampled specimens, in Japan. Only non-diapausing adults were found in Hokkaido, northern Japan, whereas diapausing adults were observed at most sites in Honshu and southward in summer. However, the non-diapausing adults were also observed in northern Honshu and at high-altitudinal sites of central Honshu. The frequency of diapause expression in northern Honshu varied greatly among populations and years. The frequency of non-diapausing adults was significantly higher when average daily mean air temperature for July was relatively low. This indicated that the populations inhabiting northern Honshu have plasticity in diapause induction, primarily in response to temperature conditions. Moreover, the coexistence of diapausing adults and non-diapausing adults at the same site was often observed. This suggested that there might be genetic variation in diapause tendency within a local population.

Key words: *Coccinella septempunctata*; summer diapause; life cycle; geographic variation; genetic variation

INTRODUCTION

In temperate climatic zones, insects are exposed to alternating periods of conditions that are favorable and unfavorable for growth and reproduction of insects. Insects therefore must regulate their life cycle to synchronize their development with the favorable periods. Many temperate insects pass severe winter by entering diapause, while there are insects undergoing summer diapause (Masaki, 1980). Both winter and summer diapause function not only to allow escape from unfavorable seasons but also to synchronize the insect's life cycle with the seasonal cycle of the climate associated with the area they inhabit. Thus, geographical polymorphism in the life cycle may be essential within intraspecific populations due to local adaptations. Therefore, a comparison of the life-cycle polymorphism among local populations may be a useful way to infer the factor of selection forces that determine the life-cycle strategy of insects (Danks, 1994).

As the ladybird beetle *Coccinella septempunc-*

tata L. is a very important predator on pest aphids, many studies on its life cycle have been conducted (see review Hodek and Honěk, 1996; Hodek and Okuda, 1997). In central Honshu, Japan, the beetle has two generations per a year, and the adult undergoes diapause in summer and quiescence in winter (Sakurai et al., 1981, 1982, 1983, 1986b, 1987a). Thus the populations from central Honshu showed a short-day photoperiodic response (Okuda and Hodek, 1983), while the reverse response was found in a population from Hokkaido, northern Japan (Okuda and Hodek, 1994). However, the geographic polymorphism of the life cycle has not been fully studied, especially in the intermediate area between the long-day and short-day type populations. Furthermore, no study has focused on the evolutionary factor of summer diapause in this species.

In the present study, we investigated the variation in summer-diapause expression of *C. septempunctata* (1) throughout Japan in one summer season, (2) at different altitudes in central Honshu, (3) in the intermediate area, northern Honshu, among

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populations and years. From these results, we discussed both proximal and evolutionary factors of summer diapause in this beetle.

MATERIALS AND METHODS

Geographic variation in diapause expression.

The field observation and sampling were conducted throughout Japan from 19 July to 11 September 1994 (Appendix 1). Since the *C. septempunctata* has a grassland-inhabiting nature, a search for both the active (non-aestivating) and inactive (aestivating) adults was made on grasslands such as dry riverbed. Active adults were collected from herbaceous plants, and inactive adults were mainly collected on bases of *Miscanthus sinensis* or within dense vegetation of *M. sacchariflorus* or *Solidago altissima*. Except for Oishi-Kougen (No. 17; alt. 800 m), all sites were located on a plain. Abundance of prey aphids that occurred at each site was classified into six grades: no aphids were found (grade 0), a few aphids were found on several plants (grade 1), a few colonies were found on several plants (grade 2), small colonies were found on more than half of plants (grade 3), large colonies were found on more than half of plants (grade 4), and aphid outbreak was found (grade 5).

Both the active and inactive adults collected were preserved in 70% ethanol until dissection. To determine the diapause status, we dissected female specimens under a binocular scope and observed them for gonad development. The degree of ovarian development was classified into five stages according to Sakurai et al. (1981), i.e., non-developing stage (stage I), follicle differentiating stage (stage II), early vitellogenic stage (stage III), mid-vitellogenic stage (stage IV), and late vitellogenic and mature egg stage (stage V). Female *C. septempunctata* has a pair of ovaries consisting of about 100 ovarioles. As their development did not synchronize, we recorded the most advanced ovariole.

Altitudinal variation in diapause expression in central Honshu. In order to examine the influence of altitude on the imaginal phase (active or inactive) of the beetle, we conducted a field study at three sites located on a mountain (Nos. 1–3) and four sites located on a plain (Nos. 4–7) from 11 to 23 July 1999 (Appendix 2). At these sites, the field census and dissection of sampled specimens were conducted in the same manner as described above.

Annual change in diapause expression in northern Honshu. In northern Honshu the field census and dissection of sampled specimens mentioned above were carried out in the summers of 1984, 1985, 1989, 1993 and 1994 (Appendix 3). The influence of air temperature on the imaginal phase (active or inactive) of the beetle was studied. The daily mean air temperature averaged for July (data from Japan Meteorological Agency, 1986, 1987, 1990, 1994 and 1995) recorded at the nearest station from each study site was used. In addition, the influence of aphid abundance on the imaginal phase was analyzed.

RESULTS

Populations with and without summer-diapause capacity

Figure 1 shows a geographical variation in the imaginal phase of the *C. septempunctata* observed in the summer of 1994. In spite of intensive searching, no inactive adults were found at any sites in Hokkaido (Nos. 1–4) (Fig. 1, left). At these four sites many eggs, larvae and pupae as well as active adults were observed. Thus, it is clear that the beetles in Hokkaido were in the reproductive phase in the summer. On the other hand, inactive adults were observed at all sites in Honshu and southward (Fig. 1, left). These adults were considered to be in summer diapause, because the development of the ovaries never exceeded stage 3 (Fig. 1, right). However, in No. 7 at Morioka located at high latitudes and in No. 17 at Oishi-Kougen located at high altitudes, active as well as inactive adults were observed (Fig. 1, left). At these two sites, though immature individuals were not found, at least some adults were not in diapause, judging from their developed ovaries (Fig. 1, right).

At the four sites in Hokkaido aphid occurrence was grade 2 or 3 (Appendix 1) on herbaceous plants *Artemisia princeps*, *Polygonum cuspidatum*, *Erigeron annuus* and *Rumex japonicus*. At the two sites of Honshu, where the active adults were observed, aphid occurrence was grade 4 on *A. princeps* at Morioka (No. 7), and grade 3 on *A. princeps* and *P. cuspidatum* at Oishi-Kougen (No. 17) (Appendix 1). The abundance of aphids may allow the reproduction of the beetles. On the other hand, even at the two sites where no active adults were found, aphid occurrence was grade 2 at Hachinohe

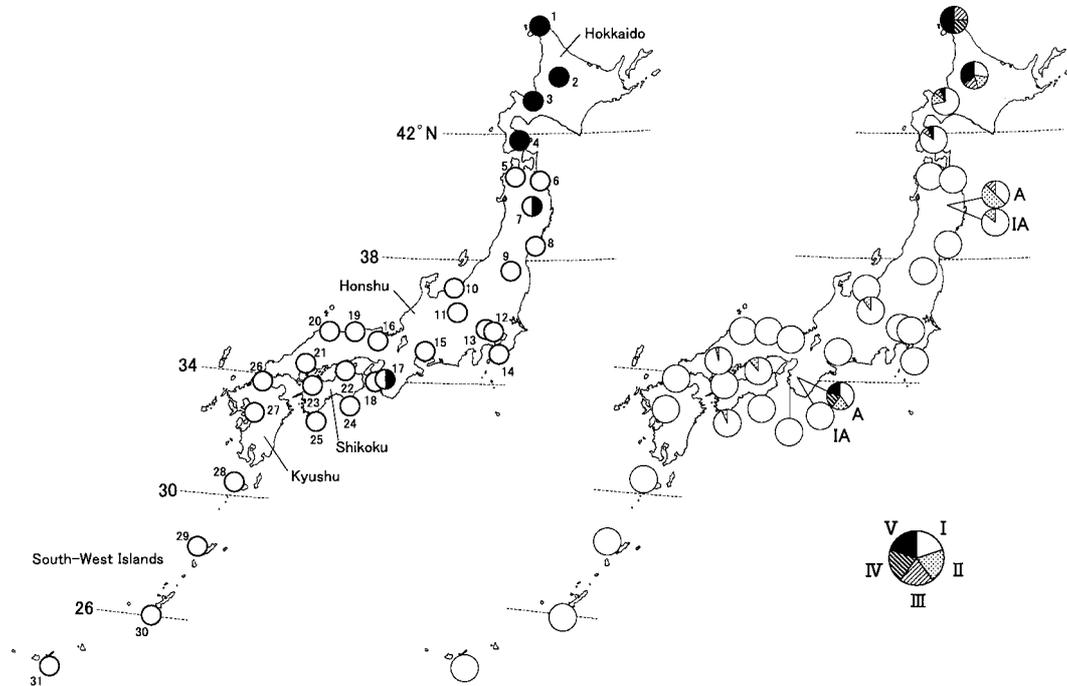


Fig. 1. Geographical variation in the imaginal phase among the *C. septempunctata* populations (left) and percent of their developmental stages of ovary (right), in summer of 1994, in Japan. ●: active adults only; ○: inactive adults only; ◐: both inactive and active adults were observed. Arabic numerals represent population codes listed in Appendix 1. Roman numerals represent stages of ovarian development: I: non-developing stage; II: follicle differentiating stage; III: early vitellogenic stage; IV: mid-vitellogenic stage; V: late vitellogenic and mature egg stage. A: active adults. IA: inactive adults.

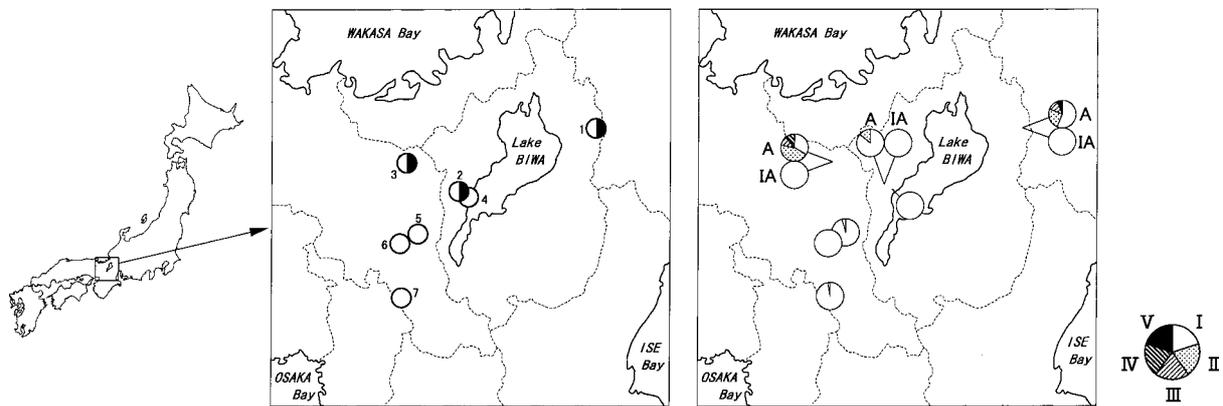


Fig. 2. Geographical variation in the imaginal phase among the *C. septempunctata* populations sampled from high (Nos. 1, 2 and 3) and low (Nos. 4, 5, 6 and 7) altitudes (left) and percent of their developmental stages of ovary (right), in summer of 1999, in Central Honshu, Japan. ○: inactive adults only; ◐: both active and inactive adults were observed. Arabic numerals represent population codes listed in Appendix 2. Roman numerals represent stages of ovarian development: I: non-developing stage; II: follicle differentiating stage; III: early vitellogenic stage; IV: mid-vitellogenic stage; V: late vitellogenic and mature egg stage. A: active adults. IA: inactive adults.

(No. 6) and Ishinomaki (No. 8) (Appendix 1).

Influence of altitude on diapause expression in central Honshu

In central Honshu no active adults were ob-

served at the four sites on the plain (Nos. 4–7), but active adults with more developed ovaries co-existed with inactive adults with undeveloped ovaries at the three sites in mountainous areas (Nos. 1–3) (Fig. 2, left and right). The frequency at

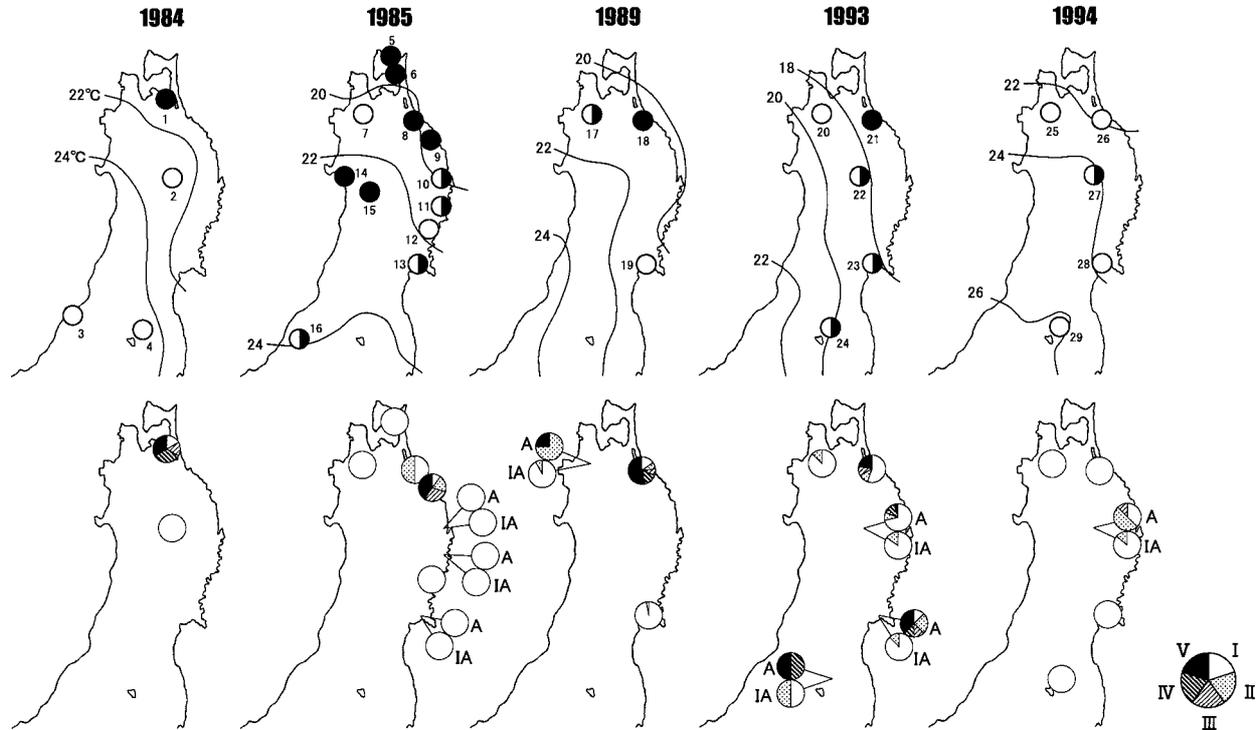


Fig. 3. Variation in the imaginal phase among *C. septempunctata* populations and years (upper), and percent of the developmental stage of the ovary (lower), in northern Honshu, Japan. ●: active adults only; ○: inactive adults only; ◐: both active and inactive adults were observed. Arabic numerals represent population codes listed in Appendix 3. Roman numerals represent stages of ovarian development: I: non-developing stage; II: follicle differentiating stage; III: early vitellogenic stage; IV: mid-vitellogenic stage; V: late vitellogenic and mature egg stage. A: active adults. IA: inactive adults. The isothermal lines represent the daily mean air temperature averaged for July.

which active adults were observed was significantly different between high (Nos. 1–3) and low (Nos. 4–5) altitudes (Fisher's exact test, d.f.=1, $p=0.029$). Therefore, altitude may influence the summer-diapause induction of the beetles.

Prey aphids were not observed at the four sites on the plain, whereas at the three sites in mountainous areas, aphid occurrence was grade 3 on *A. princeps* at Mt. Ibuki (No. 1), grade 2 on *Weigela hortensis* at Mt. Hourai (No. 2), and grade 2 on *A. princeps* and *Erigeron annuus* at Ashiu (No. 3) (Appendix 2).

Influence of air temperature and aphid abundance on diapause expression in northern Honshu

Active adults were observed at many sites of northern Honshu (Fig. 3, upper). Since eggs, larvae and pupae were also observed, and some active adults had developed ovaries (Fig. 3, lower), the beetles were obviously in the reproductive phase at these sites. On the other hand, the inactive adults

were considered to be in diapause, because the beetles never had ovaries that developed beyond stage 3 (Fig. 3, lower).

Using the data from the Japan Meteorological Agency (1986, 1987, 1990, 1994, 1995), isothermal lines of daily mean air temperature averaged for July were drawn in Fig. 3 (upper). These lines show that the summers of 1984 and 1994 were hot, and that of 1993 was cool. The frequency of adults in the imaginal phase greatly fluctuated among years even at the same site (Fig. 3, upper). For example, at Hachinohe (Nos. 8, 18, 21 and 26) only active adults were observed in 1985, 1989 and 1993, while only inactive adults were observed in 1994. A comparison between the cool summer of 1993 and the extreme hot summer of 1994 revealed that active adults occurred at four of the five sites in 1993, but only at one of the five sites in 1994.

Figure 4 shows the relationship between the frequency of diapause expression and daily mean air temperature averaged for July in northern Honshu. The frequency at which the active adults were ob-

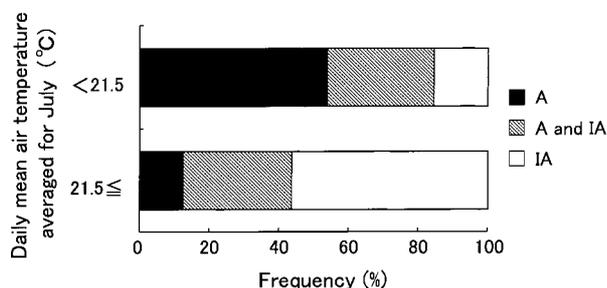


Fig. 4. Relationship between the daily mean air temperature averaged for July recorded near the observation site and the diapause expression of the *C. septempunctata* in northern Honshu. A: active adults only; IA: inactive adults only; A and IA: both active and inactive adults were observed. Statistical significance ($p=0.029$) was found by Fisher's exact test.

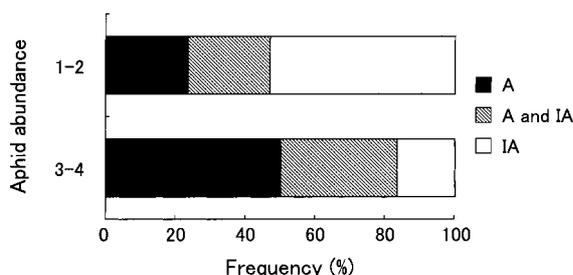


Fig. 5. Relationship between aphid abundance at each study site and the diapause expression of the *C. septempunctata* in northern Honshu. A: active adults only; IA: inactive adults only; A and IA: both active and inactive adults were observed. Statistical significance was not found by Fisher's exact test.

served was significantly higher at $<21.5^{\circ}\text{C}$, which is the average temperature for whole cases, than those of $>21.5^{\circ}\text{C}$ (Fisher's exact test, d.f.=2, $p=0.029$). A relationship between the frequency of diapause expression and aphid abundance is also shown in Fig. 5. The frequency of the active adults tended to be higher with higher aphid abundance, but this was not significant (Fisher's exact test, d.f.=2, $p=0.146$). These results suggest that the diapause induction of the beetles inhabiting northern Honshu was closely related to the ambient temperature condition at their habitat.

DISCUSSION

The results of this study showed that *C. septempunctata* did not enter summer diapause in Hokkaido with a cool summer, whereas they entered diapause in Honshu and southwards (Honshu, Shikoku, Kyushu and South-West Islands). Although we did

not investigate the effects of photoperiod on diapause induction, our results agreed with the photoperiodic response previously reported: populations from central Honshu showed a short-day photoperiodic response (Okuda and Hodek, 1983; Sakurai et al., 1986a, 1987a) and that from Hokkaido showed a reverse response (Okuda and Hodek, 1994).

Adult coccinellids in diapause status show marked anatomical changes (Hodek and Honěk, 1996); for example, very fine mid-gut (McMullen, 1967) and greatly enlarged fat bodies (Sakurai et al., 1982) and lipids (Sakurai, 1969; Sakurai et al., 1987b). The inactive adults observed in this study, not only had immature ovaries but also showed such anatomical indications (unpubl. data). Therefore, almost 100% of the adults in the inactive phase were considered to be in diapause. On the contrary, most active adults lacked such anatomical features related to the diapause status (unpubl. data). Thus, the physiological status (in or not in diapause) seemed to coincide with the behavioral phase (inactive or active phase) of the beetle.

There is no information on life cycle and voltinism of the Hokkaido *C. septempunctata* populations, except for Okuda and Hodek (1994), who reported that the Sapporo population has the long-day photoperiodic response associated with winter diapause, and that the dissected adults of the population collected in mid-October show diapausing status. The present results showed a latitudinal cline in percentage of the individuals that have developed ovaries at four sites in Hokkaido (Fig. 1; Nos. 1–4). This suggested that most of the specimens from Wakkanai (No. 1) and Asahikawa (No. 2) were overwintering-generation adults, and those from Sapporo (No. 3) and Hakodate (No. 4) were the first generation adults. It is unknown whether the first generation adults enter reproduction or diapause thereafter. If the adults enter diapause, it is considered to be a winter diapause because the summer in Hokkaido appears to have favorable conditions for reproduction and development of the beetles.

In northern Honshu, which is located in the intermediate area between the short-day and long-day type populations, the frequency of summer-diapause expression varied among years. This result provides evidence that factors other than photoperiod also greatly affect diapause induction. There

may be two possible factors influencing the diapause induction on northern Honshu populations: the ambient temperature and/or prey-aphid availability. The present study showed that the frequency of diapause expression was not significantly affected by aphid abundance, but by daily mean air temperature for July. This result suggests that the populations inhabiting northern Honshu have plasticity on diapause induction in response to ambient temperature. However, diapausing and non-diapausing adults were observed simultaneously in 31% of all the cases of northern Honshu, suggesting that food availability may partly affect the diapause induction. Thus, the populations in the intermediate area were considered to lack the photoperiodic response controlling diapause induction, or to have a plastic photoperiodic response affected by the ambient thermal and/or food conditions.

Another interpretation of the coexistence of diapausing and non-diapausing adults at the same site in northern Honshu is that there is genetic variation in diapause tendency within the local populations. It has been reported that populations of a phytophagous spider mite, *Tetranychus urticae*, derived from an intermediate area between cold and warmer regions, have a large genetic variation in winter diapause tendency (Takafuji et al., 1991; Takafuji, 1994). The mechanisms maintaining the genetic variation in the winter-diapause trait within and among *T. urticae* populations, were analyzed by Tsuda et al. (1997), who developed a mathematical model assuming three populations: the diapause population in which all individuals enter diapause in autumn, the non-diapause population that never exhibits diapause, and the plastic population that has plasticity in diapause expression. According to this model the maintenance of diapause variability was enhanced with an increasing frequency of mild winter, with increasing amounts of winter host plants, and with increase in the degree of similarity between the diapause expression (in or not in diapause) and environmental heterogeneity (absence or presence of winter host). This result was in good agreement with the case of summer diapause in the northern Honshu populations of *C. septempunctata* in the present study, because the frequency of mild summer was high, prey aphids showed high abundance, and the diapause expression (in or not in diapause) matched the environmental heterogeneity (absence or presence of prey aphids). Therefore,

genetic variation in the diapause tendency may be partly responsible for the intra-population variation in the phenotypic expression in northern Honshu.

The present study revealed that a portion of the mountain populations of central Honshu did not express diapause. This case may be a similar adaptation to cool environmental conditions as in the northern Honshu populations. The coexistence of adults with and without diapause at the one site in 1994 and the three sites in 1999 suggested that the populations might be composed of a variety of genotypes with different diapause tendencies. Although only diapausing adults were found on the plain, a few adults with slightly developed ovaries (stage 2) were observed (Fig. 1: Nos. 11, 21, 22 and 25; Fig. 2: Nos. 5 and 7). If these individuals terminate diapause faster than the other individuals, there may be genetic variability of diapause intensity in low altitudinal populations.

The altitudinal and latitudinal clines in diapause expression of Japanese *C. septempunctata* populations suggest that the summer-temperature condition is a main selective force for the summer-diapause traits. Although many temperate insects acquire a cold tolerance with winter diapause, the *C. septempunctata* do not need to acquire a high-temperature tolerance by entering summer diapause, because the high temperature condition, even 35°C, had no adverse influence on the growth and survival of the beetle at any stage (Kawauchi, 1983). Another explanation for the beetle entering summer diapause could be to escape a period with low food availability. In central Honshu and southward, few prey aphids occurred in the beetle-inhabiting grasslands in July and August (Appendix 1). This period agrees with that of summer diapause in this beetle. Diapausing adults have enlarged fat bodies and lipids stored in the body, which are used to endure the two-month starvation period (Sakurai et al., 1987b). If the beetles invested energy to develop their ovaries instead of fat bodies and lipids before summer, they might not survive the starvation period. Moreover, if the females laid their eggs in June when aphids still remain, the progeny would die out due to food shortage. Therefore, *C. septempunctata* females gain a great advantage by having the ability to arrest ovarian development before summer.

Even in Honshu the occurrence of aphids was confirmed at high altitudinal and latitudinal sites,

where non-diapausing adults were observed. This result also supports the hypothesis that the beetles enter diapause to escape the period without prey aphids. Nijjima and Kawashita (1982) suggested that food scarcity is the main factor of diapause induction in the beetle. However, the presence of aphids may not inhibit the diapause induction, because diapause-destined adults have a need to feed on substantial food for storing the nutrients such as fat bodies and lipids. Here, we propose a hypothesis with regard to a proximal factor on diapause induction in the northern Honshu populations. That is, particular temperature conditions that ensure the aphid occurrence, which are approximately lower than 21.5°C, will prevent diapause. Based on this hypothesis, the effects of temperature conditions and food supply on diapause induction need to be studied in the future.

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Appendix 1. Field observation and sampling sites throughout Japan, in 1994, and stages of ovarian development in active and inactive *C. septempunctata* females

Code ^a	Location		Latitude (°N)	Date	Grade of aphid occurrence	Adults observed ^b	Stages of ovarian development				
	Prefecture	Site					I	II	III	IV	V
1	Hokkaido	Wakkanai	45.4	Aug. 6	2	A			1	1	2
2	Hokkaido	Asahikawa	43.8	Aug. 6	2	A	3	2	2		4
3	Hokkaido	Sapporo	45.1	Aug. 5	3	A	40	8	4		4
4	Hokkaido	Hakodate	41.8	Aug. 5	3	A	11		1		1
5	Aomori	Hirosaki	40.6	Aug. 7	1	IA	26				
6	Aomori	Hachinohe	40.5	Aug. 7	2	IA	4				
7	Iwate	Morioka	39.1	Aug. 8	4	A	3	4	1		
						IA	6	1			
8	Miyagi	Ishinomaki	38.4	Aug. 9	2	IA	12				
9	Fukushima	Fukushima	37.8	Aug. 9	1	IA	4				
10	Niigata	Itoigawa	37.0	Aug. 7	0	IA	13				
11	Nagano	Matsumoto	36.2	Aug. 7	0	IA	9	1			
12	Tokyo	Chofu	35.6	July 19	0	IA	9				
13	Tokyo	Fuchu	35.7	Aug. 4	0	IA	32				
14	Chiba	Tateyama	35.0	July 20	0	IA	12				
15	Aichi	Obu	35.0	July 18	0	IA	10				
16	Kyoto	Fukuchiyama	35.3	July 26	0	IA	10				
17 ^c	Wakayama	Oishi-Kougen	34.1	July 29	3	A	2	1		1	1
						IA	8				
18	Wakayama	Shimotsu	34.1	July 27	0	IA	10				
19	Tottori	Tottori	35.5	July 26	0	IA	44				
20	Tottori	Sakaiminato	35.5	July 27	0	IA	9				
21	Hiroshima	Hiroshima	34.4	July 28	1	IA	27	1			
22	Kagawa	Utazu	34.3	July 29	0	IA	8	1			
23	Ehime	Matsuyama	33.8	July 30	1	IA	3				
24	Kochi	Muroto	33.2	Aug. 1	0	IA	9				
25	Kochi	Tosashimizu	32.8	July 31	1	IA	14	1			
26	Fukuoka	Kokura	33.8	Aug. 8	1	IA	3				
27	Kumamoto	Kumamoto	32.8	Aug. 9	0	IA	11				
28	Kagoshima	Yakushima	30.4	Sept. 11	1	IA	2				
29	Kagoshima	Naze	28.4	Aug. 28	0	IA	6				
30	Okinawa	Naha	26.2	Sept. 10	0	IA	1				
31	Okinawa	Ishigaki	24.3	Sept. 4	0	IA	3				

^aNumbers indicate locations in Fig. 1.

^bA: Active adults, IA: Inactive adults.

^cOnly this site was located on a mountain (alt. 800 m).

Appendix 2. Field observation and sampling sites in central Honshu, Japan, in 1999, and stages of ovarian development in active and inactive *C. septempunctata* females

Code ^a	Location		Altitude (m)	Date	Grade of aphid occurrence	Adults observed ^b	Stages of ovarian development				
	Prefecture	Site					I	II	III	IV	V
1	Shiga	Mt. Ibuki	760	July 23	3	A IA	9 14	4	2		1
2	Shiga	Mt. Hourai	1,150	July 22	2	A IA	6 14	1			
3	Kyoto	Ashiu	350	July 20	2	A IA	3 10	4	1	1	
4	Shiga	Shiga-cho	110	July 22	0	IA	6				
5	Kyoto	Kamigamo	110	July 17	0	IA	22	1			
6	Kyoto	Hirosawa	50	July 16	0	IA	29				
7	Kyoto	Yawata	10	July 11	0	IA	35	1			

^aNumbers indicate locations in Fig. 2.

^bA: Active adults, IA: Inactive adults.

Appendix 3. Field observation and sampling sites in northern Honshu, Japan, and stages of ovarian development in active and inactive *C. septempunctata* females

Code ^a	Year	Location		Latitude (°N)	Date	Grade of aphid occurrence	Adults observed ^b	Stages of ovarian development ^c				
		Prefecture	Site					I	II	III	IV	V
1	1984	Aomori	Noheji	40.9	Aug. 2	3	A	1		1	2	2
2		Iwate	Morioka	39.1	Aug. 2	1	IA	5				
3		Niigata	Niigata	37.9	Aug. 6	1	IA	—	—	—	—	—
4		Fukushima	Fukushima	37.8	Aug. 2	1	IA	—	—	—	—	—
5	1985	Aomori	Ohata	41.4	July 31	3	A	—	—	—	—	—
6		Aomori	Mutsu	41.3	July 31	3	A	2				
7		Aomori	Hirosaki	40.6	July 30	2	IA	14				
8		Aomori	Hachinohe	40.5	July 31	2	A	1	1			
9		Iwate	Kuji	40.2	Aug. 1	3	A	1	2	3		4
10		Iwate	Miyako	39.6	Aug. 1	3	A	1				
							IA	4				
11		Iwate	Kamaishi	39.3	Aug. 2	2	A	4				
							IA	7	2			
12		Miyagi	Kesenuma	38.9	Aug. 2	3	IA	1				
13		Miyagi	Ishinomaki	38.4	Aug. 3	2	A	1				
							IA	1				
14		Akita	Akita	39.7	Aug. 2	1	A	—	—	—	—	—
15		Akita	Kakunodate	39.6	Aug. 2	2	A	—	—	—	—	—
16		Niigata	Nagaoka	37.4	Aug. 5	2	A	—	—	—	—	—
							IA	—	—	—	—	—
17	1989	Aomori	Hirosaki	40.6	Aug. 20	3	A		3			1
							IA	23	2			
18		Aomori	Hachinohe	40.5	Aug. 20	4	A	2	1	1	2	7
19		Miyagi	Ishinomaki	38.4	Aug. 19	2	IA	36	1			
20	1993	Aomori	Hirosaki	40.6	Aug. 13	3	IA	54	8			
21		Aomori	Hachinohe	40.5	Aug. 13	2	A	5	1	1		2
22		Iwate	Morioka	39.1	Aug. 12	3	A	15	2	1	2	1
							IA	11	2			
23		Miyagi	Ishinomaki	38.4	Aug. 15	3	A	1	2	1	1	3
							IA	21	3			
24		Fukushima	Fukushima	37.8	Aug. 12	2	A				1	1
							IA	1	1			
25	1994	Aomori	Hirosaki	40.6	Aug. 7	1	IA	26				
26		Aomori	Hachinohe	40.5	Aug. 7	2	IA	4				
27		Iwate	Morioka	39.1	Aug. 8	4	A	3	4	1		
							IA	6	1			
28		Miyagi	Ishinomaki	38.4	Aug. 9	2	IA	12				
29		Fukushima	Fukushima	37.8	Aug. 9	1	IA	4				

^aNumbers indicate locations in Fig. 3.

^bA: Active adults, IA: Inactive adults.

^c—: Data were lacking.