Physiological Changes Related to Diapause of the Lady Beetle, *Harmonia axyridis* (Coleoptera: Coccinellidae)[1]

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Physiological characteristics of diapause were studied in the lady beetle, *Harmonia axyridis* Pallas. In the aestivating adults, the respiration rate decreased remarkably, but the body weight increased and mating behaviour and oviposition actively continued. This indicates that aestivation occurs at a reduced energetic state and not at a diapause state. In hibernating adults, reproduction was completely suppressed and the corpus allatum atrophied. Topical application of juvenile hormone analogue immediately induced ovarian maturation. Electrophoretic analysis of the heaemolymph of hibernating adults showed protein bands specific to diapause. These observations indicate that the hibernation of *H. axyridis* is a true diapause controlled by corpus allatum, and that their dormancy differs to that of *Coccinella septempunctata bruckii* Mulsant.

Key words: *Harmonia axyridis*, diapause, aestivation, hibernation

INTRODUCTION

Clarification of the life cycle of entomophagous lady beetles is relevant to the understanding of biological control of aphids and coccids. In Japan, *Harmonia axyridis* Pallas and *Coccinella septempunctata bruckii* Mulsant are very common aphidophagous lady beetles. Although both beetles are bivoltine, their habitats are different. The former lives in both meadows and woods, while the latter lives chiefly in meadows. It has been demonstrated in *C. septempunctata* that aestivation is true diapause controlled by corpus allatum, while the hibernation is not (Sakurai et al., 1981, 1986, 1987ab). In *H. axyridis*, physiological diapause characteristics have not yet been studied in detail (Hodek, 1973; Sakurai and Chujo, 1979). The present paper deals with physiological changes in the diapause of *H. axyridis*, showing a dormancy type quite different from *C. septempunctata*.

MATERIALS AND METHODS

Insects. *H. axyridis* adults were collected on weeds near Gifu University from 1983 to 1984. Hibernating adults were collected in Gifu Park, Omiyacho, Gifu City. Reproductive females collected in early May were allowed to lay eggs in the laboratory, and newly hatched larvae of the 1st-generation were reared in a plastic container

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(10 cm dia. ×6.5 cm depth) under natural climatic conditions. Beetles were fed on freeze-dried insect powder mixture comprised of equal weights of aphid and drone honey bee (OKADA et al., 1972) and given water. The diet was renewed once every 2 days. The population of insects in 1 container was kept below 5 in the larval stage to prevent cannibalism. Pairs of adults were reared in containers under natural climatic conditions, and occurrence of mating behaviour and oviposition was observed every day. The eggs oviposited in early September were used for establishment of the 2nd-generation colonies in the laboratory.

Measurement of respiration rate. Respiration rate was measured in individual adults by Warburg respirometer for 1 h at 25°C and expressed as μl O_2/mg wet weight/h (SAKURAI et al., 1986).

Determination of ovarian development. Ovarian development was distinguished to 5 stages according to the definition by SAKURAI et al. (1986). For determination of follicle size, the long axial length of the 1st follicle was measured under a dissecting microscope (1 unit=25 μm).

Determination of development of fat body. Development of fat body was distinguished to 3 stages; stage 1: undeveloped, stage 2: slightly developed, stage 3: well developed.

Determination of corpus allatum size. Female adults were dissected in 0.9% saline to expose the corpus allatum. Gland length and width were measured under a dissecting microscope (1 unit=25 μm). Gland area sizes were recorded (SAKURAI, 1979).

Electrophoresis of proteins of haemolymph and egg. Sample preparations and protein analysis by polyacrylamide gel electrophoresis (PAGE) were performed according to the method described previously (SAKURAI et al., 1987b). Also sodium dodecyl sulfate (SDS)-PAGE analysis in 10% gel was performed. After the electrophoresis, the gels were stained with 1.0% Coomassie Brilliant Blue in 12.5% trichloro-acetic acid.

Treatment of juvenile hormone analogue (JHA). Five μg of methoprene dissolved in peanut oil was topically applied to the sterna under the elytra of adults by microsyringe, while control was treated with peanut oil only (SAKURAI et al., 1987b).

RESULTS

Seasonal changes in respiration rate and body weight

In the 1st-generation adults emerged in mid-June, the respiration rate decreased during summer (Fig. 1A). It dropped to the lowest level in August; 0.84 μl O_2/mg/h in the male and 0.83 in the female. After September it increased significantly and reached a peak in mid-October; 2.33 in the male and 1.92 in the female. The body weight of 1st-generation adults was kept at a considerably higher level in the female than in the male and increased during summer (Fig. 1B). After mid-September, it decreased in the male, and increased remarkably in the female due to the ovarian development.

In the 2nd-generation adults emerged in mid-October, the respiration rate decreased during winter and reached a low level below 0.90 μl O_2/mg/h by April (Fig. 2A). The lowest level was in mid-January, 0.55 in the male and 0.59 in the female. The respiration rate stayed at a low level during winter. It began to increase after April and attained a considerably high level in late April. The body weight of 2nd-generation adults decreased steadily from December to early March (Fig. 2B). After May it decreased in the male, and increased remarkably in the female due to ovarian develop-
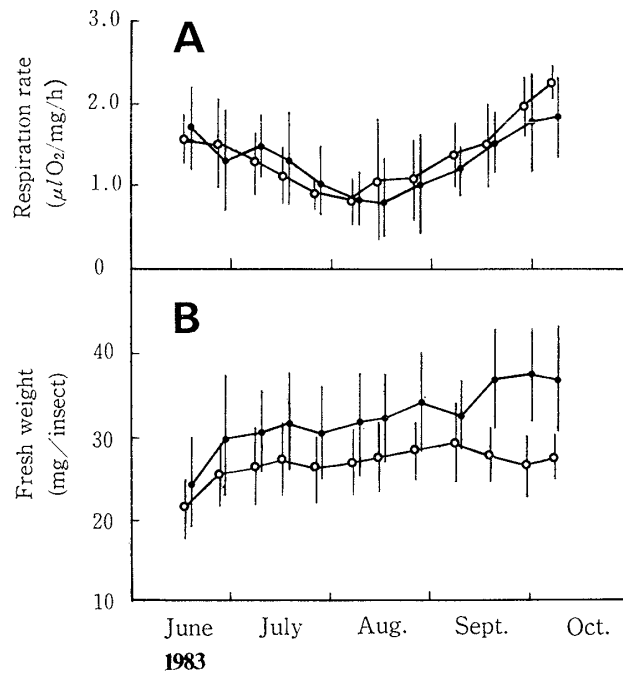
Fig. 1. Seasonal changes in respiration rate (A) and fresh body weight (B) of the 1st-generation adults, reared under natural condition. Values are mean±SD ($n=10$). ○: male, ●: female.
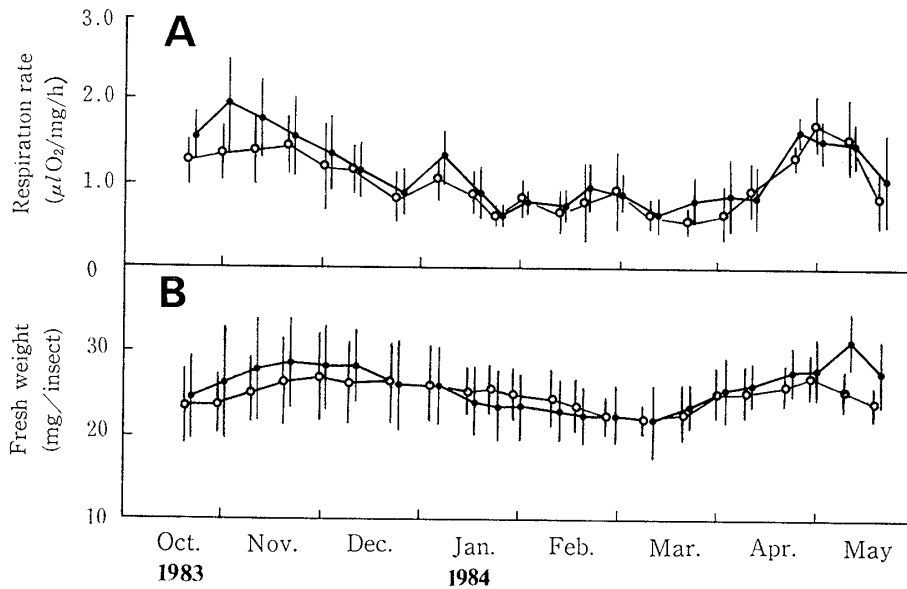

Fig. 2. Seasonal changes in respiration rate (A) and fresh body weight (B) of the 2nd-generation adults, reared under natural conditions. Values are mean±SD ($n=10$). ○: male, ●: female.

ment.   A marked sexual difference shown in the body weight of 1st-generation adults was not found in the 2nd-generation adults except May.

Seasonal changes in mating behaviour and oviposition

In the 1st-generation adults, the mating behaviour was observed after late July (Nos. 1–10 in Fig. 3A).   It occurred very frequently from mid-August to late September. Oviposition took place after early July and disappeared from late August to late September (Fig. 3B).   In the 2nd-generation 10 adults, mating behaviour and oviposition were not observed until the next spring.   Mating behaviour was again observed after late April (Nos. 11–13), but no oviposition occurred even in May.   These results demonstrated that reproductivity of females was high during summer and suppressed completely during winter.

Ovarian development during hibernation

In the 2nd-generation adults, ovarian development was markedly suppressed during winter (Fig. 4).   From November to March, the oogenic stages were mostly at stage 1 or 2 and the follicle was small in size.   Ovarian development progressed notably after April and attained the peak value in mid-May.

Fat body development during hibernation

The fat body of female adults developed notably in December and degenerated rapidly after February (Fig. 5).   Thus the fat body developed in late Autumn and degenerated during hibernation.

Electrophoretic pattern of haemolymph proteins during hibernation

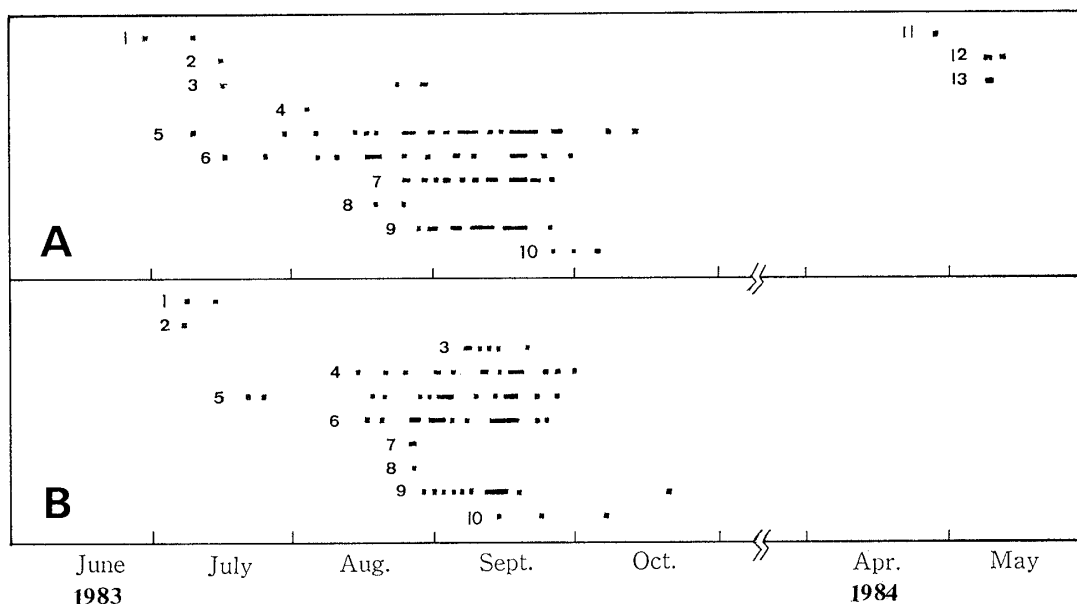Seasonal changes in heamolymph protein composition were studied by PAGE in

Fig. 3.   Seasonal changes in the mating behaviour (A) and oviposition (B) of female adults, reared under natural condition. Numerals and lines represent the individual number and day(s) in which mating behaviour and oviposition were observed, respectively. Nos. 1–10: 1st-generation, Nos. 11–13: 2nd-generation.
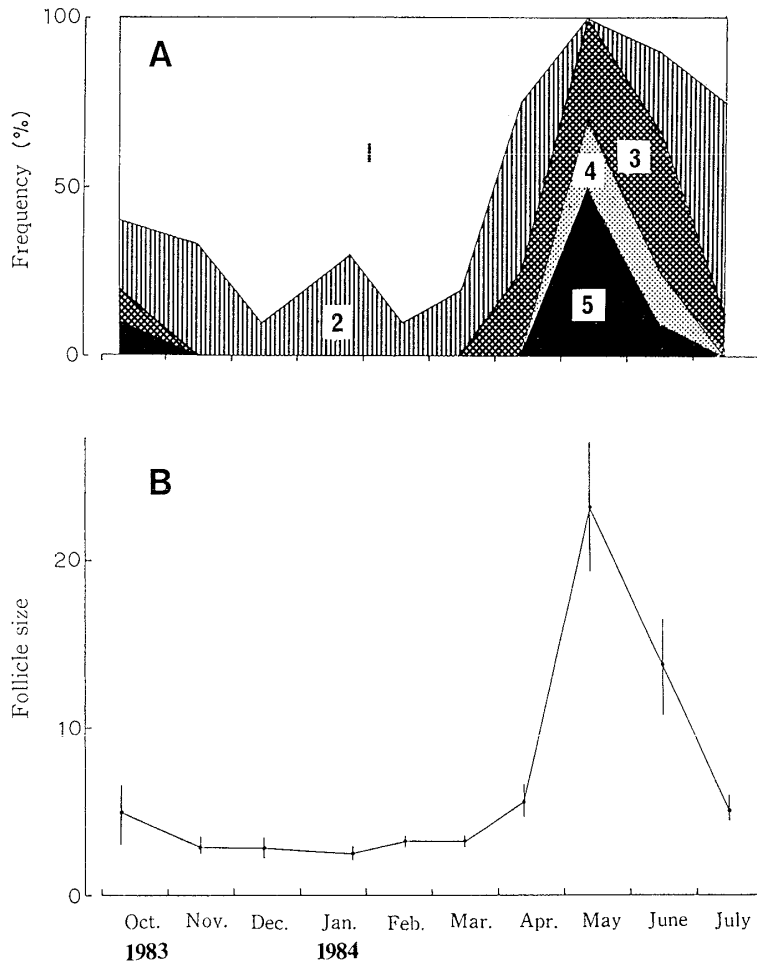
Fig. 4.   Seasonal changes in the ovarian development of the 2nd-generation female adults, collected outdoors. A: oogenic stage, B: follicle size (Size units are 40 μm and the value is mean$\pm$SD; $n=10$).
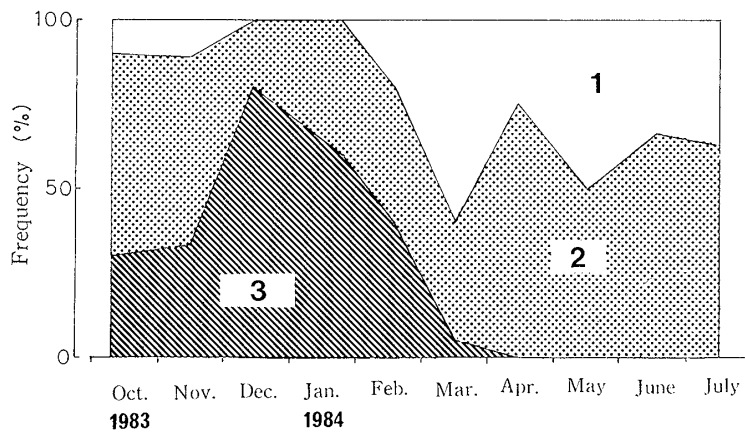

Fig. 5.   Seasonal changes in the fat body of 2nd-generation female adults, collected outdoors. Numerals indicate stage of fat body development.
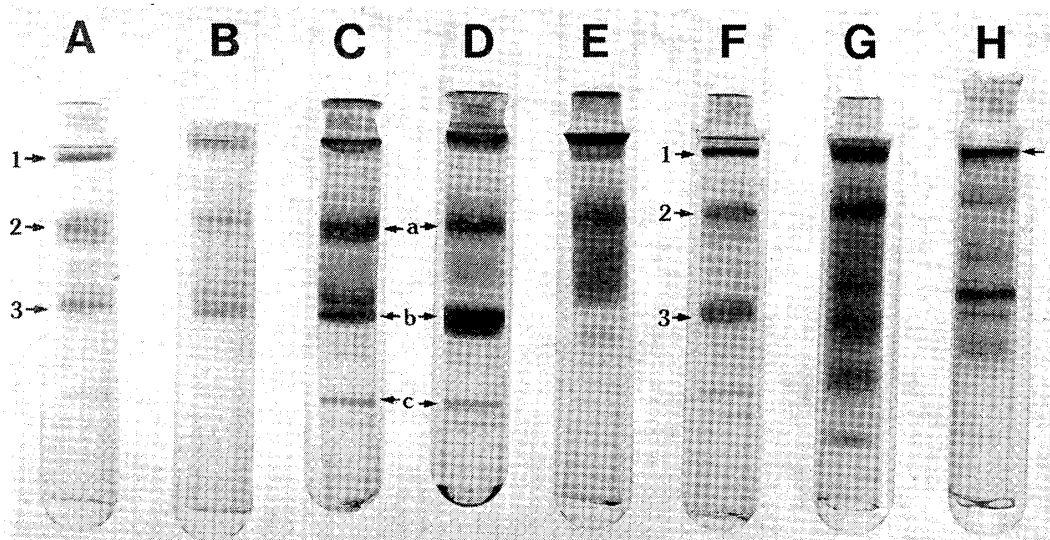
Fig. 6. Seasonal changes in polyacrylamide gel electrophoretic pattern of haemolymph proteins in the 2nd-generation female adults, collected outdoors from October, 1983 to May 1984. A–G: haemolymph, A: October 10, B: December 14, C: January 24, D: February 18, E: March 15, P: April 13, G: May 12, H: egg. Numerals indicate the bands peculiar to active adults.
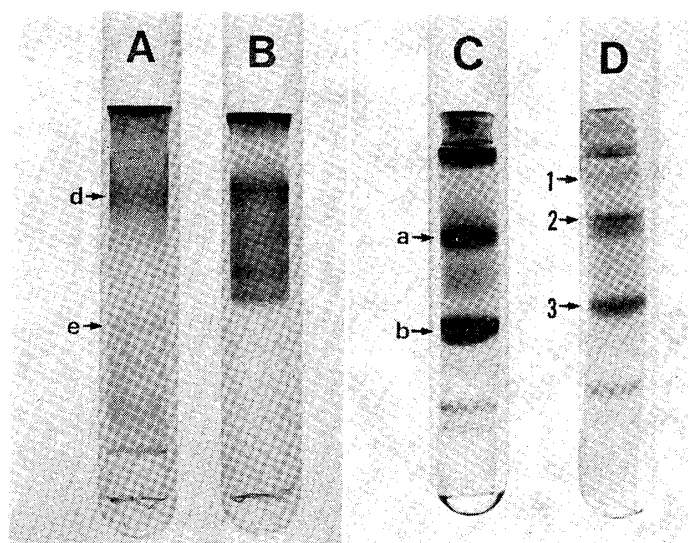

Fig. 7. Electrophoretic pattern of haemolymph proteins in the hibernating female adults. A: SDS-PAGE in mid-February, B: SDS-PAGE in mid-March. C: PAGE of adult control in mid-February, D: PAGE of JHA-treated adult in mid-February. Numerals indicate the bands peculiar to active adults.

the 2nd-generation female adults (Fig. 6). The 3 bands (1, 2 and 3) were detected in October (Fig. 6A). These bands disappeared in January and February (Fig. 6C, D). Bands 1 and 2 reappeared in March (Fig. 6E), and band 3 did so in April (Fig. 6E). Bands a, b and c were detected in January and February (Figs. 6C, D). These bands disappeared after March (Fig. 6E). In May, Band 1 was stained more intensively
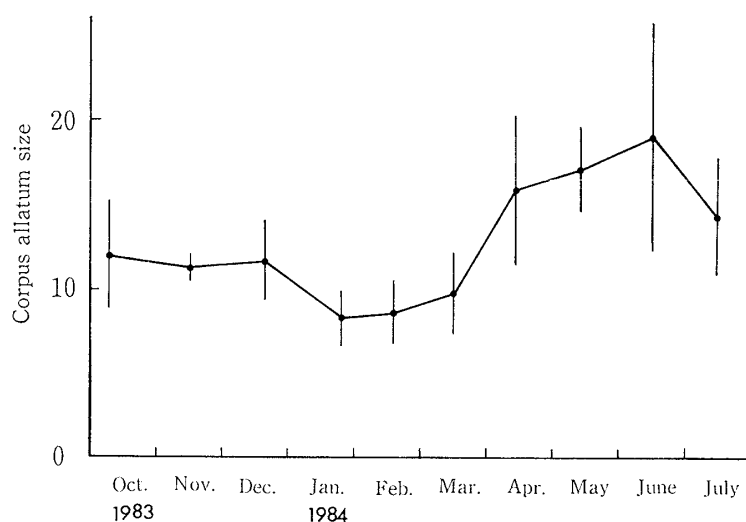
Fig. 8. Seasonal changes in the size of corpus allatum of the 2nd-generation female adults, collected outdoors. Values are mean±SD ($n=10$). Size units are $(25 \ \mu m)^2$.

Table 1. Effect of juvenile hormone analogue (JHA) on the ovarian development of hibernating female adults

Treatment	No.	Oogenic stages					Follicle size[a] (mean±SD)
		1	2	3	4	5	
Peanut oil	8	3	4	1			6.7±3.6
JHA	8			1	3	4	21.5±9.8

Beetles were treated with 5.0 μg methoprene on 24 January, 1984 and reared under 10L–14D at 25°C. Ovarian development was examined 7 d after JHA treatment.

[a] Size units are 25 μm.

and numerous bands appeared (Fig. 6G). Since the mobility of band 1 coincided with the main component of yolk protein (Fig. 6H), this band protein is assumed to be the latter's precursor, vitellogenin. The proteins recovered in bands a, b and c are associated with hibernation.

The SDS-PAGE analysis of haemolymph protein demonstrated the presence of bands d and e in the hibernating adults (Fig. 7A). Band e was faintly stained. These bands were not detected in the March adults (Fig. 7B). Molecular weights of bands d and e proteins were estimated as about 400,000 and 60,000, respectively.

Change of corpus allatum size during hibernation

Seasonal change of corpus allatum size in the 2nd-generation female adults is shown in Fig. 8. Gland sizes were very small during January and February and became very large from April to June. This indicated that the endocrine function of corpus allatum was suppressed during hibernation.

Effect of juvenile hormone analogue (JHA) on ovarian development and haemolymph protein of hibernating adults

The ovarian development of hibernating adults was stimulated markedly by JHA-

treatment (Table 1). In JHA-treated females the oogenic stages were mostly 4 and 5, while in control the stages were 1 and 2. The follicle size of JHA-treated females was also 3 times larger than that of the control. The PAGE analysis of haemolymph protein demonstrated that in JHA-treated females the a and b bands disappeared, while the bands 1, 2 and 3 appeared (Fig. 7B).

DISCUSSION

In *H. axyridis* during summer the respiration rate decreased, but the body weight increased and oviposition occurred. This indicates that the adults continue active preying behaviour and sustain higher reproductivity during mid-summer. During summer, the respiration rate in *C. septempunctata* decreased to about 1/7 of the level in late spring, and ovarian development was completely suppressed, exhibiting a typical physiological situation for diapause (Sakurai et al., 1986). This indicates that the 1st-generation adults of *H. axyridis* do not enter diapause during summer, although the metabolic level declines considerably. In the 2nd-generation adults of *H. axyridis*, the respiration rate decreased gradually after November, but the body weight increased by December, accompanying a marked development of fat body. This may indicate deposition of lipid and glycogen in the fat body as an energy source for hibernation, similar to the mechanism in *C. septempunctata* (Sakurai et al., 1987c; Sakurai and Chujo, 1979).

In the hibernating female adults of *H. axyridis*, ovarian development was completely inhibited and the corpus allatum atrophied. Topical application of JHA induced immediate ovarian development. This indicates that the hibernation of this beetle occurs at true diapause stage caused by the decline of corpus allatum activity. Electrophoretic analysis of haemolymph proteins demonstrated that some proteins are specific to diapause, and detected only in hibernating adults. With termination of hibernation, the diapause-specific proteins disappeared, while the protein corresponding to vitellogenin appeared. A similar electrophoretic profile was observed in *C. septempunctata* during aestivation (Sakurai et al., 1987b).

It has been demonstrated in *C. septempunctata* that aestivation occurs at the true diapause stage and that hibernation does not (Sakurai et al., 1986, 1987a, b), whereas in *H. axyridis* the hibernation occurs exactly then. In central and western Europe, both beetles are monovoltine and enter diapause during winter (Hagen, 1962; Hodek, 1973). The difference in diapause type between the species and regions might be due to their respective life strategies. In Japan, *C. septempunctata* lives mainly in meadows throughout the year, while *H. axyridis* lives in both meadows and woods. In summer no prey is available in the former to promote aestivation; it is available in the latter. *H. axyridis* migrates from the feeding habitat to the hibernating site in late autumn (Obata, 1986). To understand the details of the life cycle of this beetle, the environmental factor(s) regulating the diapause should be clarified in further study.

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