

Change in Electrophoretic Pattern of Haemolymph Protein in Diapause Regulation of the Lady Beetle, *Coccinella septempunctata bruckii* (Coleoptera: Coccinellidae)¹

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In *Coccinella septempunctata bruckii* found specifically in Japan, diapause regulation was studied by the electrophoretic pattern of haemolymph protein. Specific protein bands were found respectively with induction of aestivation and hibernation. With termination of dormancy, these specific bands disappeared and a band corresponding to vitellogenin (Vg) appeared in the females. The bands specific to diapause were also found in females reared at 25°C under long photoperiod after hatching. Topical application of juvenile hormone analogue to aestivating females revealed the Vg band and caused disappearance of the diapause specific bands. Observation indicates that a summer condition affect the corpus allatum activity of adults and causes a change in the electrophoretic pattern of haemolymph protein characteristic of diapause.

INTRODUCTION

The lady beetle, *Coccinella septempunctata bruckii* MULSANT found specifically in Japan is basically bivoltine, and the 1st-generation adults aestivate while the 2nd-generation hibernates (SAKURAI et al., 1983; SAKURAI and TAKEDA, 1986). It has been indicated that the aestivation is true diapause controlled by the corpus allatum, while the hibernation is not (SAKURAI et al., 1981, 1986). The summer climate might be the primary factor inducing diapause (SAKURAI et al., 1987). The present study deals with change in the electrophoretic pattern of haemolymph protein related to induction and termination of dormancy in *C. septempunctata bruckii*, which elucidates the physiological condition during diapause.

MATERIALS AND METHODS

Insects. *C. septempunctata bruckii* adults were collected in the field in Kakamigahara City, Gifu Prefecture (SAKURAI et al., 1986). For the rearing experiment, 1st-generation beetles were reared on aphids at 25°C after hatching under short-(10L–14D) or long-photoperiod (16L–8D) (SAKURAI et al., 1987).

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Electrophoresis of haemolymph and tissue proteins. The haemolymph was collected by cutting the posterior legs and the oozing adults were put in a test tube containing 40% sucrose water. Female tissues were homogenized with 40% sucrose water. After centrifuging the homogenate at 10,000 g at 4°C for 10 min, the supernatant was used for analysis. The samples of haemolymph and tissue extract were kept at -30°C until analyzed. Analysis of protein was done by polyacrylamide gel electrophoresis. The 7.5% separating gel and 3.75% stacking gel were prepared in a glass tube (5 × 70 mm) (DAVIS, 1964). Haemolymph sample corresponding to 1 insect was placed in each glass tube. Electrophoresis was done in 0.049 M Tris-0.384 M glycine buffer (pH 8.6) for 2 hr at a constant current of 1.5 mA per gel. Gels were stained in 0.05% amido black and destained in 7.0% acetic acid. Relative concentration of the protein band in the gel was measured densitometrically by chromatoscanner (Toyo, DMU-33C) at 620 nm.

Treatment of juvenile hormone analogue (JHA). Five micrograms of methoprene dissolved in 0.5 μ l peanut oil was topically applied to the sterna under the elytra of adults by microsyringe, while controls were treated with peanut oil only.

RESULTS

Change of electrophoretic pattern of female haemolymph protein during aestivation

In young adults collected in late May, 4 main bands (bands 2, 3, 4 and 5) were found, of which bands 3 and 5 were densely stained (Fig. 1A). In late June bands 2, 4 and 5 disappeared, while bands a, c, d and g were newly found (Fig. 1B). In late July bands b and f were found, and bands a and b were stained very densely (Fig. 1C). In late August all bands were faintly stained (Fig. 1D). In late September bands 1, 3 and 4 were distinct and band 2 was faint (Fig. 1E). Thus bands a, d and g appeared with the induction of aestivation.

Change of electrophoretic pattern of female haemolymph protein during hibernation

In young adults in mid October, 4 main bands were found as in late May (Fig. 2A). In late November bands 2, 4 and 5 disappeared while bands e and g appeared; bands

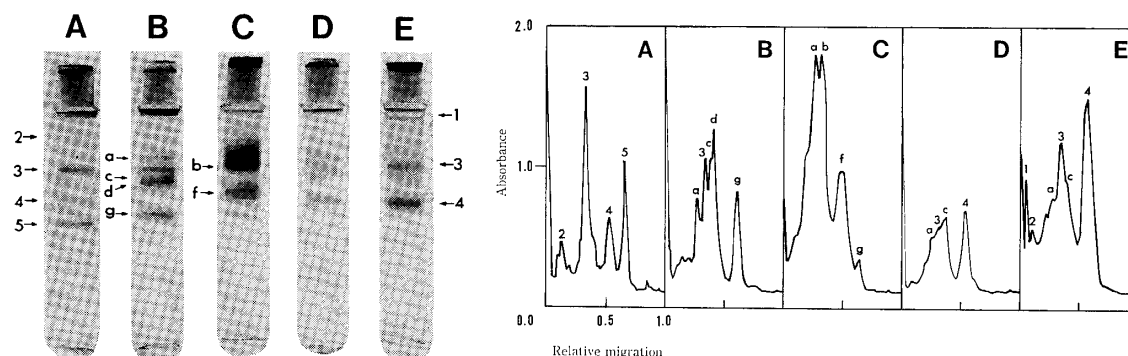


Fig. 1. Electrophoresis of haemolymph protein in 1st-generation female adults. Figure demonstrates the electrophoretic pattern and its densitometric scan (same applies to other figures). Adults were collected in 1980: A, May 23; B, June 30; C, July 29; D, August 30; E, September 30.

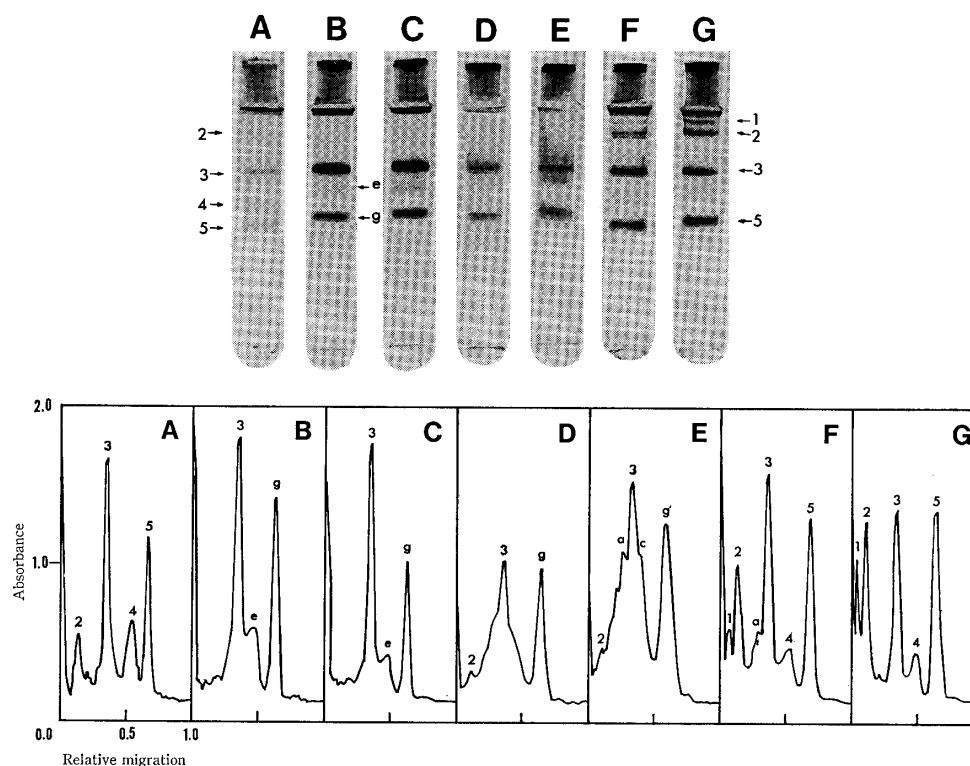


Fig. 2. Electrophoresis of haemolymph protein in 2nd-generation female adults. Adults were collected in 1980 and 1981: A, October 20; B, November 20; C, December 17; D, January 16; E, February 17; F, March 16; G, April 17.

3 and g were stained very densely (Fig. 2B). In mid December the band pattern was almost the same as in November (Fig. 2C). In mid January the stainability of band 3 decreased and band 2 was faint (Fig. 2D). In mid February the band number and its stainability both increased (Fig. 2E). In mid March band 1 in addition to the 4 main bands found in young adults in October were observed (Fig. 2F). In mid April bands 1 and 2 were distinctly seen (Fig. 2G). Thus bands e and g appeared with induction of hibernation.

Electrophoretic pattern of female tissue protein and sex difference of haemolymph

In reproductive adults collected in mid May, the sex difference was apparent with band 2 found distinctly in the female while very faintly in the male (Fig. 3A, B). The protein corresponding to band 2 was also found in the ovary and female fat body (Fig. 3D, E). Mobility of band 2 coincided with the main component of yolk protein (Fig. 3C). Hence this band might have been protein corresponding to the pre-yolk protein, vitellogenin.

Effect of JHA-treatment on electrophoretic pattern of haemolymph protein in aestivating adults

Aestivating adults were treated with JHA (methoprene) in mid July. In control adults of both sexes, 4 main bands were found and their band pattern was characteristic of the aestivation (Fig. 4A, D). One week after JHA-treatment, bands 1 and 2 appeared in the females but not in the males (Fig. 4B, E). Two weeks after JHA-treatment,

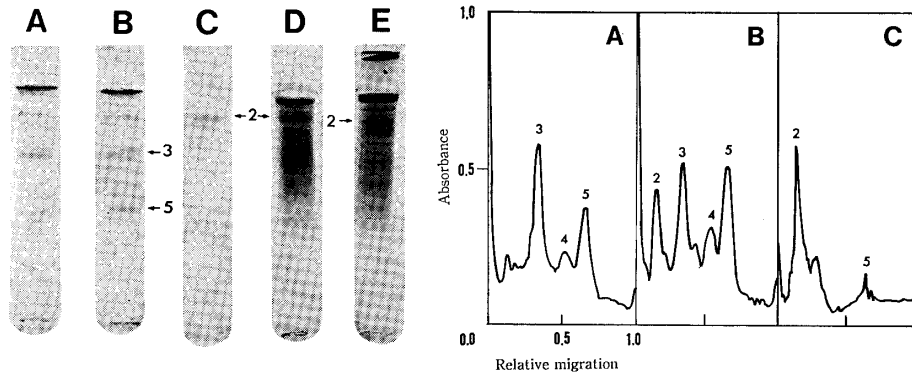


Fig. 3. Electrophoresis of protein of haemolymph (A and B) and female tissue (C-E). Adults were collected on May 15. A: male B: female, C: mature egg, D: ovary, E: fat body.

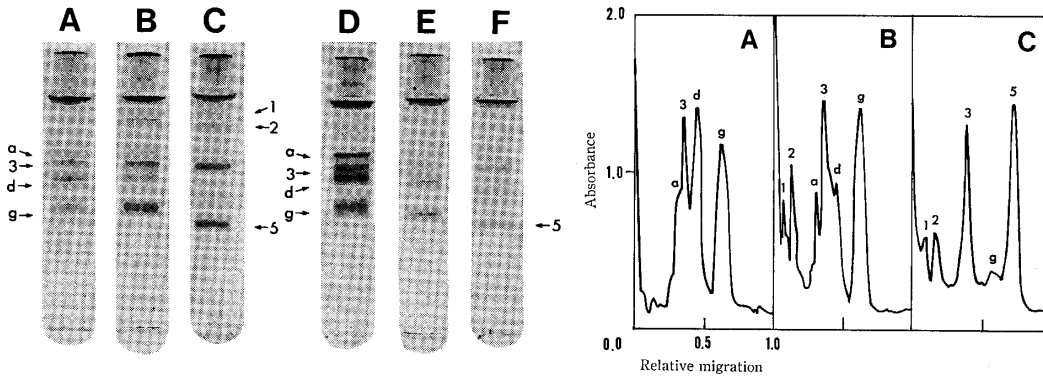


Fig. 4. Electrophoresis of haemolymph protein in diapausing adults treated with JHA. Methoprene treatment was applied to adults on July 13. A-C: female, D-F: male. A and D: control on July 20. B and E: JHA-treatment on July 20. C and F: JHA-treatment on July 27.

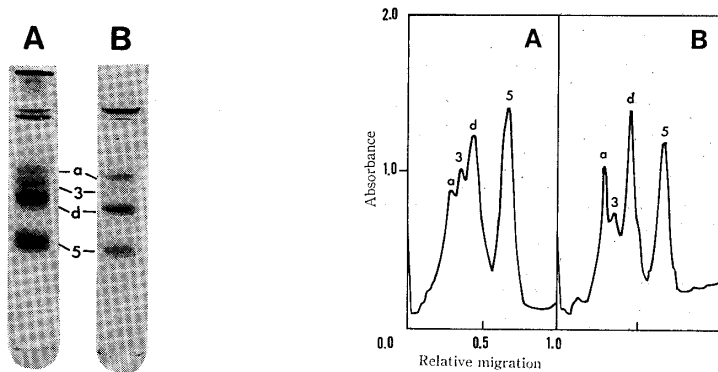


Fig. 5. Electrophoresis of haemolymph protein in female adults reared at 25°C under different photoperiods throughout life. A: 10L-14D, B: 16L-8D. Adults were analyzed 30 days after emergence.

bands a, d and g disappeared, while band 5 appeared in both sexes in place of band g (Fig. 4C, F). The band pattern after JHA-treatment was a little different from that in September after aestivation but very like that in spring after hibernation. Ovarian maturation was observed 2 weeks after JHA-treatment. Thus JHA stimulates termination of aestivation.

Comparison of electrophoretic pattern of haemolymph protein between females reared under short- or long-photoperiod at 25°C

In females 30 days old reared at 25°C after hatching under a short-(10L-14D) or long-photoperiod (16L-8D), bands a and d specific to the aestivation were found, but band 2 was lacking (Fig. 5). Band 3 was stained much more densely in the short photoperiod females than in the long photoperiod females. Observation indicated that both female groups were in diapause.

DISCUSSION

In the Gifu district the 1st-generation adults of *C. septempunctata bruckii* emerge in May and enter aestivation after June, while the 2nd-generation individuals emerge in October and enter hibernation after December (SAKURAI et al., 1986). After entering dormancy, the main protein bands except for band 3 disappear while specific bands appear in the female haemolymph. Band d or e might be protein appearing in relation to the induction of either aestivation or hibernation, and band g is common to both dormancy periods. With termination of dormancy, these specific bands disappear while band 2 appears. Band 2, which is presumed to be vitellogenin (Vg) in Fig. 3, is found distinctly after hibernation but not after aestivation. Hence Vg synthesis might not be as active in autumn females as in spring females. This coincides with the tendency in oögenesis (SAKURAI et al., 1983, 1986).

In *C. septempunctata bruckii* aestivation is true diapause controlled by the corpus allatum, while hibernation is not (SAKURAI et al., 1986). As to diapause regulation, JHA-treatment to aestivating females caused appearance of protein bands 1 and 2 and disappearance of the bands specific to diapause in the haemolymph (Fig. 4). This indicates that juvenile hormone (JH) stimulates diapause termination as well as Vg synthesis. Also, the band 1 seems to be protein corresponding to chromoprotein. The band pattern observed after JHA-treatment was a little different from that in September after diapause termination. Such difference might be ascribed to artificial diapause termination by JHA. The action of JH to stimulate Vg synthesis has been demonstrated in *C. septempunctata* L. (ZHAI et al., 1984). In diapausing adults of *Leptinotarsa decemlineata*, JH-treatment caused disappearance of diapause specific protein in parallel to the appearance of Vg in the female haemolymph (DE LOOF and DE WILDE, 1970), whereas in *C. septempunctata bruckii*, JHA caused appearance of Vg prior to disappearance of diapause specific protein.

Adults reared at 25°C under long photoperiod throughout their life enter diapause directly, while those under short photoperiod oviposit and then enter diapause (SAKURAI et al., 1987). In the present study, the protein bands specific to diapause were found in the haemolymph of females reared under both conditions. This demonstrates that females reared under short photoperiod at 25°C enter diapause after oviposition. Thus, high temperature might inhibit the metabolic activity of adults and finally cause dia-

pause. It has been indicated that a summer condition, i.e., long photoperiod and high temperature is the primary factor affecting diapause induction of this beetle (SAKURAI et al., 1987). This was confirmed in the present study.

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