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Role of Juvenile Hormone in the Control of Summer Diapause in Adult Poecilocoris lewisi (Heteroptera: Scutelleridae)【Research report】

青春激素在紅條綠盾背椿象成蟲夏季休眠的調控角色【研究報告】

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Abstract

A radiochemical method was adopted to analyze in vitro products of the corpora allata (CA) of adult females in Poecilocoris lewisi, which showed an adult summer diapause under long-day conditions. Synthetic activity of juvenile hormone (JH) by the CA increased earlier in nondiapause than in diapause adult females. When the CA of a nondiapause adult female was implanted, or a JH analogue, pyriproxyfen, was applied to diapause adult females, their ovaries developed. Therefore, JH plays a crucial role in the hormonal control of adult summer diapause in P. lewisi. Moreover, because ovaries developed to a stage slightly advanced from the diapause stage before the increase in JH-synthetic activity occurred in nondiapause adults, the possibility of the involvement of other factors in the control of ovarian development is also suggested.

摘要

紅條綠盾背椿象雌成蟲在長日照環境下，會進行夏眠。本研究利用一種放射性化學分析法，在體外檢測進入夏眠的紅條綠盾背椿象雌成蟲咽喉側腺合成之青春激素。非夏眠雌成蟲咽喉側腺加速合成青春激素的作用比夏眠者發生的早。將非夏眠雌成蟲的咽喉側腺移植至正在夏眠的雌成蟲，或予以注射青春激素類似物 (pyriproxyfen)，均會引起雌成蟲的卵巢發育。此項結果顯示青春激素在紅條綠盾背椿象夏眠的控制上，扮演關鍵的角色。此外，紅條綠盾背椿象雌成蟲在青春激素合成作用增高之前，其卵巢即發育至一特定階段，而將進入夏眠的雌成蟲，其卵巢發育在此時期較為落後。因此可能有其他因子參與卵巢發育的控制，一併在文中討論。

Key words: juvenile hormone, corpus allatum, adult diapause, summer diapause
關鍵詞: 青春激素、咽喉側腺、成蟲休眠、夏季休眠

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Role of Juvenile Hormone in the Control of Summer Diapause in Adult *Poecilocoris lewisi* (Heteroptera: Scutelleridae)

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ABSTRACT

A radiochemical method was adopted to analyze *in vitro* products of the corpora allata (CA) of adult females in *Poecilocoris lewisi*, which showed an adult summer diapause under long-day conditions. Synthetic activity of juvenile hormone (JH) by the CA increased earlier in nondiapause than in diapause adult females. When the CA of a nondiapause adult female was implanted, or a JH analogue, pyriproxyfen, was applied to diapause adult females, their ovaries developed. Therefore, JH plays a crucial role in the hormonal control of adult summer diapause in *P. lewisi*. Moreover, because ovaries developed to a stage slightly advanced from the diapause stage before the increase in JH-synthetic activity occurred in nondiapause adults, the possibility of the involvement of other factors in the control of ovarian development is also suggested.

Key words: juvenile hormone, corpus allatum, adult diapause, summer diapause

Introduction

Adult diapause in female insects is characterized by suppression of ovarian development, and the cessation of secretion of juvenile hormone (JH) by the corpus allatum (CA) has been shown to be the key factor in this suppression in many insects (Denlinger *et al.*, 2005). In Heteroptera, removal of the CA suppresses ovarian development in nondipapause females (Hodková, 1977; Kotaki and Yagi, 1989; Morita and Numata, 1997), and implantation of an active CA or application of JH or its analogue induces ovarian development in diapause females (Hodková, 1977; Numata and Hidaka, 1984a; Kotaki and Yagi, 1989). From these results, adult diapause has also been considered to be induced by a lack of JH in heteropterans. Measurement of the activity of JH secretion by the CA is one way to prove this hypothesis. CA activities have been measured in many species by the radiochemical assay originally developed by Ptatt and Tobe (1974) for the migratory locust, *Locusta migratoria*. In this method, a CA was cultured *in vitro*

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in a medium containing L-[methyl-^{3}H] methionine, and the labeled products released into the culture medium were analyzed by thin-layer chromatography (TLC). Kotaki (1993) applied this method to *Plautia crossota stali* (Heteroptera, Pentatomidae), and showed that a product in a fraction isolated by TLC had JH activity, although the chemical structure of the product was not identified. The CA activity of synthesizing this product in the JH fraction was higher in reproductive adult females than in diapause ones, supporting the hypothesis that adult diapause is induced by the lack of JH (Kotaki, 1999). All studies conducted to date on the hormonal mechanism of adult diapause in the Heteroptera have focused on winter diapause with longer duration and higher intensity, and there is no report on the role of JH and the CA in adult summer diapause in Heteroptera.

Poecilocoris lewisi (Heteroptera: Scutelleridae) has both a winter nymphal diapause and a summer adult diapause. The former is induced by short-day conditions, whereas the latter is induced by long-day conditions but terminates spontaneously in a month in which neither temperature nor photoperiod was changed (Tanaka *et al*., 2002). In the present study, we compared the *in vitro* synthetic activity of JH by the CA between reproductive and diapause adult females, and examined the effects of implantation of a CA and application of a JH analogue on the ovarian development in diapause adult females to discuss the endocrine control of adult summer diapause in *P. lewisi*.

Materials and Methods

Insects

Adults and fifth instar nymphs of *P. lewisi* were collected in the Botanical Gardens of Osaka City University, Katano, Japan (34.8°N, 135.7°E), and their progeny were used for the experiments. Nymphs and adults were reared at 25 ± 1°C on raw peanuts and water (Tanaka *et al*., 2002) with the addition of 0.05% sodium L-ascorbate and 0.025% L-cysteine. Nymphs were reared under long-day conditions of 16 h of light and 8 h of darkness (LD 16:8) that prevent nymphal winter diapause. After emergence, adults were kept under a photoperiod of LD 13:11, which induces prompt reproduction (reproductive conditions), or under a photoperiod of LD 16:8, which induces reproductive diapause (diapause conditions). Under the former conditions, females promptly develop ovaries and begin to lay eggs, whereas under the latter conditions they enter summer reproductive diapause (Tanaka *et al*., 2002).

Radiochemical assay

The radiochemical assay for measuring JH synthesis by the CA chosen was based on the method used for *Pla. c. stali* (Kotaki, 1993, 1996), which was slightly modified from that used for *L. migratoria* (Ptatt and Tobe, 1974; Ferenz and Kaufner, 1981). A corpus cardiacum-CA complex (CC-CA) was excised from adult females under a stereomicroscope in minimum essential medium (MEM, Gibco BRL), which contained Hanks' salts and L-glutamine without sodium bicarbonate and which was supplemented with 20 mM HEPES and 5 nl/ml Tween 8 (pH 7.2), on ice. A single CC-CA was incubated in a siliconized glass tube containing 30 μl MEM supplemented with 40 μg/ml L-[methyl-^{3}H] methionine (with a specific activity of 2.59-3.145 TBq/mmol, NEN) for 3 h at 30°C. After incubation, 100 μl hexane was added to extract the synthesized products. An aliquot of 50 μl of the upper hexane phase was collected and applied to a silica TLC plate (Merck). Unlabeled JH III (Sigma) and methyl-(2E)-6, 7-10, 11-bisepoxyfarnesoate (JHB_3) were also mounted on different lanes on the TLC plate as markers. JHB_3 was synthesized according to Richard *et al*. (1989). The

TLC plate was developed with a mixture of hexane and ethyl acetate (1:1). After development, spots of the markers were visualized under UV light (280 nm). Each lane was sectioned every 5 mm, and the radioactivity was determined in a scintillation liquid, ACS II® (Amersham) with a liquid scintillation counter, LS 6500 (Beckman). The Rf value is the distance the spots travel from the starting line divided by the distance the solvent front travels from the starting line.

Determination of ovarian stages

The developmental stage of the ovaries of adult females was classified into four stages under a stereoscopic microscope: stage I, no yolk was deposited in oocytes; stage II, a small amount of opaque yolk was observed in oocytes; stage III, yellow yolk had clearly accumulated in oocytes; stage IV, mature eggs were observed (slightly modified from Numata and Hidaka, 1984b).

Implantation of the CA

A CC-CA or CA was excised with a small piece of aorta by fine forceps and scissors from a female 14-17 days after emergence under reproductive conditions, and was immediately implanted into the abdomen of a recipient adult female 2 days after emergence under diapause conditions. As a control, only a piece of the aorta was implanted. Twelve days after implantation, i.e., 14 days after adult emergence, the recipient females were dissected, and the development of their ovaries was examined.

Application of a juvenile hormone analogue

A juvenile hormone analogue, 4-phenoxyphenyl (*RS*)-2-(2-pyridyloxy) propyl ether (pyriproxyfen standard, 100.0% by FID-GC, Wako), was purchased commercially. Five microliters of acetone containing 0.1, 1.0, or 5.0 μg of pyriproxyfen was topically applied to the abdomen of females 2 days after adult emergence under reproductive diapause conditions. Only acetone was applied to control insects. Twelve days after hormone treatment, i.e., 14 days after adult emergence, these females were dissected and the development of their ovaries was examined as for females after CA implantation.

Results

Products synthesized by the CA *in vitro*

The radiolabelled products synthesized *in vitro* by the CC-CA were examined in adult females of various ages under both reproductive and diapause conditions. In females 14 days after adult emergence under reproductive conditions, high radioactivity occurred only in one fraction whose Rf value was 0.53 and was between those of JH III and JHB_3 (n = 5). Figure 1A shows TLC separation of the products in the adult female with the highest radioactivity. Under diapause conditions, however, radioactivity was low for all Rf values for 15 days from adult emergence (total n = 7 at various ages). Figure 1B shows TLC separation of the products of an adult 15 days after emergence. In *Pla. c. stali*, a product of the CA with an Rf value between those of JH III and JHB_3 based on TLC showed JH activity and was therefore regarded as native JH (Kotaki, 1996). Moreover, high radioactivity also occurred in a fraction with similar Rf values in four other heteropterans (Kotaki, 1993; Hodková *et al.*, 1996). Therefore, the CA in reproductive *P. lewisi* mainly synthesizes the product with an Rf value of 5.3, which is probably native JH in this species. In the following experiments, radioactivity for this Rf value was regarded as an indicator of JH-synthetic activity in *P. lewisi*.

Comparison of JH-synthetic rates and ovarian development under reproductive

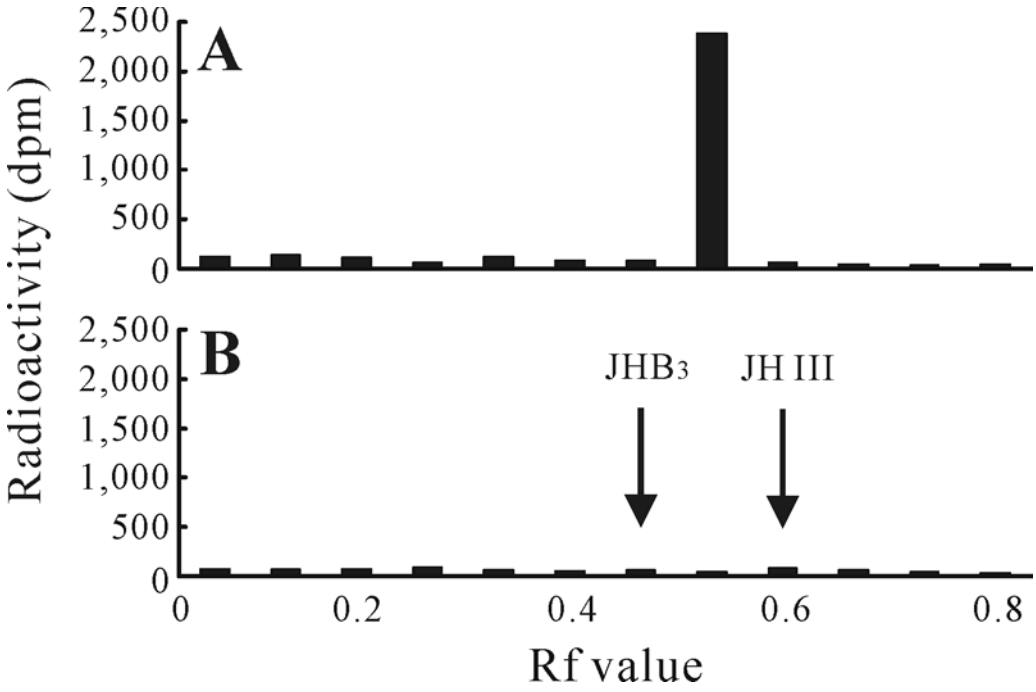

Fig. 1. Thin-layer chromatography of hexane extracts of products synthesized *in vitro* for 3 h at 30°C by the corpora allata from a reproductive female 14 days after adult emergence (A) and a female in summer diapause 15 days after adult emergence (B) of *Poecilocoris lewisi*. Radioactivity derived from L-[methyl-^{3}H] methionine is shown as a function of the Rf value. Arrows indicate the Rf values of JH III bisepoxide (JHB_3) and JH III. Note that in the reproductive adult female high radioactivity occurred in the Rf value between those of JHB_3 and JH III.

and diapause conditions

JH-synthetic activity and ovarian stages were examined during the course of development under both reproductive and diapause conditions (Fig. 2). Two days after adult emergence, ovaries were in stage I, and the CA synthesized a low level of JH *in vitro* under both conditions. Under reproductive conditions, a small amount of yolk had been deposited in oocytes in some insects at 6 or 10 days after adult emergence, although the JH-synthetic activity was still low. Under these conditions, JH-synthetic activity increased between 10 and 12 days after adult emergence, and all insects had ovaries in stage II-IV 12 days after adult emergence. Fourteen days after adult emergence, seven of ten insects had mature eggs, and some of them had already begun to lay eggs. Under diapause conditions, however, all insects showed low JH-synthetic activity, and had ovaries in stage I, 6, 10, or 14 days after adult emergence, except one individual. These insects were regarded as being in diapause. Then, JH-synthetic activity gradually increased, and the proportion of insects with developing ovaries also increased. There was no significant difference in JH-synthetic activity between the reproductive and diapause conditions 2, 6, and 10 days after adult emergence ($p > 0.05$, by the Mann-Whitney U test), although the CA synthesized significantly higher amounts of JH under reproductive

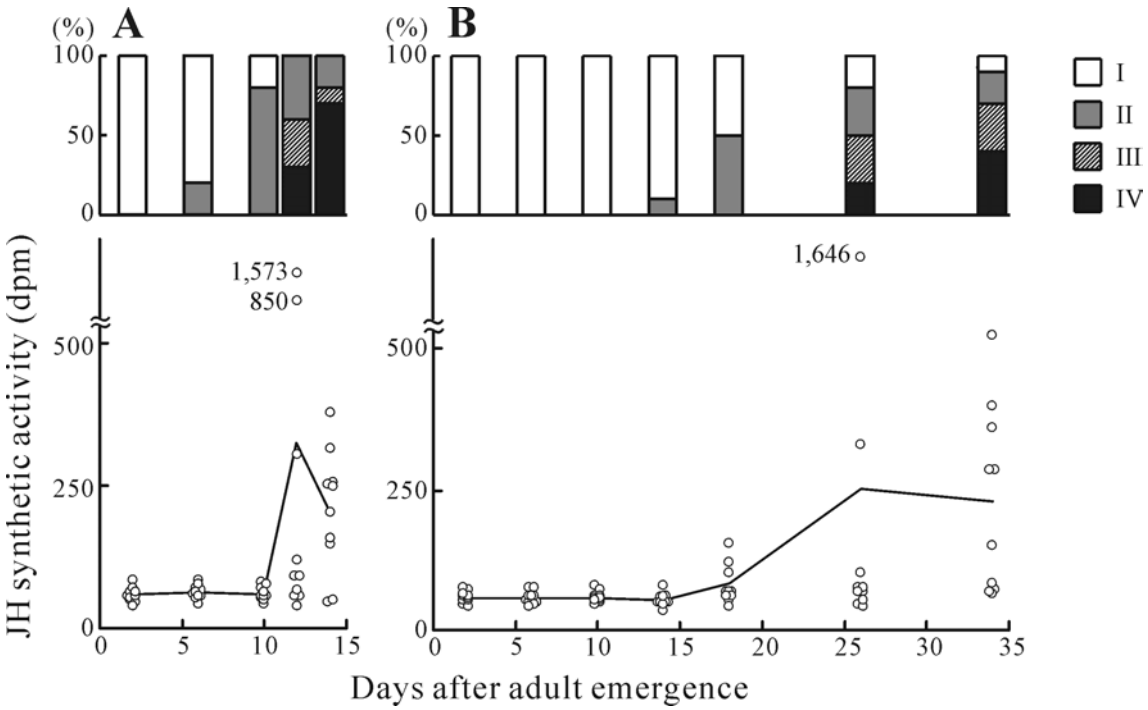

Fig. 2. Ovarian development and *in vitro* JH-synthetic activity of adults of *Poecilocoris lewisi*. A, Females transferred from LD 16:8 to LD 13:11 at adult emergence at 25°C; B, females kept under LD 16:8 in the nymphal and adult stages at 25°C. n = 10 for each. Upper panels show ovarian stages: stage I, no yolk was deposited in oocytes; stage II, a small amount of opaque yolk was observed in oocytes; stage III, yellow yolk had clearly accumulated in oocytes; stage IV, mature eggs were observed. Lower panels show the JH-synthetic activity. The CC-CA was incubated for 3 h at 30°C, and JH synthetic activity was expressed as the amount of the radiolabel detected in the JH-active fraction after TLC separation of the CC-CA products *in vitro*.

conditions than under diapause conditions 14 days after adult emergence ($p < 0.01$, by the Mann-Whitney U test).

Effect of CA implantation on ovarian development

Implantation of a single CC-CA from a reproductive adult induced vitellogenesis in most diapause adults, and complete ovarian development in some of them (Fig. 3). The proportion of reproductive adults, i.e., females with ovaries in stage II, III, or IV, was significantly higher after implantation of a CC-CA than in intact insects ($p < 0.001$ by Fisher's exact probability test). Implantation of only a CA also induced ovarian development, although the number of insects was much smaller.

Effect of a JH analogue on ovarian development

Topical application of pyriproxyfen, a JH analogue, induced vitellogenesis in most diapause adults, and complete ovarian development in some of them (Fig. 4). The proportion of reproductive adults was significantly higher after application of 1.0 and 5.0 μg pyriproxyfen than in control insects ($p < 0.01$ and < 0.001, respectively, by Fisher's exact probability test).

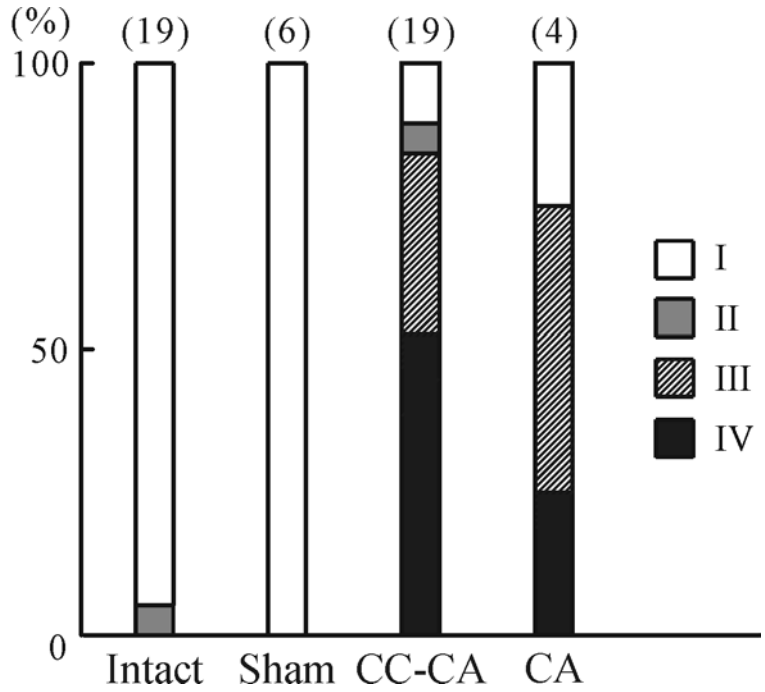

Fig. 3. Effect of CC-CA or CA implantation on ovarian development in diapause adults of *Poecilocoris lewisi*. The CC-CA and CA were excised 14 days after adult emergence from reproductive females which had been transferred from LD 16:8 to LD 13:11 at adult emergence at 25°C. These organs were implanted 2 days after adult emergence into females which had been reared under LD 16:8 in the nymphal and adult stages and which were destined to enter summer diapause. As a sham operation, a piece of aorta was implanted. Ovarian stages of the recipients were examined 12 days after implantation (see the legend for Fig. 2). The numerals above the columns indicate the numbers of insects.

Discussion

The CC-CA of reproductive adult females in *P. lewisi* cultured with L-[methyl-^{3}H] methionine synthesized a radiolabelled product, for which the Rf value was 0.53 and between those of JH III and JHB_3 by TLC (Fig. 1A). Radioactivity predominantly occurred in a fraction of TLC with a similar Rf value in *Pla. c. stali* and *Pyrrhocoris apterus* (Kotaki, 1993, 1996; Hodková *et al*., 1996), and the product in this fraction shows JH activity in *Pla. c. stali* (Kotaki, 1996). Therefore, we regarded the product in this fraction to be native JH in *P. lewisi*. It is necessary to examine whether this product has JH activity in *P. lewis*,*i* in order to discuss its role. Moreover, the chemical structure of this product remains unidentified not only in *P. lewisi* but also in *Pla. c. stali* and *Pyr. apterus*. The Rf value of this product differs from those of JH I, JH II, JH III, JHB_3, and methyl farnesoate, and therefore the structure differs from any known JHs (Kotaki, 1993, 1996; Hodková *et al*., 1996). Chemical identification of the JH molecule in these insects is required. Nevertheless, we assumed that this product is native JH in *P. lewisi*.

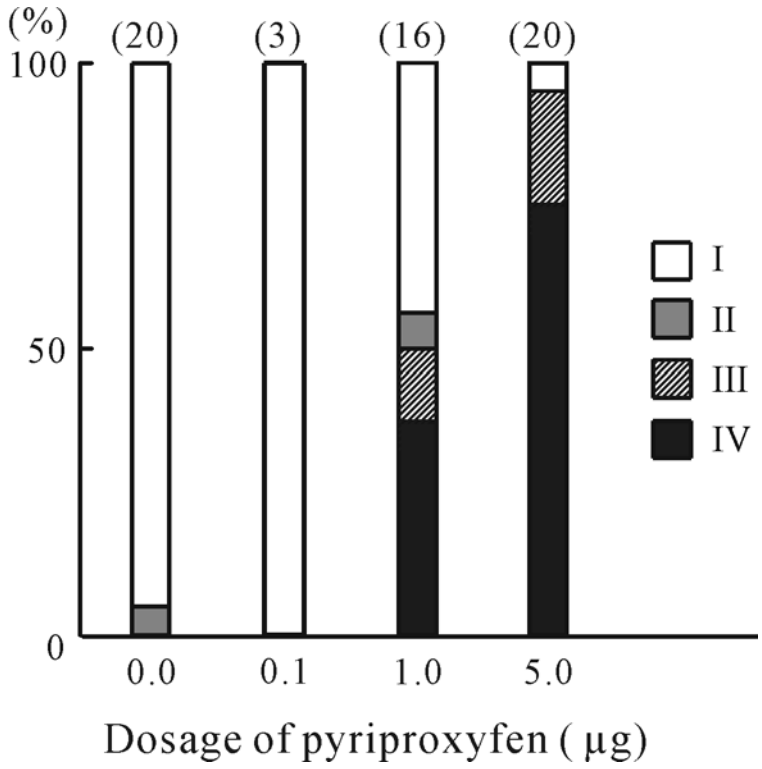

Fig. 4. Effect of a juvenile hormone analogue, pyriproxyfen, on ovarian development in diapause adults of *Poecilocoris lewisi*. Pyriproxyfen dissolved in acetone was topically applied 2 days after adult emergence to females which had been reared under LD 16:8 at 25°C and which were destined to enter summer diapause. Ovarian stages of the recipients were examined 12 days after hormone application (see the legend for Fig. 2). The numerals above the columns indicate the numbers of insects.

The increases in JH-synthetic activity and ovarian development were both earlier under reproductive conditions than under diapause ones. Moreover, implantation of a CA of a nondiapause adult female and topical application of a JH analogue induced ovarian development under diapause conditions. It was concluded, therefore, that the low activity of the CA in secreting JH is a major factor inducing adult summer diapause in *P. lewisi*. The role of JH in adult winter diapause has been documented in many insects including some species of Heteroptera, e.g., *Eurygaster integriceps* (Burov *et al*., 1972; Panov and Kryuchkova, 1977), *Pla. c. stali* (Kotaki and Yagi, 1989; Kotaki, 1999), *Pyr. apterus* (Hodková, 1976, 1977; Hodková *et al*., 1996), and *Riptortus clavatus* (Numata and Hidaka, 1984; Morita and Numata, 1997). However, the lower JH-synthetic activity of the CA in diapause adults has been shown only in *Pla. c. stali* (Kotaki, 1999), and the present results showed the lower JH-synthetic activity of the CA during summer diapause in *P. lewis*. Winter diapause in *Pla. c. stali* and summer diapause in *P. lewis* basically depend on the same hormonal control mechanism.

In *P. lewisi*, ovaries reached stage II, which is slightly advanced from the ovarian stage in diapause adults, before the increase in JH-synthetic activity

occurred under reproductive conditions (Fig. 2A). Therefore, we cannot attribute the difference in ovarian development between diapause and reproductive adults only to the difference in JH-synthetic activity as measured by the present method. One possibility for explaining these results is that ovarian development from stage I to II is induced by a small amount of JH secretion from the CA, and the present method was not sensitive enough to detect this. In *Pla. c. stali*, the addition of precursors of JH, i.e., farnesoic acid or farnesol, in the culture medium of CA remarkably increased the radioactivity in the JH-active fraction in TLC (Kotaki, 1996). In the present study, we did not add these precursors of JH, and it is probable that the addition of the precursors would have increased the sensitivity.

Another possibility is that a factor other than the lower JH-synthetic activity of the CA is responsible for keeping the ovaries in stage I during diapause, but implantation of an active CA and high dose of a JH analogue overrode this suppression. In *Pla. c. stali*, transection of the nerve between the brain and the CC-CA in diapause adults first caused ovarian development, and afterwards an increase in JH synthesis was observed (Kotaki, 1999). In the bumblebee, *Bombus terrestris*, in which adult diapause is related to low JH titers in the hemolymph, diapause termination occurs earlier than the increase in JH production rates (Larrere *et al*., 1993). Although adult diapause of the blow fly *Protophormia terranovae* is related to low rates of JH production, removal of the pars lateralis in the brain caused ovarian development but no significant increase in JH synthesis in diapause adults (Shiga *et al*., 2003). In these insects as well as *P. lewisi*, therefore, other factors may also contribute to the suppression of ovarian development in diapause adults in addition to the low rate of JH synthesis.

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青春激素在紅條綠盾背椿象成蟲夏季休眠的調控角色

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摘　要

紅條綠盾背椿象雌成蟲在長日照環境下，會進行夏眠。本研究利用一種放射性化學分析法，在體外檢測進入夏眠的紅條綠盾背椿象雌成蟲咽喉側腺合成之青春激素。非夏眠雌成蟲咽喉側腺加速合成青春激素的作用比夏眠者發生的早。將非夏眠雌成蟲的咽喉側腺移植至正在夏眠的雌成蟲，或予以注射青春激素類似物 (pyriproxyfen)，均會引起雌成蟲的卵巢發育。此項結果顯示青春激素在紅條綠盾背椿象夏眠的控制上，扮演關鍵的角色。此外，紅條綠盾背椿象雌成蟲在青春激素合成作用增高之前，其卵巢即發育至一特定階段，而將進入夏眠的雌成蟲，其卵巢發育在此時期較爲落後。因此可能有其他因子參與卵巢發育的控制，一併在文中討論。

關鍵詞：青春激素、咽喉側腺、成蟲休眠、夏季休眠。