Sexual Difference in the Termination of Adult Diapause of the Leaf Beetle, *Gastrophyta atrocyanea*
Motschulsky (Coleoptera: Chrysomelidae)1,2

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The leaf beetle, *Gastrophyta atrocyanea* feeds selectively on dock, *Rumex obtusifolius* and has a univoltine life cycle with adult diapause. There have been few reports on obligate adult diapause compared with the facultative diapause induced by photoperiodism. However, interesting reports of studies have revealed that the females of *G. atrocyanea* oviposit eggs after mating whenever diapause is inhibited or broken by juvenile hormone analogue (JHA), but those eggs cannot hatch (Ashida, 1980). The unhatchability is due to the reproductive impotency of the males which require exposure to high temperature followed by treatment at low temperature or JHA for diapause termination (Ashida and Kamehisa, 1981). Ashida (1981) also found that short exposure to high temperature induced early diapause termination in the female and late diapause termination in the male.

These observations suggest that JHA controls oogenesis and special elements of behaviour but does not control spermatogenesis, and that exposure to high temperature is essential for spermatogenesis. Although very little is known about the male in adult diapause, Ashida’s findings center on the most significant factor in the relationship between reproductive system and diapause. Our studies of adult diapause in *G. atrocyanea* aimed at the analysis of the specific mechanism of diapause development of the obligate type. In the present experiments a noticeable difference in diapause termination between female and male was investigated under some definite conditions.

**MATERIALS AND METHODS**

Larvae on the foliage of *R. obtusifolius* were collected in the field of Iwate University in May 1985 and reared on fresh leaves of dock in a plastic container (25 × 13 × 20 cm) at 25°C. After emergence from the soil (the first day of adult life), the adults fed on the leaves for a week and then burrowed into the soil to enter diapause. Diapausal adults were divided into two groups, one of which was reared at 5°C and the other at 15°C for 120–135 days. The adults chilled at 5°C for 120 days were transferred to two temperature conditions, 25°C and 15°C. To the ventral side of these adults 1 µg of methoprene (JHA) dissolved in 1 µl of acetone was topically applied every day until they emerged from the soil and fed fresh dock leaves. The number of beetles emerging from the soil was counted and fresh dock foliage was provided daily.

A distinction between female and male adults was observed under a dissecting microscope by the pull of elytra on the body. According to the difference of the tergum colour (female, orange; male, dark brown), adults which had been reared at 5°C and 15°C for 120–135 days were divided into two groups by sex and subjected to JHA application. For histological observation, adult testes were extirpated in saline water and fixed in 6.25% glutaraldehyde in cacodylate buffer (pH 7.2) for about 2 hr at 5°C. Each sample was embedded in Epon-A after dehydration and

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2 This is the first report of a serial study, “Regulation of Obligate Type in Insect Adult Diapause.”
Fig. 1. Diapause termination induced by methoprene in the female and male of *G. atrocyanea*. Adults were chilled at 5°C for 120 days after diapause initiation and then divided into female and male groups. Fifty adults in each group were treated daily with methoprene throughout incubation at 25°C.

**Table 1. Effect of chilling and mild temperature on diapause termination in the female and male of *G. atrocyanea***

<table>
<thead>
<tr>
<th>Sex</th>
<th>Cumulative percentage of adults above the soil throughout incubation at 15°C</th>
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<tbody>
<tr>
<td></td>
<td>0-day^a</td>
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<tr>
<td>Female (103)^b</td>
<td>0</td>
</tr>
<tr>
<td>Male (163)</td>
<td>0</td>
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</table>

^a Female and male groups were transferred to 15°C after chilling at 5°C for 120 days following diapause initiation.

^b Numerals in parentheses denote the number of adults.

^c Two beetles emerged from the soil and fed only two days. One of them burrowed again into the soil on the 17th day and the other showed no mating behaviour when it met reproductive females.

Fig. 2. Cross-section of testis in the males of *G. atrocyanea*. A: chilled at 5°C for 135 days since diapause initiation. ×70. B: chilled at 15°C for 120 days after diapause initiation. ×140.

A cross-section (3 µm) was observed after staining with Mayer's hematoxylin.

**RESULTS AND DISCUSSION**

As the chilling at 5°C for 120 days was insufficient to terminate diapause, JHA was topically applied to female and male groups daily after incubation at 25°C. Special elements of behaviour such as feeding, mating and oviposition were adopted as indices of diapause termination. As shown in Fig. 1, cumulative percentage of the adults emerging from the soil soon increased in the females and attained 50% 2 days after JHA treatment. The males, however, did not come out even 4 days after JHA treatment but thereafter did gradually, and by 6.5 days half of them were found above the soil. All the males had emerged from the soil and fed on fresh dock leaves by 10 days after the incubation. When female and male groups were transferred to the condition of 15°C after chilling at 5°C for 120 days, more than half of the females emerged from the soil but almost all the males remained in the soil for 15 days (Table 1). There was a great delay in diapause termination of males induced by JHA or mild temperature after chilling at 5°C as compared to females.

The females emerging from the soil started to oviposit eggs 5 days after the incubation but those eggs did not hatch when mated with males applied with JHA after chilling at 5°C for 120 days. When mated with males whose diapause was broken by JHA after chilling at 15°C for 120 days, the females...
oviposited normal eggs. Histological observation showed that the testes of adults chilled at 5°C for a long period were immature at the stage of pre-spermiogenesis and no sperm was found (Fig. 2). Abundant sperm was found in the testes of adults chilled at 15°C for a long period. These results suggest that the reproductive system of males develops irrespective of the phase of diapause termination.

The relation between photoperiodism and hormonal control and oogenesis has been characteristic of adult diapause of the facultative type (de Wilde et al., 1959; de Wilde and de Boer, 1961; de Kort, 1981; Kurihara, 1967; Kono, 1980, 1982, 1986). A significant reproductive system was found exclusively in adult diapause of the obligate type in the leaf beetle, G. atrocyanea. When the adult diapause of this insect was inhibited or terminated by JHA, oogenesis developed normally but oviposited eggs could not hatch (Ashida, 1980; Ashida and Kanehisa, 1981). Ashida’s studies elucidated that the un hatchability was due to immature testes and that exposure to high temperature was essential for spermatogenesis. In addition, it has been proposed that spermatogenesis of leaf beetles requires a long time in univoltinism and is finished at emergence or occurs immediately after emergence in multivoltinism (Ashida and Matsuda, 1983).

The present study in adult diapause of G. atrocyanea clearly revealed sexual differences in diapause termination with the treatment of JHA or mild temperature to adults chilled at 5°C for 120 days (Fig. 1 and Table 1); Ashida (1981) mentioned this fact following his experiment utilizing both high and low temperatures. Histological observation elucidated that the spermatogenesis of this insect might be independent of the phase of diapause termination (Fig. 2). The relationship between sex and diapause development in adults of this insect could be regarded as the typical mechanism for adult diapause of the obligate type. It remains to be elucidated how temperature and JHA induce the two different responses to diapause termination. Experimental details on this will be given in following reports.

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REFERENCES