Studies on the ovarian development in *Coccinella septempunctata bruckii* Mulsant

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Introduction

The seven spotted lady beetle, *Coccinella septempunctata* L. has very wide geographical distribution and several types of voltinism according to latitude, and these voltinisms were classified into four types (Hagen 1962). In north European region this species shows univoltinism with a winter diapause. *Coccinella septempunctata bruckii* Mulsant which destributes commonly in Japan is a subspecies of the European species. In Tokyo region they usually produce two generations in a year and their reproductive seasons are spring and autumn after winter- and summer-diapause when they stop ovipositing. The diapause seemed to be the key point of labotatory culture of the lady beetle all year round and many studies on prevention or artificial reduction of the diapause have been done. Hodek and Cerkasov (1961) determined that long photoperiod and high temperature inhibited diapause. Hamalainen and Markkula (1972) also reported that several generation cycled in a year under long photoperiod condition in Finland, where the beetle showed only one generation in the field.

In this study the usefulness of an artificial diet (drone honeybee powder) was examined and some environmental or hormonal treatments were attempted in order to improve reproduction of the species.

Materials and methods

The lyophilyzed drone honeybee brood (DP) was used as an artificial diet. The rearing method was basically the same as for *Harmonia axyridis*. Adults emerged from pupae collected from the field in Tokyo region were exposed to constant long day (16L 8D), short day (12L 12D) or darkness (DD) condition at 20 or 25°C. A pair of female and male was confined in a 9-cm petridsh and mainly supplied with DP. Some species of aphids such as *Myzus persicae*, *Achyrthosiphon pisum* and *Aphis rumicis* were used for

food to investigate nutritional factor. These aphids are should be suitable food for the coccinellids, because both adults and larvae of the beetle are very common predators of these aphids in the field. Eggs laid by females were counted everyday and foods were given sufficiently. The ovarian development of females except ones which began to lay eggs, was checked by dissection after 30 days from the beginning of each treatment. The development of ovaries was classified into four stages (A-D) in the same way of *Harmonia axyridis* (Niijima and Takahashi 1980). The most matured stage which has matured eggs was symbolized by A and the most immatured stage with empty ovarioles was D. Egg-laying female was marked as Ⓐ.

To investigate hormonal effects on the ovarian development, 2 μl acetone solution of a junvenile hormone analogue (ALTOSID: Otsuka Pharm. Co.) was topically applied to abdominal cuticule of newly emerged female. Contrlos received acetone only. The treated beetles were fed on DP and incubated at 25°C in the dark condition.

Results and discussion

Successive rearing on DP in dark condition

Drone powder (DP) used in this experiment as diet was effective for many aphidophagous insects such as *Harmonia axyridis*, *Menochilus sexmaculatus*, etc. (Matsuka et al. 1972, Niijima 1979). On the other hand, *C. septempunctata bruckii* has been reared on DP with little success in the first stage of experiment (Matsuka et al. 1972). But in this study the species could be reared on DP alone for four generations and the larval development was not so inferior to ones fed on aphids (Table 1). These results were obtained from the method of "forced feeding", in which larvae were deposited directly onto DP when the diet was renewed. In the "unforced feeding" (control) the larvae were deposited at random in the dish and their feeding was not minded. The forced feeding resulted in body weight comparable to field insects and 10 to 50% heavier than larvae in the unforced feeding group. This suggested that DP might be deficient in some sort of feeding stimulant rather than nutritive composition for this species. Recent research in China (Plant Protec

Table 1. Development of *Coccinella septempunctata bruckii* fed on drone powder

Generation	Larval & pupal period (days)	Emergence ratio (%)	Adult body-weight (mg+SD)	
			female	male
G_1	24.8	65	34.6±5.1	27.8±2.8
G_2	26.5	34	34.4±3.5	25.2±3.7
G_3	25.5	53	33.2±3.7	29.4±3.4
G_1 (aphids)	19.4	80	37.4±5.9	33.1±5.2

Table 2. Oviposition and longevity of *Coccinella septempunctata bruckii* fed on drone powder under dark condition

Generation	No. of females tested	Longevity (days)	Preoviposition period (days)	Oviposition period (days)	No. of eggs laid
P*	8	255.6 (155—397)**	144.8 (64—212)	61.1 (2—187)	171.4 (44—439)
G_1	12	141.6 (64—236)	89.4 (35—157)	22.2 (1—112)	64.0 (11—185)
G_2	8	137.9 (119—157)	72.6 (47—104)	18.3 (1— 59)	67.4 (12—187)
G_3	10	114.4 (104—148)	66.3 (43—101)	8.7 (1— 42)	47.0 (11—190)

* : Collected from the field at pupal stage in autumn.
** : Figures in parentheses represent minimums and maximum.

Gr., 1975) reported that the body weight of adults fed on fresh drone brood did not differ greatly from ones fed through the forced feeding in our experiment. The fact also suggested that moisture in the diet took play a role as an important physical or stimulative factor.

The reproduction by the females was not good although they survived over 100 days. The preovipositional period was long but shortened as increasing generation (Table 2). Maeta (1965) reared this species on aphids and observed long preovipositional period and their first and second generations were 60–86 and 24–47 days respectively. Therefore the long preovipositional period shown in Table 2 was not considered to be caused by only nutritional insufficiency of DP. However the number of eggs laid was fewer than that deposited by aphid-feeding females and decreased through generations, probably because of undernourishment from DP. Addition of some stimulative substances or improvement of physical condition, especially moisture content, would increase ingestion and result in better result.

Dietary and environmental factors influencing oviposition

The beetles collected in spring were exposed to three photoperiodic conditions and fed on DP or aphids to investigate influence of food and photoperiodic factors (Table 3). In both cases of DP and aphid, photoperiodic influence was not so notable though short day condition (12L 12D) slightly promoted ovarian development than others. On the other hand dietary factor showed conspicuous influence and even in DD condition 75% of females began oviposition in a month whereas no female laid eggs with DP feeding.

As compared with spring samples, results with females collected in autumn were shown in Table 4. They were supplied with only DP and exposed to each photoperiod in two temperature conditions. In both long and short photoperiodic conditions at 25°C some oviposition was observed within a month and the rate was slightly higher in short day than in long day condition as well as spring sample. As concerns with the ovarian

Table 3. Influence of photoperiod and diet on ovarian development in *Coccinella septempunctata bruckii* collected in spring*

Diet	Photo-period		No. of females tested	Stage of ovaries Ⓐ(%)	A	B	C	D	Preoviposition period (pays±SD)	No. of eggs per female**
DP	16L	8D	27	0(0)	0	1	4	22	——	——
	12L	12D	26	3(12)	0	1	4	18	18.0± 4.1	31.0±22.5
	D	D	24	0(0)	2	0	4	18	——	——
Aphid	16L	8D	9	6(67)	0	0	0	3	13.5± 2.2	311.2±298.2
	12L	12D	18	14(78)	0	0	0	4	15.6± 5.6	810.6±558.0
	D	D	12	9(75)	0	0	0	3	15.0± 5.5	516.3±484.1

* : Rearing temperature was 25°C.
** : No. of eggs laid within 30 days after beginning of oviposition.

Table 4. Influence of photopepiod and temperature on ovarian development in *Coccinella septempunctata bruckii* collected in autumn*

Tempepature (°C)	Photo-period		No. of females tested	Stage of ovaries Ⓐ(%)	A	B	C	D	Preoviposition period (days±SD)
25	16L	8D	29	2(7)	2	2	3	20	29.0± 0.0
	12L	12D	28	4(14)	2	0	4	18	29.3± 0.5
	D	D	28	0(0)	2	2	4	20	——
20	16L	8D	26	0(0)	0	1	11	14	——
	12L	12D	27	0(0)	1	0	5	21	——
	D	D	28	0(0)	0	0	2	26	——

* : DP was supplied in this experiment.

development, the autumn sample made faster progress than spring ones and the result coincides with the observation by Maeta (1965). Hirano et al. (1982) indicated that high temperature induced summer diapause. As shown in Table 4 ovarian development tends to delay at low temperature (20°C). In the field at middle or end of May when the reproduction of the species almost ceases, atomospheric temperature is lower than 25°C in Tokyo region. But 25°C is not farmful to their reproduction because they oviposited at this temperature when aphids were supplied (Table 3). Hirano et al. (1982) also suggested that long photoperiod induced summer diapause. In the results of the experiment such tendency was recognized slightly in comparison with short photoperiod but it is not clear. Adult females of the first generation, when fed with aphids, began to oviposit after short preoviposition period under long day condition although day length in May in Tokyo region is already long. These results did not coincide with the observation by Hirano et al. Hodek and Ruzicka (1979) described that the plasticity of life cycle in European species to be due to a great extent to the plymorphisms in photoperiodic response. The disparity would be due to variantion of photoperiodic sensibility as suggested by Hodek. But even under DD condition nearly half females began laying eggs when aphids were supplied at adult stage (Table 5 and also Table 3). So Ovarian maturation

Table 5. Influence of food on ovarian development in adult and larval stage of *Coccinella septempunctata bruckii* collected in spring

Photo-period		Food		No. of females tested	Stage of ovaries					Preoviposition period (days±SD)	No. of eggs per female(±SD)*
		larva	adult		Ⓐ(%)	A	B	C	D		
16L	8D	aphids	aphids	13	10(77)	0	0	0	3	8.5±1.7	989.6±571.8
〃	〃	aphids	D P	18	1(6)	0	0	0	17	16	23
D	D	D P	D P	23	0(0)	0	1	6	16	——	——
〃	〃	D P	aphids	18	8(44)	0	0	2	8	22.9±4.9	333.5±258.4

* : Eggs laid in 30 days from beginning of oviposition.

Table 6. Effect of JHA on developmant of ovary in *Coccinella septempunctata bruckii* fed on drone powder at 25°C

JHA	Dose per female	No. of females tested	Stage of ovaries					Preoviposition period in days (Max.—Min.)	No. of eggs laid*	Hatching ratio (%)
			Ⓐ(%)	A	B	C	D			
ALTOSID	200 (μg)	19	5(26)	3	3	4	4	15.4(10—29)	29.6	11.5
	100	23	5(22)	2	3	6	7	16.2(9—28)	49.2	37.0
	10	19	2(11)	0	0	9	8	19.0(15—23)	161.0	71.4
CONTROL (acetone)	—	20	0(0)	0	1	2	17	——	——	——

* : No. of eggs laid in 30 days from beginning of oviposition.

of this species seems largely on suitable food especially in adult stage.

Hormonal effects on ovarian development

In order to augment oviposition capacity a juvenile hormone analogue, ALTOSID was topically applied. As showh in Table 6 ovarian development of beetles was promoted disinctly by the JHA even under bad nutritional and DD condition. As amount of JHA was increased the preoviposition period was reduced while control females did not produce any eggs in 30 days. Wang et al. (1977) also treated European species with JHA and their preoviposition period was shortened by 4-5 days. But the number of eggs obtained with JHA treatment in our experiment and their hatchability reduced as dose of JHA increased. Furthermore, many larvae that hatched from the eggs laid by treated females died during the lst instar. It was found that ovarioles of the females treated with JHA developed irregularly and the oviposition did not continue. Thus influence of JHA remains in the next generation, so JHA treatment is not useful to obtain next generation in combination with DP.

Summary

Coccinella septempunctata bruckii which is subspecies of European species was reared

under various condition in order to improve development and reproduction and some factors influencing on ovarian development were discussed.

Four successive generations were reared with drone powder (DP) as a diet by some improvement of rearing method.

The most important factor to prevent diapause was considered to be satisfactory food. Photoperiod acted as a secondary factor for oviposition in this species and short days (12L 12D) was slightly more effective than 16L 8D and DD.

A juvenile hormone analogue (ALTOSID) treatment promoted development of ovaries and the preovipositional period was reduced. But the oviposition was temporary and hatchability was very low. Then JHA treatment could not be used to obtain next generation with DP.

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摘　　要

ナナホシテントウの卵巣発育に関する研究

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我国に棲息しているナナホシテントウ，*Coccinlla septempunctata bruckii* Mulsant はヨーロッパに広く分布するコモンナナホシテントウ，*C. septempunctata* L. の亜種であり，我国では夏眠と冬眠の２つの生殖休止期をもつ．本実験では雄蜂児粉末を用いて本種の改良飼育を行ない，特に休眠を起こさせずに卵巣発育を継続させる条件について検討を行なった．

その結果，本種は雄蜂児粉末のみで４世代の飼育が可能であり，供試幼虫を餌の上に置いてやることでより大きな個体を得ることができた．

卵巣発育に関する要因では，ヨーロッパ種で言われている光周期は二次的なもので，餌条件が最も重要であることが明らかとなった．またコモンナナホシが長日条件下で卵巣発育が継続されるのに対し，本種では短日条件下の方がむしろ有効であった．

さらに幼若ホルモン様物質（ALTOSID）によって明らかに卵巣発育が促進され，餌条件が多少悪い条件下（雄蜂児粉末飼育）においても産卵前期の短縮が認められた．しかしその産卵には連続性はなく，孵化率も低かった．また次世代において若齢期の死亡が目立ち，雄蜂児粉末との組み合わせは不適と考えられた．