

Title	Phenology of Two Sympatric Phytophagous Ladybirds of <i>Henosepilachna vigintioctomaculata</i> Complex in and near Sapporo, Northern Japan (Coleoptera: Coccinellidae) (With 5 Text-figures and 3 Tables)
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Citation	北海道大學理學部紀要 = JOURNAL OF THE FACULTY OF SCIENCE HOKKAIDO UNIVERSITY Series . ZOOLOGY, 20(3): 313-328
Issue Date	1976-10
Type	bulletin
URL	<a href="http://hdl.handle.net/2115/27613">http://hdl.handle.net/2115/27613</a>
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**Phenology of Two Sympatric Phytophagous Ladybirds  
of *Henosepilachna vigintioctomaculata* Complex  
in and near Sapporo, Northern Japan  
(Coleoptera: Coccinellidae)<sup>1)</sup>**

By

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(With 5 Text-figures and 3 Tables)

Two forms of phytophagous ladybirds belonging to *Henosepilachna vigintioctomaculata* complex (henceforth abbreviated as Hv-complex) are distributed in and near Sapporo; namely, Hokkaido form of *Henosepilachna vigintioctomaculata* (Motschulsky) (abbreviated as V-II)<sup>2)</sup> and Sapporo form of *H. pustulosa* (Kôno) (P-III)<sup>2)</sup>. The relation of these two forms, as well as other members of Hv-complex, has been analysed from various points of view, e.g., morphology (Watanabe and Sakagami 1948; Eharà 1952, 1953; Katakura 1974b), food habits (Watanabe and Sakagami op. cit.; Kurosawa 1953, 1954a, b; Sakagami and Yamaguchi 1954; Watanabe and Suzuki 1965; Hinomizu 1976), cytology (Yosida 1948; Takenouchi 1955), etc. However, comparative studies on the biology of V-II and P-III have rather been ignored except for some experimental studies of food habits cited above. In the present paper, I will describe and compare only the general trend in life cycles of V-II and P-III in the vicinity of Sapporo, based upon periodic field observations made since 1971.

Before going further, I wish to express my sincere gratitude to Dr. Sh. F. Sakagami for his constant advices and encouragements throughout the present study and critical reading of the manuscript. Cordial thanks are also due to Dr. H. Hasegawa, Hokkaido National Agricultural Experiment Station, who gave me many facilities to the surveys in the experimental station at Tsukisamu, and to Mr. K. Nakamura, Entomological Laboratory, Kyoto University, who kindly allowed me to cite his unpublished data.

#### Areas Surveyed and Methods

In and near Sapporo, P-III mainly depends upon a thistle species, *Cirsium kamtschaticum* Ledeb. (Compositae). *Caulophyllum robustum* Maxim. (Berberidaceae) and another thistle species, *Cephalonoplos setosum* (Bieb.) Kitam., are

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1) Contributions to the knowledge of *Henosepilachna vigintioctomaculata* complex. V.

2) Usage of these scientific names and abbreviations follows Katakura (1974a, b).

*Jour. Fac. Sci. Hokkaido Univ. Ser. VI, Zool. 20 (3), 1976.*

also eaten when they grow together with *C. kamtschaticum*. On the other hand, the host plant of V-II is virtually restricted to potato (*Solanum tuberosum* L., Solanaceae) in rural areas. In forests, V-II depends upon *Schizopepon bryoniaefolius* Maxim. (Cucurbitaceae) which was recently reported as one of its native host plants (Katakura 1975). In order to confirm the seasonal trend of life cycles, the following surveys were performed from May to October, 1975, with some populations of P-III and V-II. The localities concerned, all at relatively low altitudes, are mapped in Fig. 1.

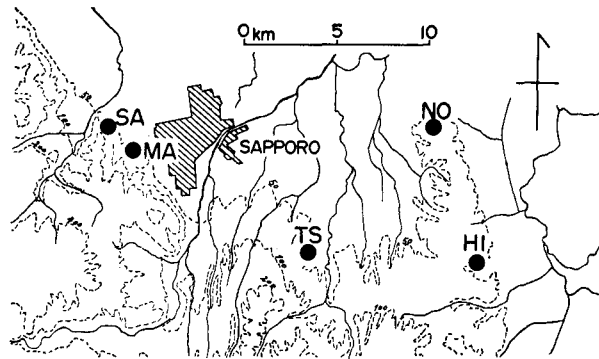


Fig. 1. Areas surveyed. SA: Sankakuyama, MA: Maruyama, TS: Tsukisamu, NO: Nopporo, HI: Hiroshima.

*Weekly census of adults and egg masses:* The weekly census was undertaken for A) P-III in a secondary broad leaved forest in Tsukisamu, B) V-II in a small household vegetable garden at Tsukisamu, and C) V-II in a primary broad leaved forest reserved at Maruyama, with the procedures explained below:

A) P-III at Tsukisamu: An area of ca.  $30 \times 40$  m with more than 200 shoots of *C. kamtschaticum*, ca. 120 shoots of *C. setosum* and 100 shoots of *C. robustum* was selected in the forest. On each census day all host plants were carefully examined and the number of adults were recorded. The egg masses were counted for about the half of *C. kamtschaticum* and every shoot of *C. robustum* and *C. setosum*.

B) V-II at Tsukisamu: The survey field, being about 300 m apart from A and adjacent to a part of the same forest, contained 264 shoots of potato. Adults and egg masses on every potato plant were counted on each census day. Other plants in the field, such as egg plants (120 shoots), tomatoes (58 shoots), pumpkins (3 shoots), cucumbers (72 shoots), etc., were also examined especially in the earlier and later seasons, when potatoes were yet or already not available.

C) V-II at Maruyama: Three subareas were chosen in an area where *S. bryoniaefolius* grew abundantly. A 30 minutes census was performed for both adults and egg masses on the plants in each subarea (in total 90 min. per day).

The survey was accompanied with adult census of another phytophagous ladybird, *Epilachna admirabilis* Crotch, the result of which is reported elsewhere (Katakura 1976). In addition, adults and egg masses of V-II alone were censused at a small patch of *S. bryoniaefolius* (Patch A) outside those subareas. Another small patch (Patch B) discovered on June 19 was subsequently added to the surveying schedule.

In order to avoid duplicated count, egg masses were marked when discovered throughout the census. Newly discovered adults received different color marks with quick drying inks or lucker paints according to census dates.

*Examination of ovarian development:* Ten several or less adult females were periodically sampled at D) P-III feeding upon *C. kamtschaticum* in a mixed forest at Nopporo, E) P-III upon *C. kamtschaticum* and *C. setosum* in a secondary forest at Sankakuyama, and F) V-II in several fields at Hiroshima. Ovarian development of each individual was examined and classified by Kurihara's system as follows (for further description on ovarian development, see Maki *et al.* 1964 or Kurihara 1967):

Stadium 1: No oocyte in vitellarium; Stadium 2: One or two oocytes in vitellarium; Stadium 3: Three oocytes in vitellarium, the undermost one yet without chorion; Stadium 4: Three oocytes in vitellarium, the undermost one with chorion, or sometimes falls into calyx; Degenerated ovaries: Oocytes in degeneration or already degenerated.

The following descriptions are mainly based upon these periodical surveys, but some other results obtained during 1971-1975 in the vicinity of Sapporo are also incorporated when necessary.

### Phenology of V-II and P-III

1. *Life cycle of V-II.* The results obtained by periodical surveys are summarized in Figs. 2 and 3. P-III simultaneously observed in the census at Tsukisamu and Maruyama, being included in these figures, is referred to the next section.

1.1. *V-II in field conditions:* Phenology of V-II in field conditions is given in Fig. 2 by seasonal change of the number of adults and egg masses censused at Tsukisamu and of ovarian development in Hiroshima. The number of egg masses counted is probably underestimated. Because eggs are mainly laid on the lower leaves of potato but such lower leaves were artificially covered with soil twice (June 19 and 25) by the owner of the field in order to keep the stems stable. Further, some egg masses of P-III might be involved. After the time when nearly all shoots of potato withered the adults found on potato and those on other plants are shown separately in the figure. In earlier seasons such adults were so negligible (1♀, May 29; 2♂♂1♀, June 6; 1♂, June 12) that were not separately mentioned in the figure. Probably these autumn individuals involved the immigrants from other potato fields adjacent to the surveyed field. Therefore, Fig. 2-B does not

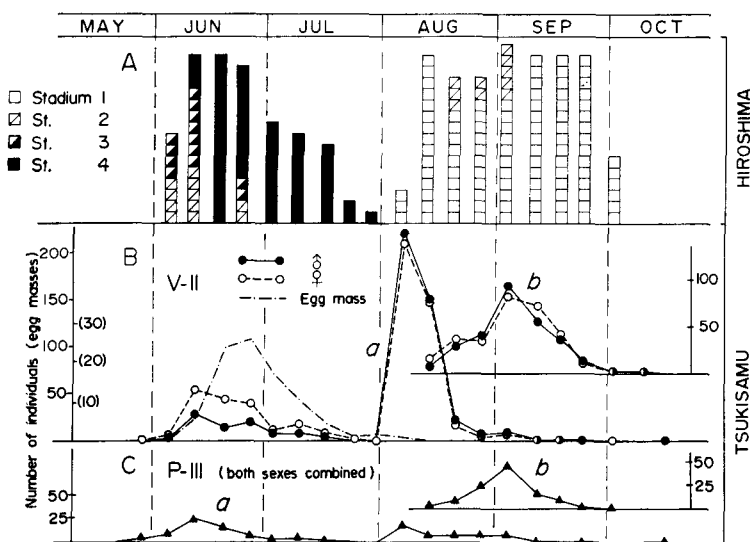


Fig. 2. Phenology of V-II in field condition shown by ovarian change at Hiroshima (A) and seasonal fluctuation of adults and egg masses at Tsukisamu (B), together with seasonal fluctuation of adult P-III simultaneously observed at Tsukisamu (C). In B and C, *a* shows the individuals counted on potato and *b*, those on other plants.

show the seasonal trend of the number of adults and egg masses of V-II born in the field surveyed.

In Tsukisamu, the first post hibernating adult was observed on May 29 on a leaf of elder. At this time, potato was just at sprouting and no V-II could be observed on it. A few adults were observed on potato on June 6. The number of post hibernating adults attained the maximum in the next week (June 12). Thereafter, they gradually decreased and nearly disappeared in late July, though the marking experiment revealed the presence of a few individuals in early or mid August (the final observation of post hibernating adults: August 14, one female marked on June 27). Oviposition took place mainly from mid June to early July. The larvae appeared from late June. The first observations of each instar larva and pupa are as follows: June 20 (1st instar), June 27 (2nd instar), July 3 (3rd), July 10 (4th) and July 25 (pupa). An explosive and synchronous emergence of new adults took place in early August. Since most potato plants were withered up until mid August, the new adults dispersed to other crop plants. The number of individuals counted on potato suddenly decreased in mid August, while that on other plants gradually increased from mid August to early September. Further, most adults censused on August 14 were observed at small crevices at soil surface near the roots of potato forming small aggregations. Such facts suggest the aestivation of some new adults of V-II, as is known in one of the southernmost

populations of *H. vigintioctomaculata* in Japan (Iwao 1970). New adults disappeared in late September.

Ovarian examination revealed the following facts: 1) Ovarian development began soon after the arrival of post hibernating adults of V-II at potato. 2) Mature ovaries (Stadium 4) were observed between mid June to late July. 3) No individual having degenerated ovaries was detected. 4) Ovaries of some new adults slightly developed in late August or early September, but probably degenerated before they enter hibernation.<sup>1)</sup>

Above mentioned seasonal trend is more or less common in the field populations of V-II in and near Sapporo (cf. Kurosawa 1960). Some additional comments are given on the appearance of post hibernating adults. In the vicinity of Sapporo, a few post hibernating adults are generally discovered in late May, or rarely even in mid May on the leaves of trees and herbs growing around fields. In early June when potato plants grew to about 5 cm high or a little more, they enter the fields and settle there. On calm days in early June, one can find post hibernating adults of V-II flying from the surrounding forests to potato fields. An example is given in Table 1 based upon a periodical survey made at Sankakuyama in 1972. The number of V-II observed in the forest was very small, but seemingly increased toward the end of May. At the beginning of June when the potato of the field grew about 10 cm high, a number of V-II were discovered on weeds and elders around the field, mostly at the boundary between the forest and the field. Later, I could find only one individual of V-II in the forest and boundary from mid June to early August. Some individuals were again observed at the boundary on August 17-23, and one individual was discovered in the forest on August 23 and

Table 1. Number of individuals of V-II counted at Sankakuyama in 1972 along a path penetrating the forest adjacent to a potato field and at the boundary between the field and the forest. No individuals found both at the path and boundary on April 25, 28; May 6, 8, 10; June 29; July 2, 4, 6, 21, 26, 31; August 5. No observations made on June 5 at the path. Individuals discovered at boundary were collected, but those in forest not.

Date	Forest	Boundary*	Date	Forest	Boundary*
May 13	1	0	Aug. 17	0	6
23	3	0	22	0	8
26	4	0	23	1	14
29	3	0	Sep. 2	1	0
Jun. 2	2	24	12	0	0
5	-	9			
6	0	2			
24	1	0			

\* Some individuals observed at the margin of the field other than boundary are included.

1) Ovaries degenerated at Stadium 2 could hardly be separable from Stadium 1.

September 2, respectively. In this case, most post hibernating adults appeared in early June, though a few individuals were exceptionally already observed from mid May. Individuals observed at the boundary in late August is probably on the way of dispersion from potato to other plants due to the withering of the former.

**1.2. V-II in forests:** Unfortunately the population density of V-II in Maruyama was very low. Further, a remarkable difference was observed among the results of the censuses at Patches A and B and of the 90 minutes census by unknown reasons (Fig. 3). However, the combination of these censuses is regarded tentatively as showing trend general to V-II in forests or on *S. bryoniaefolius*. The similar seasonal change, though less clear, was observed at Patch A during May to October in 1974. Fragmentary observations in other areas (cf. Katakura 1975) also suggest a similar cycle. *S. bryoniaefolius* sprouted in mid May at Maruyama, but no V-II could be found until May 28. Post hibernating adults reached the maximum level in early or mid June. They rather rapidly decreased in mid July though some were confirmed at least to early September (final observation: September 3, one female marked on June 11). Oviposition took place from mid June to mid July. New adults mainly appeared between late August and mid September and entered hibernation in late September or early October. Although the seasonal sequence of each immature stage could not clearly be detected because of the low density, miscellaneous observations of the final instar larvae and pupae support the emergence of new adults in late summer to autumn.

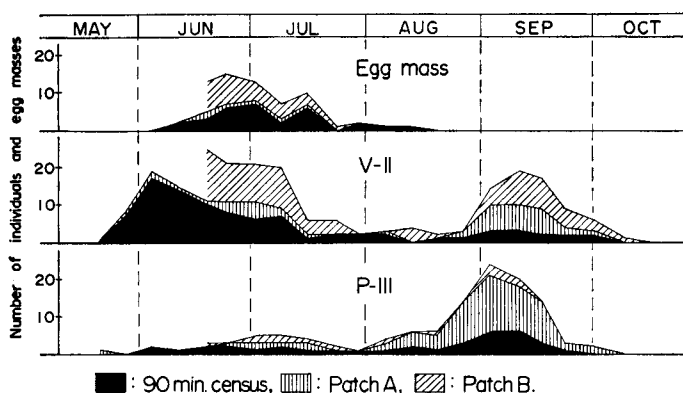


Fig. 3. Phenology of V-II and P-III on *S. bryoniaefolius* at Maruyama. Both sexes combined. Further explanations in text.

## 2. Life cycle of P-III.

**2.1. P-III on the host plants in forests:** Seasonal fluctuation of the number of adults and egg masses at Tsukisamu are presented in Fig. 4, together with

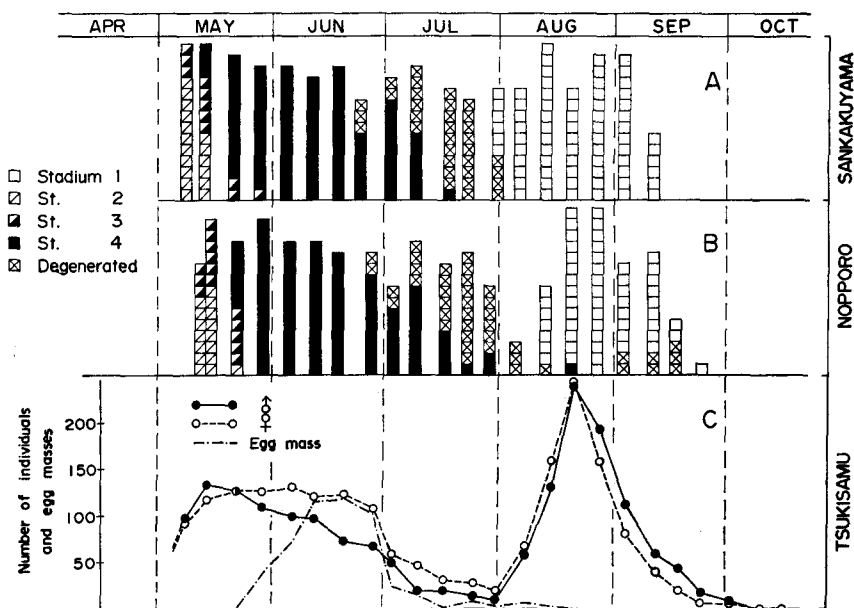


Fig. 4. Phenology of P-III in forests by ovarian change at Sankakuyama (A) and Nopporo (B) and seasonal fluctuation of adults and egg masses at Tsukisamu (C).

ovarian change in Nopporo and Sankakuyama populations. Numerous post hibernating adults were observed at the first census (May 8) at Tsukisamu. Since only a few adults were found at the preliminary observation made on May 3, it is evident that most post hibernating adults arrived at the host plants in early May. The number of individuals reached the maximum in mid May and gradually decreased from mid May to late July in males. On the other hand, females kept a high level during early May to late June and suddenly decreased in July. Anyway, most post hibernating adults disappeared in late July. Oviposition took place mainly from late May to the end of June, but fractionally also in July to early August. The larvae appeared from early June. The first observations of each larval instar and pupa are: June 12 (1st instar), June 28 (2nd instar), July 3 (3rd), July 10 (4th) and July 24 (pupa). Newly emerged adults soon after the last moult from pupae have soft and pale elytra. Such adults were first observed on July 31 at Tsukisamu. The presence of pupal exuviae is another evidence of the emergence of the adults of the new generation. Seasonal fluctuation of new adults showed unimodal pattern with a peak on August 20. New adults virtually disappeared in late September.

Ovarian development of Nopporo and Sankakuyama populations basically well coincided with the oviposition curve at Tsukisamu. Ovaries were still not matured in early May. Mature ovaries were observed from May 22 at Nopporo and



from May 13 at Sankakuyama. Nearly all individuals examined of both populations had mature ovaries at the end of May. Individuals having degenerated ovaries appeared in late June and increased in July. Individuals with entirely immature ovaries (Stadium 1), apparently belonging to the new generation, were observed from July 31 at Sankakuyama and from August 15 at Nopporo. As far as my observations go, new adults enter hibernation without any change of ovaries. Interestingly, some individuals having degenerated ovaries were observed even in mid September at Nopporo, though not detected at Sankakuyama. The fat bodies of these individuals developed well like other individuals of the new generation. The survival of some post hibernating adults in early September was also confirmed at Tsukisamu by the recapture of marked individuals (final observation: September 18, one female marked on July 17). Such facts suggest a possible second hibernation by some adults of P-III, which is known in *H. vigintioctomaculata* in the Eastern Siberia (Ivanova 1962) and *H. pustulosa* at Asiu in Kyoto (Nakamura, pers. comm.). As given in Fig. 4, some local differences were noted on the phenology. The first appearance of post hibernating adults and beginning of oviposition were slightly earlier at Sankakuyama than at Tsukisamu and Nopporo. Emergence of new adults was earlier in the order of Sankakuyama > Tsukisamu > Nopporo. These differences are possibly due to different topographical and thermal conditions of habitats, such as either faced to south or north, and either exposed or shaded.

**2.2. P-III in fields and on *S. bryoniaefolius*:** Some P-III were simultaneously observed with V-II at Maruyama and at the field of Tsukisamu (cf. 1). The behavior of these P-III on the host plants of V-II is briefly mentioned below. In and near Sapporo, *C. kamschaticum*, the most important host plant of P-III, generally withered up in mid August. After withering, new adults of P-III feed mainly upon rosettes of the plant (cf. Ehara 1952). But the rosette does not always develop in every shoot. In particular case, rosettes of a given area were virtually unavailable for P-III. Therefore, P-III suffers a food shortage in autumn. Under such situation, some adults are often discovered on various plants growing in and around the forest where P-III lives. The seasonal distributions of P-III on *S. bryoniaefolius* (Fig. 3) and on the plants other than potato (Fig. 2-Cb) seem to be produced by such immigration of new adults. In both cases, P-III concentrated on such plants in autumn while very scarce in earlier seasons (less than five individuals at every census from late May to early August at Maruyama; 1♂2♀♀ on May 29, 1♀ on June 12 and 1♂ on June 20 at Tsukisamu). On the other hand, the seasonal distribution of P-III in potato field of Tsukisamu (Fig. 2-Ca) showing a bimodal pattern with peaks on mid June and early August suggests breeding of some P-III in the potato field. Probably the egg masses counted in the field involved some of P-III, of which oviposition period covered the first peak. Such invasion of some P-III into potato fields is often observed (cf. also Kurosawa 1953, Sakagami and Yamaguchi 1954), but is restricted to the fields adjacent to the forests inhabited by P-III.

The reversed case, coexistence of V-II and P-III on *C. kamtschaticum* or other host plants of P-III, were not observed in the census of P-III at Tsukisamu except for the following two cases: One male of V-II on a leaf of a coltsfoot beside *C. setosum* on June 12 and another male on *C. setosum* on August 28. In both cases, V-II rested on the leaves, but did not feed upon them.

### Discussions

The seasonal life cycle trend of V-II in fields, V-II in forests and P-III given above are schematically summarized in Fig. 5. V-II and P-III have basically the same annual cycle, both spend one generation per year and hibernate by adult stage. Some differences, recognized between the field and forest populations of V-II, and between V-II and P-III are discussed below, together with a brief consideration on the isolating mechanisms between V-II and P-III.

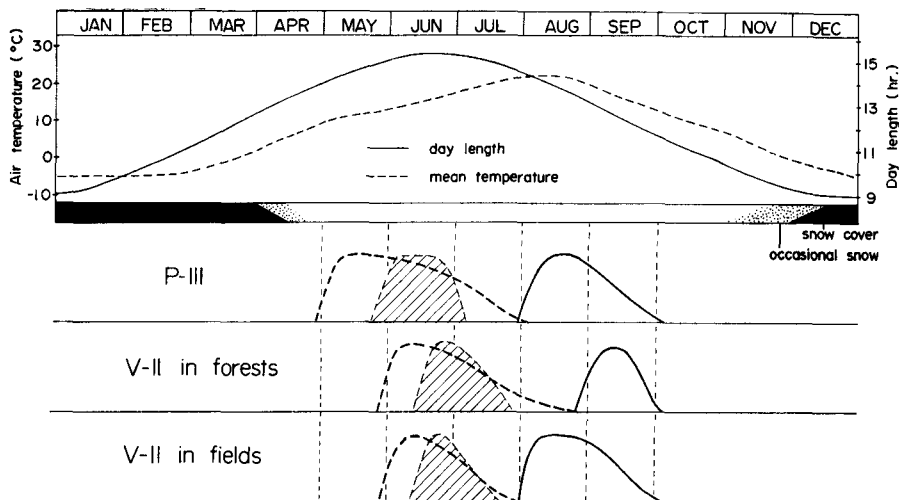


Fig. 5. Schematic life cycle trends of P-III and V-II in forests and V-II in fields, together with the change of day length and mean temperature trend of 30 years from 1941 to 1970 at Sapporo. Thick broken line: Post hibernating adults. Thick line: New adults. Thin broken line and hatching: Oviposition period.

I. *Comparison of life cycle between field and forest populations of V-II.* The active season, i.e., the period from the appearance of post hibernating adults to the disappearance of new adults, of the field and forest populations is similar with each other, both approximately from the end of May to late September. Differences between the two are summarized and discussed as follows:

A) Decrease of post hibernating adults of the forest population is rather gradually from mid June to mid August while that of the field population

Table 2. Number of individuals of P-III and V-II infested by a tachinid, *Medina* sp., observed in the course of ovarian examination. A: Total number examined. B: Number of individuals infested by *Medina* sp. \*: Individuals infested by a nematomorphan.

Date collected	P-III				V-II	
	Nopporo (forest)		Sankakuyama (forest margin)		Hiroshima (open field)	
	A	B	A	B	A	B
May 8-12	10		15	1	-	
13-14	14		14		-	
21-22	12		13		-	
28-29	14		12		-	
Jun. 4-5	12		12		8	
11-12	12		11		15	
18	11		12		15	
24-27	11		11	2	14	
Jul. 2-3	9	1	12	1	15	5+1*
9	12		12		13	5
17-18	10		10		9	2
23	11		9		2	
29-31	8		10		1	
Aug. 5-6	3		15	5	3	
13	8		15	1	15	
20	15		15	5	15	2
27	15		15	2	15	2
Sep. 3	11	1	14	1	16	
11	11		10	3+1*	15	
17-18	5		3	3	15	
24	1		-		15	
Oct. 2	-		-		7	1
Total (% infested)	214	2(0.9)	240	24(10.0)+1*	208	17(8.2)+1*

relatively rapid from mid June to late July. Consequently, the oviposition period and emergence of new adults in the forest population is slightly longer than the field population, though the difference is rather quantitative. Post hibernating adults are still observed at least in mid August in field conditions. Since degenerated ovaries was not detected in the field population of V-II while frequently observed in P-III in forests, elimination of sexually matured adults of V-II by certain factors in field conditions is assumed. Although no precise analysis was made on the mortality factors of V-II and P-III adults, the role of a tachinid fly (*Medina* sp.) may be important in the present case. The number of individuals of P-III and V-II infested by the fly, observed in the course of the ovarian examination, is given in Table 2, though such individuals are omitted in Figs. 2 and 4. The field population of V-II was attacked by the fly especially in July. Sankakuyama population of P-III was also attacked by the fly, but the

parasitism mostly restricted to new adults and in late summer and autumn. Nopporo population of P-III was rarely attacked by the fly throughout the active period. The period of a high ratio of parasitism in V-II coincided well with the period of rapid decrease of the post hibernating adults of V-II. The different ratio of infestation between Nopporo and Sankakuyama populations of P-III may be also partly responsible for the scarcity of individuals having degenerated ovaries in Sankakuyama population during late summer and autumn (cf. 2.1). This table also suggests that parasitism of the fly is frequent in open fields and forest margins while scarce amid forests. If this assumption is valid, the forest population of V-II feeding on *S. bryoniaefolius* may show a low ratio of parasitism as P-III in Nopporo.

B) New adults of the forest population emerged from late August to mid September while those of the field population did in early August. In other words, larval period of the field population is shorter than that of the forest population. Three explanations are possible. Namely, the difference of larval period is brought by: i) different thermal condition of the habitats, ii) different host plants of the populations and iii) adaptation of V-II to the life cycle of potato, i.e., the elimination of individuals still in larval stage at withering or cropping of the potato<sup>1)</sup>. Among them, the effect of thermal condition is most plausible. Thermal condition seems considerably different between the two habitats. Temperature on or near forest floor is generally lower than in field at the same time and place (cf. Yoshino 1961). Further, as forests are mainly composed of deciduous broad leaved trees in the vicinity of Sapporo, the difference of thermal condition must increase from spring to summer by the growing foliage. Since larval period of insects is generally shortened by higher thermal conditions, the presumed difference of thermal condition may force to shorten the larval period of V-II in field conditions. To test two other factors, namely the shorter larval period of V-II feeding on potato than on *S. bryoniaefolius* and the shorter larval period of the field population by the adaptation to the life cycle of potato, the larvae of both field and forest populations of V-II were reared either with potato or *S. bryoniaefolius*. Each egg mass was divided into two groups and each half was reared with potato or *S. bryoniaefolius* in laboratory. Although the results given in Table 3 are still of preliminary nature, no marked difference of larval period is observed between field and forest populations and between the individuals reared with potato and those reared with *S. bryoniaefolius*. The differential survival in some lots (P-1, P-2, S-3) also cannot be explained by the afore mentioned assumption. These cases possibly suggest some technical failures in experiment.

## II. Comparison of seasonal life cycle trend between V-II and P-III. The

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1) As previously reported (Katakura 1975), so far discovered populations of V-II feeding on *S. bryoniaefolius* in forests are fairly isolated from the nearest field populations. Therefore, gene exchange between the field and forest populations seems rare. Further, one of the forest populations (Maruyama) has been continued for twenty years.

Table 3. Rearing experiment of the field and forest populations of V-II with two different host plants.

Egg mass No. <sup>1)</sup>	Reared with <sup>2)</sup>	No. of individuals tested	No. of individuals reached each stage <sup>3)</sup>						Hatched on	Larval period <sup>4)</sup>
			I	II	III	IV	P	A		
P-1	P	20	20	20	20	20	16	16	June 25	29-31
	S	20	20	20	20	11	8	30-34		
P-2	P	13	13	13	13	12	11	8	June 23	30-33
	S	13	13	13	13	12	12	28-30		
S-1	P	8	8	8	8	7	7	7	June 28	30-32
	S	7	7	7	7	7	7	31		
S-2	P	12	12	12	12	11	11	11	July 1	28-29
	S	13	13	12	12	12	11	11		28-29
S-3	P	14	14	14	14	7	7	7	July 3	28
	S	13	13	13	13	12	12	27-29		

1) P: Collected from potato at Hiroshima. S: from *S. bryoniaefolius* at Maruyama.

2) P and S: Reared with potato and *S. bryoniaefolius*, respectively.

3) I-IV: 1st to 4th instar larvae. P: Pupae. A: Adults.

4) Days from hatching to emergence of adults.

most remarkable difference is observed on the active period of P-III and V-II. The active period of P-III ranged from the beginning of May to the end of September while that of V-II from the end of May to the end of September. The end of the period approximately coincides but the beginning is delayed in V-II about four weeks than in P-III. Some other differences observed, such as the oviposition period and emergence of new adults, are probably caused by the difference in the appearance of post hibernating adults. The difference in the oviposition period was also noticed by Kurosawa (1953). The difference in the time of appearance of post hibernating adults is probably specific to V-II and P-III and regarded as results of adaptations of V-II and P-III to respective host plants. As mentioned earlier, *C. kamtschaticum*, the main host plant of P-III, sprouts in mid or late April but tends to become insufficient after mid August by withering. Therefore, completion of larval growth until early August is essential for P-III. On the other hand, *S. bryoniaefolius*, being considered as one of the native host plants of V-II, sprouts in mid May. It provides sufficient food and shelter for V-II throughout the active period. The life cycle of V-II is quite adaptive from such point of view and the appearance of post hibernating adults of V-II in earlier season like P-III is vain.

Life cycle differences, such as observed between V-II and P-III, may also be discovered between other sympatric forms of *H. vigintioctomaculata* and *H. pustulosa*. The situation is more complicated when so called 'Towada form' (feeder of *Caulophyllum robustum*) is taken into account. The 'Towada form',

hitherto discovered from several localities in Honshu and from the southernmost part of Hokkaido, is sympatric with both *H. vigintioctomaculata* and *H. pustulosa* at least at the northernmost Honshu (Towada: Yasutomi 1974) and the southernmost Hokkaido (Katakura, unpubl.). Further comparative studies on the phenology of these sympatric forms are necessary for the thorough understanding of the biology of Hv-complex.

III. *A consideration on the isolating mechanisms between V-II and P-III.* As mentioned above, P-III and V-II are sympatric, being separated on respective host plants and maintain the life cycle difference. Although taxonomic status of V-II and P-III must be clarified through comparisons with related allopatric forms, they indubitably represent distinct biological entities. There must be some isolating mechanisms between P-III and V-II. Takenouchi (1955), who studied meiotic process of hybrids between several local populations of *H. pustulosa* and those of *H. vigintioctomaculata*, found no cytological abnormality in the hybrids between male P-III and female V-II. Further, Yasutomi (1966) reported that V-II and P-II (Typical form of *H. pustulosa* distributed in the central part of Hokkaido) successfully produced fertile  $F_1$  hybrids under laboratory conditions. P-III and V-II probably produce, at least partly, fertile hybrids, though further precise investigation is needed especially as to the survival and characteristics of hybrids. The life cycle difference between P-III and V-II may decrease the chance of the hybridization, but is not so enough to separate them completely. Previous information clearly shows that V-II could not complete its growth with *C. kamschaticum*, the main host plant of P-III (Kurosawa 1953, Sakagami and Yamaguchi 1954, Watanabe and Suzuki 1965, Hinomizu 1976). On the other hand, the preference of P-III for *S. bryoniaefolius*, the native host plant of V-II, is hitherto not studied except by Kurosawa (1953). According to him, 80% of first instar larvae of P-III could become adults with *C. kamschaticum* while 40% with *S. bryoniaefolius*. Further, the first instar larvae of P-III put on fresh leaves of *C. kamschaticum* immediately fed upon the leaves, but those put on fresh leaves of *S. bryoniaefolius* once removed from the leaves. They walked around for a while as if searching for their 'legitimate food', then they 'returned' the leaves and started the feeding (Katakura, unpubl.). Judging from these facts, probably P-III cannot complete its growth with *S. bryoniaefolius* under natural condition though the adults can feed on it. The result in Fig. 3 seems to support this assumption. Therefore, it is probable that V-II and P-III are separated with each other by the host plant specificity, as far as *C. kamschaticum* and *S. bryoniaefolius* are considered as host plant of P-III and V-II, respectively.

On the other hand, all so far published studies revealed a high acceptability for potato not only by V-II but also by P-III (Watanabe and Sakagami 1948, Sakagami and Yamaguchi 1954, Watanabe and Suzuki 1965, Hinomizu 1976). Given potato under laboratory conditions, both P-III and V-II can complete the growth. Therefore, isolation by the host plants is not supported as far as laboratory experiments show. Nevertheless, only V-II does actually succeed to

enter potato fields and settle there. The density of P-III observed in potato fields is very low, being restricted to the fields adjacent to forests where they live. Some factors must inhibit the entrance of P-III into potato fields. Relating to this point, Sakagami and Yamaguchi (1954) stressed the importance of the stability of the habitat of P-III. They mentioned, "The thistle provides continuously from spring until hibernation the suitable food and shelter for P-III in contrast with the potato, which is usually cropped out at the mid summer. Moreover, the population density of P-III at their habitat hitherto recorded is seemingly not so enough high to necessitate the migration into other places. Therefore, the mere conservatism to the isolated suitable habitats may act considerably, if not completely, as an inhibiting factor for the dispersion of adults into the potato fields." Their assumption is rejected by the presence of V-II feeding upon *S. bryoniaefolius* in forest. *S. bryoniaefolius* provides food and shelter sufficiently for V-II throughout the active period. Further, as far as my observations go, the population density of V-II on *S. bryoniaefolius* is generally lower than even that of P-III on thistles, though the density in Maruyama, reported in the present paper, seems exceptionally low. Moreover, as mentioned above, the thistle (*C. kamtschaticum*) tends to become insufficient to maintain a high density of P-III in autumn. Probably the difference between the active period of V-II and P-III is of considerable importance for an alternative explanation. Post hibernating adults of P-III appear in early May. They find *C. kamtschaticum* and other host plants already grew to some degree and settle on them. Potato sprouts in early June, about one month after the appearance of P-III. At this time the host plants of P-III are still abundant and, therefore, migration of P-III to the potato may rarely be occurred. Hinomizu (1976) reported that post hibernating adults of P-III collected from *C. kamtschaticum* showed a higher preference for thistle than potato. Conditioning of P-III to *C. kamtschaticum* in spring season is also not excluded. If some P-III could complete their growth with potato, they should meet the same situation in the next spring. On the other hand, there is no discordance between the appearance of post hibernating adults of V-II and sprouting of potato. V-II appeared in early June can discover immediately, or only after a short delay, the sufficient food, i.e. potato as well as *S. bryoniaefolius*.

Naturally the above mentioned speculation cannot explain the isolating mechanisms completely. As was reported in the present paper, some adults of P-III are found with V-II on both potato and *S. bryoniaefolius*. There are chances of hybridization. Partial introgression may occur especially in potato fields. In the course of the present study, I actually observed two cases of copulation between P-III and V-II, one on July 10 (V-II ♂ × P-III ♀) on potato at Tsukisamu and another on July 16 (V-II ♂ × P-III ♀) on *S. bryoniaefolius* at Maruyama. These individuals were collected and reared but unfortunately both females died off without oviposition. Final solution of these problems is remained in the future.

### Summary

1. Based upon field observations made in the vicinity of Sapporo, life cycle of two closely allied sympatric ladybirds, Hokkaido form of *Henosepilachna vigintioctomaculata* (V-II) and Sapporo form of *H. pustulosa* (P-III) are described.

2. The active period, the period from the appearance of post hibernating adults to the disappearance of new adults, of V-II ranges from the end of May to the end of September. On the other hand, the active period of P-III ranges from the beginning of May to the end of September. Such life cycle difference is considered as specific to each form.

3. The emergence of new adults of V-II takes place in early August in the field population (feeding on potato), while from late August to mid September in the forest population (on *S. bryoniaefolius*). This difference is presumably caused by different environmental condition of the habitats.

4. Prereproductive isolating mechanisms between P-III and V-II are briefly discussed. In forest, P-III and V-II may be separated by the host plant specificity, namely P-III preferring for *C. kamtschaticum* and V-II for *S. bryoniaefolius*, while in field (on potato), a time lag in the appearance of post hibernating adults may be responsible, at least partly, to segregate P-III and V-II.

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- 1) Single asterisk: Papers written in Japanese.
  - 2) Double asterisk: Ditto, with English summary.
  - 3) †: Paper not directly accessible.
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