Title	Variation Analysis of Elytral Maculation in Henosepilachna vigintioctomaculata Complex (Coleoptera, Coccinellidae) (With 6 Text-figures and 4 Tables)
Author(s)	KATAKURA, Haruo
Citation	北海道大學理學部紀要, 19(2), 445-455
Issue Date	1974-04
Doc URL	http://hdl.handle.net/2115/27570
Туре	bulletin (article)
File Information	19(2)_P445-455.pdf



## Variation Analysis of Elytral Maculation in *Henosepilachna* vigintioctomaculata Complex (Coleoptera, Coccinellidae)<sup>1)</sup>

By

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(With 6 Text-figures and 4 Tables)

Two closely related sympatric species of phytophagous lady beetles,  $Henosepilachna\ vigintioctomaculata\ (Motschulsky)\ and\ H.\ pustulosa\ (Kôno)\ (henceforth abbreviated respectively as <math>Hv$  and Hp), are found in Japan, distributed mainly in the central to northern regions. The distinction between two species is clear in typical forms by external morphology, especially leg coloration, and food plants in natural conditions, the former preferring the potato while the latter mainly thistles. In addition to them, however, there is another puzzling form called Western Tokyo form (abbreviated Ht). This form is morphologically similar to Hp while resembles Hv in food plants and is distributed in the southern Kantô district, where both Hv and Hp have not been recorded. Moreover, both Hv and Hp, especially the latter, show a conspicuous differentiation in some external features as dealt with in a coming paper. Thus, these beetles,  $Henosepilachna\ vigintioctomaculata\ complex\ (=Hv-complex)$ , offer a complicated but interesting problem from the standpoint of speciation.

Since 1971 I have participated in comparative studies of Hv-complex in Hokkaido, where the local differentiation of Hp is most conspicuous both in morphology and biology. In the course of these studies some differences were noticed between Hv and Hp as to the appearance of anomalies in elytral maculation. The present paper deals with these anomalies in Hv-complex, compiling new items of information on Hp and Hv obtained by myself and those by previous authors on Hv and Ht.

The nomenclature of these beetles is still confused. Some authors, Yasutomi (1954, 1966) and Tsuneki and Kubo (1960), regarded Hv and Hp as subspecies of the same species. Li and Cook (1961) considered them as those showing mere intraspecific variation, even not worthy to be ranked subspecifically. Further, Chûjô (1968) suggested that the type series of

<sup>1)</sup> Contributions to the knowledge of Henosepilachna vigintioctomaculata complex. I. Jour. Fac. Sci. Hokkaido Univ. Ser. VI, Zool. 19 (2), 1973.

Epilachna niponica Lewis (generally accepted as a synonym of Hv) involved some specimens of Ht and Honshu form of Hp. Moreover, according to Yasutomi (personal communication), the types of Lewis included Ohnuma form of Hp and Hokkaido form of Hv. In this and subsequent papers, however, Hp and Hv are treated tentatively each as distinct species and the name 'vigintioctomaculata' is used for Hv, until the final solution would be achieved.

Before going further, I wish to express my sincere gratitude to Dr. Shôichi F. Sakagami, Zoological Institute, Faculty of Science, Hokkaido University, for his pertinent guidance through the present study, and to Prof. Mayumi Yamada, Zoological Institute, Faculty of Science, Hokkaido University, for his reading through the manuscript. Cordial thanks are also due to Dr. Chihisa Watanabe, Entomological Institute, Faculty of Agriculture, Hokkaido University, who kindly offered an opportunity to study the specimens preserved in Entomological Institute, Hokkaido University.

These lady beetles show no extreme polymorphism of elytral pattern such as famous in Harmonia axyridis (Pallas). The variation is thus exhibited mainly by the coalescence and evanescence of elytral maculation. In Europe variation analysis of another Henosepilachna species, H. chrysomelina (Fabricius), was undertaken on the size and location of the spots (Zarapkin 1937), but so far not on the coalescence and evanescence. Variation of the size and location of the spots are also found in Hv-complex (Ikemoto 1955, Kawabe 1947, Koyama 1962, Kurisaki 1932, Tsuneki 1960, Yasutomi 1973), but not yet precisely analyzed. Moreover, the dark specimens of Hp sometimes possess an additional black stripe along the elytral margins, either partly or entirely. Li and Cook (1961) reported

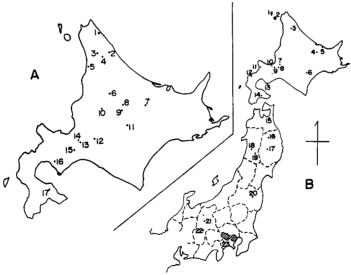


Fig. 1. Provenance of the samples examined. A, H. pustulosa. B, H. vigintioctomaculata and Western Tokyo form. In H. pustulosa and H. vigintioctomaculata, each locality is shown with the code number and in Western Tokyo form the area of provenance is given by hatching.

Table 1. Localities and numbers of specimens examined.

Table 1. Localities	and numbers of speci	mens examine	ed.
Locality (code number)	Collecting date	Number of specimens examined	Reference
H. pustule	osa (Hp, all from Hok	kaido)	
Asajino (1)	16 V 1973	51	Katakura
Utanobori (2)	18 V 1972	57	"
Teshio Nakagawa (3)	10 IX 1971	137	"
KamiOtoineppu (4) I	23 VIII 1961	216	"
$\Pi$	10 IX 1971	300	"
Shosanbetsu (5)	20 V 1973	68	"
Shiokari (6)	17 VII 1971	109	"
Onneyu (7)	4 VIII 1972	45	"
Sôunkyô (8)		40	"
Yukomanbetsu (9)	15 VII 1971	105	"
Kamuikotan (10)	4 VIII 1972	77	"
Tomuraushi (11)	VII 1949	50	"
Tsugitate, near Yûbari (12)	25 V 1972	123	"
Hiroshima, near Sapporo (13)	24 VI 1971	114	"
Nopporo, near Sapporo (14)	1 VI 1971	145	"
Eniwa valley (15)	17 VI 1971	83	"
Nakajima, Lake Tôya (16)	29 VIII 1972	140	,,
Ohnuma (17)	30-31 VIII 1972	150	"
H. v.	igintioc to maculata (Hv)	I	I
Rebun Island (1)	25 VII 1951	101	Katakura
Rishiri Island, Oshidomari (2)	16 VII 1972	109	"
KamiOtoineppu (3)	13-17 VI 1972	117	" "
Kitami (4)	1946	2, 113	Kawabe '47
Abashiri (5)	1946	1, 629	"
Obihiro (6) A	1945	1, 414	",
B	1946	1, 781	"
MinamiHoromui (7)	1946	1,070	",
Hiroshima (8)	24 VI 1971	1,070	Katakura
Sapporo (9)	21 /1 10/1	121	I wookura
A. Experimental farm of			
Hokkaido University, Ia	1945a	1, 393	Kawabe '47
Ib	1945b	3, 022	ILWWOOD II
$\widetilde{\mathbf{Ic}}$	1945c	2, 781	,,,
ĬĬ	1946	3, 401	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,
B. Sôen I	1945	702	, "
II	1946	1,433	"
C. HigashiSapporo	1945		<b>"</b>
D. Yamahana	1945	1,825	",
KamiTeine, near Sapporo (10)	1946	3,487	<i>"</i>
Tomari (11)	1945	297	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,
Harauta, Shimamaki (12)	1945 1 IX 1972	586	Katakura
Muroran (13)	1 IA 1814	102	Zatakura
A. Funamichô	1946	723	Kawabe '47
B. Wanishi	1946	2, 283	Kawabe 41
Nanae, near Hakodate (14)	1945	2, 265 357	,,
Timeso, Hour Tradounio (17)	1010	991	1 "

Table 1. (continued)

-	abic 1. (commuca)				
Locality (code number)	Collecting date	Number of specimens examined	Reference		
Honshu					
Noheji, Aomori Pref. (15)	1945	173	Kawabe '47		
Kozuya, Iwate Pref. (16)	1946	310	"		
Iwate, Iwate Pref. (17)	X 1951	1, 265	Sasaki and Nakakarumai '53		
Kariwano, Akita Pref. (18)	2 VIII 1954	253	Ikemoto '55		
Sakai, Akita Pref. (19)	IX 1952	298	n		
Kôriyama, Fukushima Pref. (20)	IX 1952	187	"		
Seba, Nagano Pref. (21)	31 VII 1954	103	"		
Ina, Nagano Pref. (22) Ia	30 VII 1952	247	"		
Ib	20 VIII 1952	255	"		
II	8 VIII 1953	115	"		
$\mathbf{III}$	28 VII 1954	25	"		
Aone, Kanagawa Pref. (23)	15 VII 1953	111	"		
Manchuria					
Chuhô (24)	1944	202	Kawabe '47		
Wes	stern Tokyo form (Ht)				
Tokyo (1) MusashiKoganei, western suburbs		68, 026	Inoue '52		
of Tokyo (2) I	VII-VIII 1949	977	Ikemoto '55		
ı ` `II	22 VII 1951	47	"		
Fuchû, western suburbs of Tokyo					
(3) I	23 VII 1951	241	"		
II	VI-VII 1952	162	,,		
III	VII-VIII 1953	40	,,		
ĪV	1 VIII 1954	126	,,		

that this anomaly was also found in the dark specimens of Hv from Burma and China. In the present paper, however, these anomalies are omitted and only the coalescence and evanescence of spots are dealt with.

The previous contributions quoted for comparison are of Ikemoto (1953), Inoue (1952), Kawabe (1947) and Sasaki and Nakakarumai (1953). The provenance of each sample and the number of examined specimens cited are given in Table 1, together with those of the samples of Hv and Hp examined by myself, approximately arranged from north to south. The localities cited are mapped in Figure 1, with the code numbers given in Table 1. Concerning Hv, the localities cover the most part of its distribution range. On the other hand, no samples of Hp from Honshu have still been studied and Ht has so far examined only with some populations inhabiting Tokyo and the vicinity. Besides these samples given in the table, Ikemoto examined seven samples from Hokkaido. But his result generally coincides with that given by Kawabe, who precisely analyzed the variation trend based upon the samples from diverse localities of Hokkaido. Further, Ikemoto also examined a sample of Hv from Shiojiri, Nagano Prefecture, Honshu, but the specimens examined are very few and the anomaly tendency is not so much different from that in Seba, near Shiojiri. Therefore, these results were omitted from the consideration. Kurisaki (1932) first studied the variation of elytral spots of Hv based upon the samples

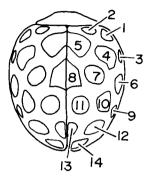


Fig. 2. Elytral maculation of *H. pustulosa*. Each spot is numbered according to Kurisaki's system.

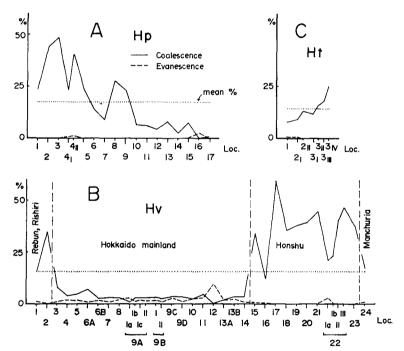


Fig. 3. Frequency distribution of anomalies shown by the percentage ratios of the total number of specimens examined. Localities are arranged approximately from north to south. Mean percentage of anomalies are shown with dotted lines.

from Saghalien, Hokkaido and Honshu. But the variation tendency is not given in each locality separately, so that this pioneer work cannot be used here.

In the following descriptions, each elytral spot is numbered following the system by Kurisaki (Fig. 2). Evanescence is shown with these numerals and coalescence by the combinations.

1. Frequency of total anomalies. There are found three types of anomalies, coalescence, evanescence and their combinations in the same specimen. Aside the combination type, which is very rare in any locality or form, the frequency distributions of other anomalies are shown in Figure 3, coalescence and evanescence given separately, and localities approximately arranged from north to south. Even if more than one anomaly appeared in the same individual, they were not separately counted.

Hp (Fig. 3A): Evanescence is very rare while coalescence is quite frequent with a remarkable local difference. The comparison of Figures 1A and 3A shows a northward increase of coalescence. Concerning the populations in Honshu detailed studies are still absent but in my preliminary study 16 anomalies, 15 cases of coalescence and only one evanescence, were observed among 120 specimens collected from 16 localities. The percentage ratio of coalescence in all specimens combined, 12.5%, is approximately comparable to that in localities 6 or 7, given in Figure 3A, but a considerable fluctuation of frequency seems to occur among localities.

Hv (Fig. 3B): Except Rishiri and Rebun Islands the anomalies are rare in the mainland of Hokkaido, being always less than 10%, but interestingly both coalescence and evanescence appear at most localities. On the other hand, the samples from Rishiri, Rebun, Honshu and Manchuria seldom exhibit evanescence in spite of a quite high frequency of anomalies with a marked local difference.

Ht (Fig. 3C): Evanescence is very rare. The frequency of coalescence is lower than in Hv in Honshu but higher than in Hv in Hokkaido, comparable to that in Hp in localities 5 to 9.

In some localities, samples collected in different years were examined as follows: Hp, 1) KamiOtoineppu (code number Hp-4); Hv, 1) Sapporo (Hv-9) A and B, 2) Ina (Hv-22); Ht, 1) Musashi-Koganei (Ht-2), 2) Fuchû (Ht-3). The year to year difference was remarkable in Hp 1), Hv 2) and Ht 2) while virtually absent in Hv 1) and Ht 1), that is, except Ht, being higher in the localities with higher frequencies of anomalies. Probably the sample from a given locality with higher anomaly frequency would exhibit a larger fluctuation of the frequency.

The specimens examined in the present study involve all morphological forms of Hp so far known from Hokkaido and all forms of Hv hitherto recognized in Japan. Correspondence of form differentiation to the frequency of anomalies does not occur in Hp, but conspicuous in Hv, between two forms, Hokkaido and Honshu forms. A third form recently discovered from Rishiri and Rebun Islands (Katakura 1973) is characterized by the frequency of anomalies higher than in Hokkaido form, rather resembling Honshu form as is obvious from Figure 3.

2. Frequency of particular anomalies. Inoue and Ikemoto reported that most cases of evanescence appeared in spots 13 and 14 in Hv in Hokkaido and spot 7 in Ht. But the frequency of evanescence is so low in most samples that only coalescence is described and discussed here. Each coalescence is counted as one

Table 2. Frequency of predominant types of coalescence of H. pustulosa in each locality shown with the code number.  $t_1$ , total frequency of predominant types in each locality.  $t_2$ , total frequency of each type. T, total number of all types. Nakajima (Loc. 16) is omitted because no coalescence is observed.

Type of	Locality										+							
acalogopae	2	3	41	411	5	6	7	8	9	10	11	12	13	14	15	17	$\mathbf{t_2}$	
13-14	8	25	50	35	93	13	15	3	9	12	3	3	1	9	3	5	0	287
7-11	4	3	20	5	42	0	0	2	4	15	1	0	3	0	0	0	0	99
3-4	0	0	9	13	3	0	0	0	0	0	1	0	0	0	0	0	0	26
12-14	0	1	1	0	1	1	0	1	0	1	0	0	0	0	0	0	0	6
10-12	0	0	0	0	3	1	0	0	0	0	0	0	0	0	0	_1	0	5
t,	12	29	80	53	142	15	15	6	13	28	5	3	4	9	3	6	0	423
Other types combined	0	0	0	1	1	3	0	0	0	1	0	0	3	0	0	1	1	11
T	12	29	80	54	143	18	15	6	13	29	5	3	7	9	3	7	1	434

unit. Therefore, the total frequency of coalescence is higher than the number of specimens examined, because more than one case of coalescence can appear in the same specimen.

Hp: Throughout various localities five types of coalescence are predominant, which are 13-14, 7-11, 3-4, 12-14 and 10-12. Frequency of these types in each locality are shown in Table 2, together with the sum of other types combined. The relative ranks of five predominant anomalies in all specimens combined fairly correspond to the ranks in localities with higher anomaly frequencies. For instance, type 13-14 is top-ranked in 13 localities and second-ranked in two (9 and 12). The second-ranked type 7-11 occupies the same rank in localities, 1, 2, 3, 4-II, 7, 8 and 10, but the top in two localities where 13-14 is the second-ranked. The other three types, 3-4, 12-14 and 10-12, are less frequent than 13-14 and 7-11 in most localities. Therefore, the rank order shown by Table 2, 13-14 $\gg$ 7-11>3-4>12-14 $\rightleftharpoons$ 10-12, is regarded as a trend general to Hp in Hokkaido. Among 16 Hp specimens from Honshu examined by me, 13-14 was also predominant, appearing in 12 specimens, while 7-11, the second-ranked in Hokkaido, was not detected. This suggests that type 13-14 is a coalescence predominantly appearing in Hp.

Hv: According to Kawabe, predominant types of coalescence in various localities are as follows:

Districts (locality, code no.)

Eastern Hokkaido (Abashiri 4, Kitami 5, Obihiro 6) 3-4, 7-11, 11-12, 13-14,

Central Hokkaido (Sapporo 9)

Southern Hokkaido (Tomari 11, Muroran 13)

Southernmost part of Hokkaido (Nanae 14)

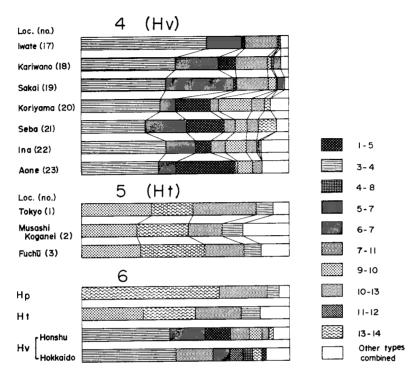
Types in the order of frequency
3-4, 7-11, 11-12, 13-14,
3-4, 6-7, 11-12, 13-14.

Table 3. The relative frequency of predominant types of coalescence in *H. vigintiocto-maculata* in Hokkaido. The result by Kawabe (1947) is given with percentage ratio, and that by the present study in absolute number. (Total number of specimens examined are shown in parentheses). (Harauta, Loc. 12, omitted because no coalescence appeared there).

Type of coalescence	Hokkaido Kawabe	Rebun (101)	Rishiri (109)	KamiOtoineppu (117)	Hiroshima (121)			
comicsconce	(1947)	Katakura (1973)						
3-4	45.0%	14	32	3	2			
7-11	17.8	1	0	1	0			
6-7	8.4	0	6	0	0			
11-12	6.4	0	4	4	1			
4-8	4.6	0	0	0	0			
13-14	4.6	0	0	0	0			
1-5	2.1	1	0	0	0			
Others combined	11.1	1	1	2	0			

Tôhoku, Honshu (Noheji 15, Kozuya 16) Manchuria (Chuhô 24) 6-7, 3-4. 6-7, 3-4.

Type 3-4 is frequent over all localities referred to and top-ranked in six localities, while other types cited exhibit some local differences. The relative frequency of these predominant types cited by Kawabe is given in Table 3. As most of his samples were taken from Hokkaido (samples of Honshu and Manchuria combined only 2.1%), the result could be interpreted as showing the trend general to Hv in Hokkaido. The frequency of each type observed by me in four localities are added to. The sample from KamiOtoineppu (code number 3) shows a slight difference. Kawabe did not examine the samples from northern Hokkaido so that it is possible that the populations in northern Hokkaido show a trend different from that reported by Kawabe. The specimens examined by me are still few in number, requiring further analysis with larger samples. Nevertheless, the sample from Rishiri shows a different tendency: Type 7-11 being frequent in eastern and central Hokkaido does not appear while 6-7 being frequent in southern Hokkaido, Honshu and Manchuria is frequent. Figure 4 presents relative frequencies of eight predominant types in several localities with percentage ratios. As like in Hokkaido, 3-4 is top-ranked in all localities while other types show considerable local differences. Type 6-7 is relatively frequent in most localities but low in Kôriyama (20) and Aone (23). Type 1-5 shows a southward increase, 7-11 an opposite trend, and 9-10 is relatively frequent in Kôriyama and Ina (22). Comparing the results with those in Hokkaido, less frequent types are quite different between two districts, while higher ranked types show similar trends except 1-5 being frequent in Honshu but rare in Hokkaido. Summarizing, type 3-4 is mostly top-ranked while 7-11, 6-7, 1-5 etc. are frequent with local difference.



Figs. 4-6. Percentage ratio of predominant types of coalescence. 4. H. vigintioctomaculata in Honshu. 5. Western Tokyo form. (4 and 5, both calculated from the results by other authors). 6. Four forms compared.

Few detailed studies exist on the populations outside of Japan. With the samples from the Continental Asia, Dieke (1947) reported the presence of one subspecies, Epilachna niponica coalescens Mader (=Henosepilachna vigintioctomaculata coalescens) (originally described as an aberration), in Szechwan Province, China and Tibet. In this subspecies, following spots are in coalescence; 1–5, 3–4, 7–8, 9–10 and 13–14. Further, based upon 177 specimens from diverse localities in Formosa, China Proper, Burma, Japan and Korea, Li and Cook (1961) reported the frequent appearance of coalescence in 1–5, 6–7, 3–4, 9–10, 13–14, though except 6–7, all found from Burma and China Proper. Closer studies with more samples remain for the future.

Ht: The relative frequency of four predominant types of coalescence recorded by Inoue and Ikemoto is given in Figure 5. These are in the descending order, 9-10, 13-14, 7-11 and 3-4. Among them the frequency varies in 9-10, 13-14 and 7-11 while relatively constant in 3-4. Other types are far less frequent.

The relative frequency of the predominant types of coalescence in Hp, Hv and Ht is presented in Figure 6 and Table 4. A clear difference is seen between

Type of coalescence	H. pustulosa	Western Tokyo	H. vigintioctomaculata (Hv)			
	(Hp)	form $(Ht)^{\circ}$	Honshu	Hokkaido		
1-5	0 %	(0.4)	11, 7	2.1		
3-4	6.0	8.9	41. 9	45. 0		
4-8	0	(0.4)	0	4.6		
5-7	0.5	(1, 6)	1.2	0.7		
6-7	0.2	0, 3	17.4	8.4		
7-11	22.8	22. 2	9. 2	17.8		
9-10	0.2	29. 5	6.3	0.8		
10-13	0.2	(0.8)	3, 3	0.1		
11-12	1.2	(0.4)	0	6.4		
12-14	1.4	(0)	0	0		
13-14	66. 1	25. 1	2.0	4.6		

Table 4. Percentage ratio of predominant types of coalescence in various forms, the most frequent types shown in Gothic. In Western Tokyo form, calculated from Ikemoto (1955) only are shown in parentheses.

Hp and Hv, Hp characterized by the predominance of 13-14, being rare in Hv. The opposite case is 3-4 predominant in Hv, both in Honshu and Hokkaido, but rare in Hp. Similar but less conspicuous contrasts are seen in 6-7, 1-5, too. The type relatively frequent in both Hv and Hp is only 7-11. Finally, Ht is intermediate between Hp and Hv by the predominance of types 9-10, being frequent in Hv in Honshu and 13-14 frequent in Hp, and an intermediate trend in 3-4.

10.4

7.0

9.5

1.4

combined

The results presented above is still of preliminary nature. Further analysis must be undertaken with samples taken from the district still unexplored, especially, Hp in Honshu and Ht. Moreover, the mechanisms governing the appearance of these anomalies, either genetic or epigenetic, are still unknown. However, it is noteworthy that the tendency to produce coalescent spots clearly differs between Hp and Hv and seemingly intermediate in Ht, which is also intermediate in other characters.

## Summary

The variation of the elytral maculation in *Henosepilachna vigintioctomaculata* complex was analyzed using the samples collected from diverse localities of Hokkaido and those studied by previous authors. Two types of anomalies, evanescence and coalescence, were observed but the former was so rare that the latter was mainly dealt with. The results are:

1. *H. vigintioctomaculata* from Rishiri and Rebun Islands and Honshu and *H. pustulosa* from Hokkaido is characterized by a higher appearance of anomalies against *H. vigintioctomaculata* in Hokkaido, and Western Tokyo form is intermediate between them.

2. Predominant types of coalescence distinctly differ between *H. vigintiocto-maculata* and *H. pustulosa*, the former is characterized by type 3–4 while the latter by 13–14. Western Tokyo form is again intermediate between *H. vigintiocto-maculata* and *H. pustulosa*.

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