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**A Comparative Study of Spermatheca in Some  
Local Populations in *Epilachna pustulosa* and**

***E. vigintioctomaculata***

**(Systematic Studies of Coccinellidae, II)**

By

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(With 5 Text-figures)

In the previous paper the writer (1952) published a comparative anatomy of the genitalia and internal reproductive organs in three Japanese species of *Epilachna*, with a special reference to their spermatheca of female. At that time, the writer pointed out that the features of spermatheca seem to be valued, to some extent, as interspecific differences among these species, as well as the coloration of legs by Watanabe and Sakagami (1948) and the curvature of elytra by Yasutomi (1952b). With respect to the external characters, there has been reported a remarkable local variability in *Epilachna pustulosa* Kôno, a problematical species, by Watanabe and Sakagami, and Yasutomi (1952b, '52d). The occurrence of such a local variability has puzzled, more and more, the problem whether *E. pustulosa* is an independent species from *E. vigintioctomaculata* or not. Furthermore, as regards the food-habits, it has been confirmed that the adults of *E. pustulosa* are attracted not only to thistles belonging to the Asteraceae, but occasionally also to some plants belonging to the Solanaceae (Inoue, 1952; Yasutomi, 1952b), though the larva feeds specially on the thistles in nature. In the present research the writer concerns with a comparative study of the spermatheca of some local forms of *E. pustulosa*, referring to the organ of *E. vigintioctomaculata*.

Before going further, the writer wishes to express his heartiest thanks to Professor Tohru Uchida for his helpful guidance and for his kindness in review of the manuscript. His gratitude is also due to Dr. C. Watanabe, Mr. S. F. Sakagami, Dr. M. Okada, Mr. K. Yasutomi and Mr. N. Koyama for their various kindnesses shown him during this work.

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### Material

The materials treated were all females. Synthesizing some informations obtained by some writers, the specimens were grouped,<sup>1)</sup> for convenience, as follows :

#### *E. pustulosa*

Type PI. Elytra provided with a tubercle at each apex, moderately curved but sometimes exceptionally sharply curved to various degree in profile ; legs almost black

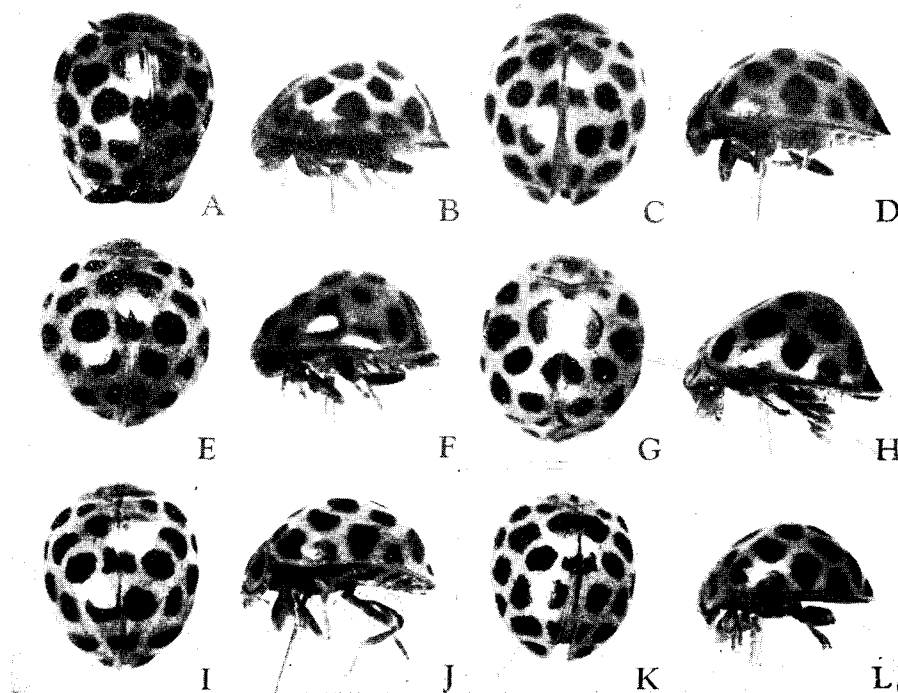


Fig. 1. Photograph of specimens, showing tubercles and curvatures of elytra (photo by Dr. M. Okada). A-H. *E. pustulosa*. A & B. Type PI. (Loc. Niinai). C & D. Type PII. (Loc. Nopporo). E & F. Type PIIIa. (Loc. Sôunkyo). G & H. Type PIIIb. (Loc. Shima-jima). I & J. *E. vigintioctomaculata*, type VI. (Loc. Nopporo). K & L. Type Xk. (Loc. Kodaira).

1) The detailed researches on the external morphology, habits, and distribution of the various local forms of *E. pustulosa* are now carried out by Dr. C. Watanabe (unpublished), and the writer's opinion on these points are greatly owe to his suggestions.

(Fig. 1, A & B; Fig. 2, A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub>; Fig. 3, A). The specimens collected about the type locality of this species are included in this group. *Specimens dissected*: Moshiri closely located to Soeushinai, the type locality, Prov. Sorachi, 2 spp., 12-14. VII. 1944, C. Watanabe leg.; Niinai, Prov. Tokachi, 4 spp., 16. VII. 1952, 8 spp., 17. IX. 1952, S. Ehara leg.

Type PII. Elytra without tubercle, moderately curved in profile; legs almost black (Fig. 1, C & D.; Fig. 2, B.; Fig. 3, B.). *Specimens dissected*: Nopporo near Sapporo, 20 spp., 17. VIII. 1952, S. Ehara leg.

Type PIIIa. Elytra without tubercle, sharply curved generally in profile; legs almost black (Fig. 1, E & F.; Fig. 2, C.; Fig. 3, C.). *Specimens dissected*: Sôunkyo near Asahigawa, 20 spp., 1. IX. 1952, S. Ehara leg.

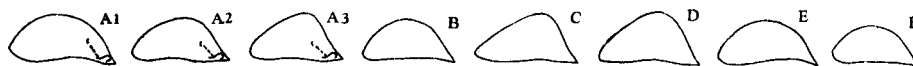


Fig. 2. Schematic figures in elytra of specimens, showing different curvature. All in profile. A-D. *E. pustulosa*. A<sub>1</sub>, A<sub>2</sub>, & A<sub>3</sub>. Type PI. (t. tubercle). B. Type PII. C. Type PIIIa. D. Type PIIIb. E. *E. vigintioctomaculata*, type VI. F. Type Xk.

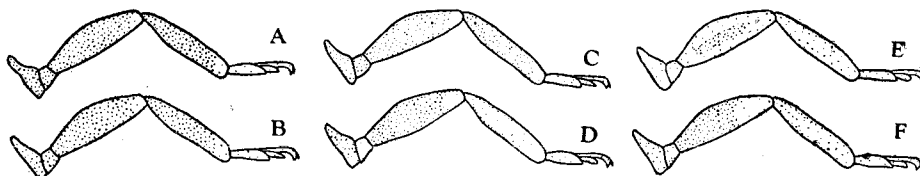


Fig. 3. Hind legs of different specimens. A-D. *E. pustulosa*. A. Type PI. B. Type PII. C. Type PIIIa. D. Type PIIIb. E. *E. vigintioctomaculata*, type VI. F. Type Xk.

Type PIIIb. All characters are almost equal to PIIIa except the tibia which is rather reddish brown, however, the specimens of Honshu are expediently separated from those of Hokkaido, PIIIa (Fig. 1, G & H.; Fig. 2, D.; Fig. 3, D.). *Specimens dissected*: Shima-jima Strain (breded by K. Yasutomi in laboratory; emerged out in Dec., 1952, from the specimens collected by S. Asahina at Shima-jima in Nagano Pref. on Aug. 3, 1952), 5 spp., Doai, Gumma Pref., 2 spp., 15. V. 1952, K. Yasutomi leg.

#### *E. vigintioctomaculata*

Type VI. (Fig. 1, I & J.; Fig. 2, E.; Fig. 3, E.). *Specimens dissected*: Nopporo near Sapporo, 20 spp., 11. IX. 1952, S. Ehara leg.; Niinai, Prov. Tokachi, 15 spp., 17. IX. 1952, S. Ehara leg.

Type VII. *Specimens dissected*: Ueda, Nagano Pref., 3 spp., IX. 1952, 2 spp., 9. IV. 1953, N. Koyama leg.

*E. vigintioctomaculata*<sup>1)</sup> shows not so remarkable variations in different districts

1) It was suggested by Maki (1951) that the mandibles of *E. vigintioctomaculata* are slightly different in the population of Hokkaido from that of Honshu.

as *E. pustulosa*, though slightly divergent between the specimens of Hokkaido (type VI) and Honshu (type VII) as regards the coloration of legs; those of Hokkaido are somewhat darker than those of Honshu.

#### *Undetermined specimens*

Type Xk. The specimens from Kodaira near Tokyo, collected by Mr. K. Yasutomi from potato and labeled by him as *E. vigintioctomaculata*, are temporarily treated to be grouped in the type Xk<sup>1)</sup> by the present writer, with a query on the identification of species. For, in these specimens not only their legs are rather blackish, but also their elytra are of a considerably high brightness<sup>2)</sup>. These features of the type Xk are rather similar to those of *E. pustulosa*. The type Xk is probably one of the transitory forms between the two species in question. The specimens which Yasutomi (1952c) described under the name of *E. vigintioctomaculata* from Fuchû, Asakawa, etc. near Tokyo as allied to *E. pustulosa*, seem to be possibly identical with the present specimens of the type Xk (Fig. 1, K & L.; Fig. 2, F.; Fig. 3, F.). *Specimens dissected*: Kodaira near Tokyo, 15 spp., IX. 1952, K. Yasutomi leg.

#### Observations

The specimens fixed and preserved in about 70% alcohol were dissected under binocular microscope. The spermatheca were frequently examined in a weak solution of KOH in order to clarify its outline by melting away the muscles covering it. Generally, the spermatheca becomes gradually paler in colour from the apex to the base (Fig. 4, D-K.). So far as the writer observed, among the seasonal stages of specimens, there occur scarcely the differences in coloration and shape of spermatheca. As the organ of the specimens just emerged out is yet pale in colour, such the specimens were excluded at examination. A tendency of the difference of coloration of the organ among the types of various local populations can be seen in Table 1. The degree of size of the apex of spermatheca, the apical index (S), is shown as a ratio of the widest breadth (m, in Fig. 4, C) to the length of the line (n, in Fig. 4, C), which is drawn perpendicularly from its apical point to the former. In this case the index seems to be the most convenient. The value of S was measured in each specimen from the figure drawn with camera-lucida.

#### *E. pustulosa*

Type PI. The spermatheca of the specimens belonging to the type PI is generally moderate brown in colour, with the exception of two pale brown ones. The apex is more or less enlarged. The mean value of S is 1.9 (Fig. 5, A).

Type PII. The spermatheca is brown in colour in various degree, including

1) After Mr. K. Yasutomi (personal communication), this type seems to represent the dominant type of so-called "*E. vigintioctomaculata*" in the vicinity of Tokyo. In this paper, however, the writer enlisted the only specimens which possess the typical characteristics shown in the types VI and VII, to *E. vigintioctomaculata*.

2) It was pointed out by Yasutomi (1952c) that the degree of brightness in elytra is in use, to some extent, in distinguishing *E. pustulosa* from *E. vigintioctomaculata*.

3 dark brown ones which have not been found in other types of *E. pustulosa*. In the type PII, the apex is generally enlarged. The mean of S was accounted 1.8 (Fig. 5, B).

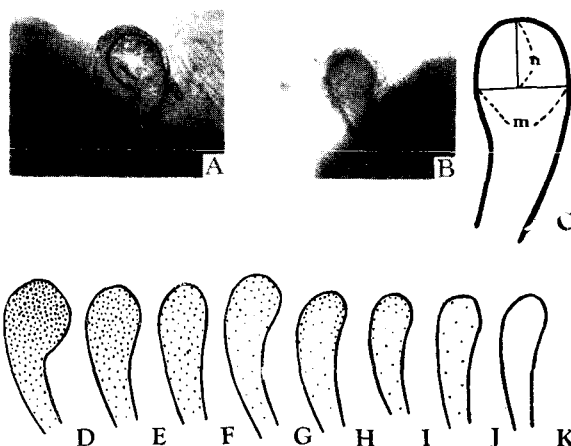


Fig. 4. Spermatheca. A & B. Photomicrographs (photo by Mr. H. Nakahara).  $\times 70$ . A. *E. pustulosa*, PI. B. *E. vigintioctomaculata*, VI. C. Showing the method of measuring the apical index,  $S. m/n=S$ . D-K. Showing the scala of the coloration of spermatheca (Schematic). D. Dark brown. E. Moderate brown. F. Pale brown. G, H & I. Apex and margin alone showing various shades of brown, other parts yellowish or typically white. J. Yellowish white. K. Typically white.

Table 1. Variation in the coloration of spermatheca among the specimens examined (cf. Fig. 4).

Scala of coloration	Type PI	PII	PIIIa	PIIIb	VI	VII	Xk
From white to yellowish white	0	0	0	0	14(40%)	4(80%)	0
Apex and margin brown, other part yellowish or typically white	0	0	0	2(29%)	10(29%)	0	3(20%)
Pale brown <sup>1)</sup>	2(14%)	7(35%)	7(35%)	5(71%)	7(20%)	0	12(80%)
Moderate brown <sup>1)</sup>	12(86%)	10(50%)	13(65%)	0	4(11%)	1(20%)	0
Dark brown	0	3(15%)	0	0	0	0	0
Total specimens examined	14	20	20	7	35	5	15

1) The "dark brown colour" used in the writer's previous paper is divided into two scalae of moderate brown and dark brown colour in this paper.

Type PIIIa. The spermatheca is moderate or pale brown in colour. Its apex is generally enlarged. The mean of S was accounted 1.9 (Fig. 5, C).

Type PIIIb. So far as the writer's observations go, the spermatheca of the specimens belonging to this type is generally paler in colour than that in any other types of *E. pustulosa*. The apex is generally enlarged. The mean of S is 1.9 (Fig. 5, D).

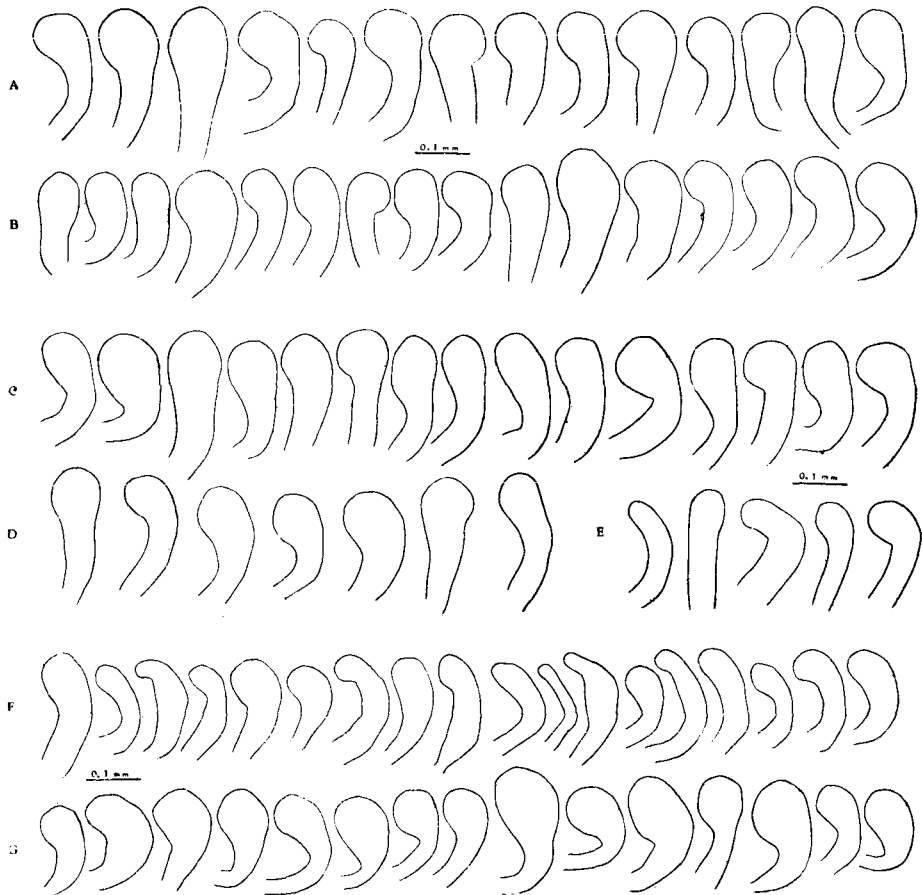


Fig. 5. Variation of spermatheca (by camera-lucida). A-D. *E. pustulosa*. A. Type PI. B. Type PII. C. Type PIIIa. D. Type PIIIb. E-F. *E. vigintioctomaculata*. E. Type VII. F. Type VI. G. Type Xk.

#### *E. vigintioctomaculata*

Type VI. The spermatheca is highly variable from white to moderate brown

in colour, white colour occurring most frequently. The apex is not enlarged, but rarely enlarged as that of *E. pustulosa*. The mean of S was accounted 1.5 (Fig. 5, F). There has been found an abnormal spermatheca which has seemingly an apical bulb. The darker spermatheca is generally much enlarged at the apex than the paler one.

Type VII. The spermatheca of the specimens belonging to this type seems to be of the type VI both in colour and in feature of apex (Fig. 5, E).

Table 2. The mean and mean-square of the apical index of spermatheca (S) in each type studied.

Value	Type	PI	PII	PIIIa	PIIIb	VI	Xk
Mean of S		1.9	1.8	1.9	1.9	1.5	1.7
Size of sample (M)		14	20	20	7	35	15
Mean square ( $u^2$ ) ( $\times 10^{-2}$ )		56.25	32.63	98.42	45.00	91.76	48.57

Table 3. The correlation of statistical difference among the types studied. Each value is a variance ratio  $F_0$ . The marks of plus and minus in parentheses show the value of difference between the components in each case: ++ and + being significant respectively below 1 and 5 % of level of significance, and - not significant.

Type	Type	PI	PII	PIIIa	PIIIb	VI	Xk
	PI		1.8 (-)	0 (-)	0 (-)	19.6 (++)	5.5 (+)
	PII	1.8 (-)		1.5 (-)	1.2 (-)	4.6 <sup>1)</sup> (+)	2.2 (-)
	PIIIa	0 (-)	1.5 (-)		0 (-)	21.1 (++)	4.9 (+)
	PIIIb	0 (-)	1.2 (-)	0 (-)		11.0 (++)	4.0 (-)
	VI	19.6 (++)	4.6 <sup>1)</sup> (+)	21.1 (++)	11.0 (++)		6.8 (+)
	Xk	5.5 (+)	2.2 (-)	4.9 (+)	4.0 (-)	6.8 (+)	

- 1) As the F-test was impossible between PII and VI from the writer's data, test of t was applicated as an approximate method in this case.

#### *Undetermined specimens*

Type XK. The spermatheca is generally pale brown in colour. The apex

is enlarged, resembling rather that of types of *E. pustulosa*, the mean of S being accounted 1.7 (Fig. 5, G).

As shown in Tables 2 and 3, the writer checked subsequently whether the differences of the size of spermatheca in these types are statistically of significance or not. The type VII alone is excluded, they being very few in number. So far as the writer's observations go, no statistical difference was detected among the types, PI, PII, PIIIa and PIIIb. The type VI is different from all other types in the size of spermatheca. On the statistical test, the type Xk is different not only from VI but also PI and PIIIa, whereas it shows no significant difference from both PII and PIIIb. The writer, however, is of the opinion that the difference of Xk from PI and PIIIa is possibly due to the errors of measurement.

As stated above, the spermatheca of *E. vigintioctomaculata* differs both in shape and in coloration from that of the specimens of every types of different local populations of *E. pustulosa* and also the questionable specimens of the type Xk. In four types of local forms of *E. pustulosa*, the spermatheca is generally identical with one another both in coloration and in shape. The spermatheca of the type Xk differs from that of *E. vigintioctomaculata* in general coloration along with the statistical test on the size of apex, and rather has some resemblance to that of *E. pustulosa* in these characters.

### Summary

1. A comparative morphology of spermatheca of several local variable forms in *Epilachna pustulosa* and *E. vigintioctomaculata* has been reported in the present paper.
2. The spermatheca seems to be admissible as a relative specific character between the two species, as well as the coloration of legs and curvature of elytra. In four types of local forms of *E. pustulosa*, the organ is generally coincided with one another both in coloration and in shape.
3. The problematical specimens (usually called "*E. vigintioctomaculata*"), feeding on the potato near Tokyo and externally transitional between *E. pustulosa* and *E. vigintioctomaculata*, are rather closely allied to the former species as regards the spermatheca.

### Literature cited

- For the papers important for this study but not given below, refer to Ehara (1952).
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