Comparative Anatomy of the Genitalia and the Internal Reproductive Organs of Ladybeetles belonging to *Epilachna* (Systematic Studies of Coccinellidae, I)[1]

By

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(*With 7 Text-figures*)

There are known in Japan the following three species of Ladybeetles belonging to the genus *Epilachna* : *Epilachna vigintioctomaculata* Motschulsky,[2] 1857, *E. pustulosa* Kôno, 1937, and *E. sparsa* (Herbst, 1786). Among them *E. sparsa*[3] is found only in southern localities, while *E. vigintioctomaculata* and *E. pustulosa* are reported only from the northern parts. With regard to the external characters, *E. sparsa* is quite distinct from two others, which are very similar to each other. Watanabe and Sakagami (1948) who studied on the external morphology, life-history and habits of the two species, found no difference in the external characters of the adults, pupae, larvae and eggs of them, except the coloration of the adult legs of them ; legs of *E. pustulosa* are black, while those of *E. vigintioctomaculata* are reddish brown. As regards the food-plants, however, there can be seen an obvious distiction ; *E. pustulosa* is chiefly attracted to the thistle, *Cirsium boreale* Kitamura (Ezo-azami) belonging to the Asteraceae, while *E. vigintioctomaculata* feeds mainly on potato belonging to the Solanaceae. In the same year Yosida compared the chromosomes of the two species in question but found no difference except slight size-variance. Yasutomi who tried the cross-breeding between them suceeded to breed F_2 between them but published no detailed result (1951,'52). Such being the case, the author took up the problem and

1) Contribution No. 271 from the Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, Japan.

2) The scientific names of species are after Watanabe (1951).

3) On the distribution of *E. vigintioctomaculata* and *E. sparsa* see Takahashi (1932) and Watanabe (1950).

made the comparative anatomy of the genitalia and the internal reproductive organs of the three Japanese species. Concerning these organs of the coccinelid-beetles several studies have been already published, and Dieke (1947) attached importance to the male and female genitalia in the taxonomic revision of *Epilachna* from Asia, Europe, and Australia.

E. vigintioctomaculata and *E. pustulosa* employed in the present study were collected by the author mainly from September to November, 1951, at Nopporo in the vicinity of Sapporo, while *E. sparsa* collected by Mr. Kiyoshi Tsuchikawa during the same season, 1951, in Misima in Pref. Sizuoka, was sent to the author for study. Immediately after collecting in the field, the beetles were fixed with 75% alcohol and preserved there. When deemed necessary, the internal reproductive organs were observed after staining with 0.2 % methylene blue solution for about two minutes. For the histological examinations of some parts Bouin's soluton was used as fixative, and serial sections were cut 10μ thick and were stained with Delafield's haematoxylin and eosin.

Before proceeding further, the author would like to express his cordial thanks to Professor Tohru Uchida for his helpful guidance given him during the course of the present work, and for his kindness in reading through the original manuscript. Moreover, his heartiest thanks are due to Mr. Shôichi F. Sakagami of our Institute for his kind criticism ; to Dr. Misao Tatewaki of the Hokkaido University for kindly determining the species of the food-plants ; to Mr. Kiyoshi Tsuchikawa of the National Institute of Genetics at Misima for his kindness in collecting some materials ; and to Dr. Chihisa Watanabe of the Hokkaido University for lending the valuable private literature.

Male Genitalia

E. vigintioctomaculata

The male genitalia[1] are composed of a tegmen and a median lobe, the former being made of two lateral lobes, an apical piece,[2] a basal piece and a tegmen strut. The tegmen is tinged with blackish brown except the tegmen strut which tends to pale brown ; both the apical piece and the lateral lobes being stout. The lateral lobes slender, almost equal in width along the whole length, though slightly widened at the base, and bluntly rounded at the apex which is covered with

1) The terms of the male genitalia applicated in this paper are mainly based on those of Sharp and Muir (1912), who used a suitable term for each part from the standpoint of comparative anatomy of the male genitalia of most of coleopterous families. The author is of the opinion that the terms of the organs should be universally used on the viewpoint of homology in all families. Verhoeff, Dobzhansky, Dieke, etc. called the lateral lobes as the paramera, the apical piece as the penis, and the median lobe as the sipho.

2) The term "apical piece" is employed here for the first time.

minute setae. The apical piece broader and stouter than the lateral lobes, narrowed gradually towards the apex, curved up in the apical part ; the ventral side provided with a lengthwise seam along the median line, the edges of the seam shift gradually outwards in the apical portion and form an oval opening within them, the dorsal side furnished with a small thin ridge like the dorsal fin of fishes (Dieke's "basal knife edge") near the base, with long setae growing except the apex and base, and with several processes resembling the saw-teeth separately arranged directed to the base in the median part ; the processes being variable in size, form, number and arrangement. The basal piece thin, nearly cylindrical, and provided

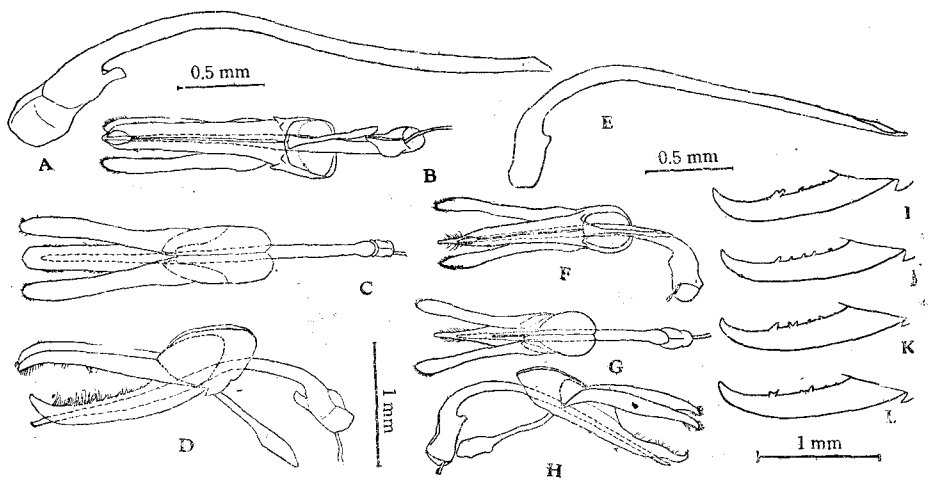

Fig. 1. Male genitalia. A-D. *E. vigintioctomaculata*. A. Median lobe (lateral view). B. Lateral view. C. Dorsal view. D. Ventral view. E-H. *E. sparsa*. E. Median lobe (lateral view). F. Lateral view. G. Dorsal view. H. Ventral view. I-L. Teeth on the dorsal side of the apical piece of *E. pustulosa*, showing individual variation.

The male genitalia were examined after boiling in KOH-solution in order to melt away the muscles and other connective tissues. Therefore, the ejaculatory ducts are drawn more slenderly in the figure than those in the natural state.

with a dark stripe running longitudinally along the median axis ; the areas adjacent to the base of the lateral lobes weakly chitinized, though variable in degree in specimens ; the lower part ending into a remarkable tooth on either side. The tegmen strut compressed, connected each with the basal and the apical piece by a connecting membrane, spreading in the apical part. The median lobe paler than the tegmen, representing a long slender tube with an enlarged base, along the median line of the ventral side, on which runs a longitudinal seam, strongly curved near the base, gradually narrowing towards the apex which is

obliquely truncate in profile, but almost equal in thickness, provided along the medain line of the dorsal side except the base with a longitudinal seam, the edges of which are in close contact each other in the apical part of the lobe but become gradually apart in the middle part outwards ; the apical half of the lobe piercing through the tegmen, with the apex exposed from the ventral oval opening of the apical piece ; the part concealed by the tegmen tinted blackish brown, while the basal naked part being pale brown except the base. The median orifice[1] indistinctly opened at the truncated apex of the median lobe. The ejaculatory duct being led through the median lobe. The internal sac[2] could not be found.

E. pustulosa

The male genitalia of this species, though closely resemble those of *E. vigintioctomaculata*, are different in colour. Each about ten male genitalia of *E. pustulosa* and *E. vigintioctomaculata* which had been preserved in 75% alcohol for about four weeks, were compared ; those of the former far darker than those of the latter.

E. sparsa

The male genitalia of this species are similar to those of *E. vigintioctomaculata*, but the following differences were observed.

They are smaller in general appearance and paler in colour than those of the latter. The apex of the lateral lobes are more or less faced each other. The apical piece narrows rapidly towards the apex which is remarkably hooked up, and deficient in teeth on the dorsal side. The basal knife edge is well developed, nearly semicircular, paler in colour than the other part of the piece. The weakly chitinized parts of the basal piece are small. The medain lobe is extremely curved and gradually flattenning towards the apex ; the edges of a longitudinal seam along the median part of the dorsal side are gradually separated from each other nearer to the apex. The slit thus formed between the edges is connected with the medain orifice, which opens near the apex of the dorsal side.

Female Genitalia (Ovipositor)

E. vigintioctomaculata

The female genitalia[3] are composed of the 9th sternite, the 9th and the 10th tergites ; represented by a flat and short tube in general appearance. Ninth tergite dark brown except the dorsal base which is blackish brown, divided longitudinally in two doubly folded parts, enveloping the sides of the 9th sternite, and scarcely reaching the apex of either the 9th sternite or the 10th tergite. Tenth

1) The spermatozoa are thought to be ejected from the median orifice into the copulatory sac of the female during coitus.

2) In the Coleoptera, the term "internal sac" is adopted for the enlarged portion of the azygos (the azygotic portion of the male genital tube).

3) The female genitalia are connected ventrally with the 6th sternite and dorsally with the 8th tergite, which are both variable in form and size interspecifically.

tergite undivided, nearly cordate, pointed bluntly, universally membranous or semi-chitinized except the peripheral area which is chitinized, with a short transverse rod-like sclerite island imbedded in the basal part of the membranous area ; the chitinized part dark brown, especially darker along the margin. Ninth sternite divided in two genital plates longitudinally, the basal part of the plates blackish brown except the inner peripheral area, the other part dark brown, the intermediate area between these parts transitional in colour, the extent of the dark brown and the blackish brown part being fairly variable among specimens, the apical edge of the plates pubescent, with a slight process, the inner margin of the plates notched shallowly near the base.

The 6th or the last sternite of the abdomen is blackish brown, of a shape of an acute angled triangle, divided longitudinally in two in the median part ; the 8th or the last tergite provided with a median dark or pale brown stripe, which is wide and variable in width and sometimes absent, the posterior margin of the tergite concave in the median part, clothed with many brown hairs, the anterior corners ended into a tooth and imbedded in the connecting membrane.

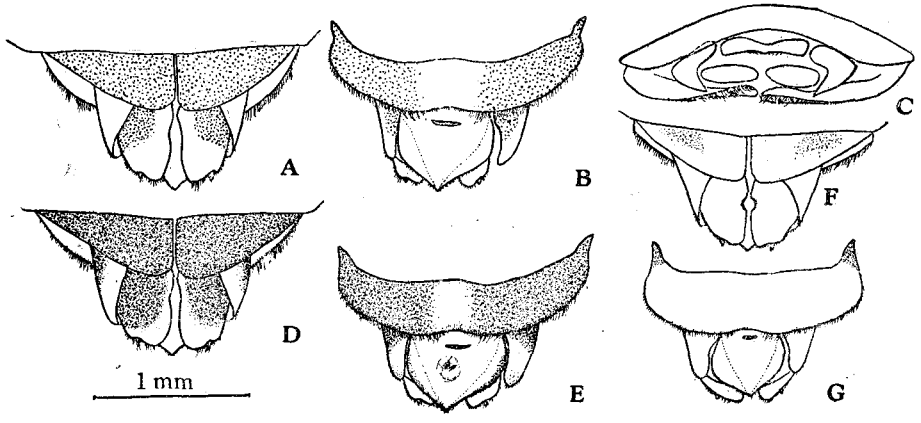

Fig. 2. Female genitalia. A-C. *E. vigintioctomaculata*. A. Ventral view. B. Dorsal view. C. Posterior view. D-E. *E. pustulosa*. D. Ventral view. E. Dorsal view. F-G. *E. sparsa*. F. Ventral view. G. Dorsal view.

E. pustulosa

The female genitalia of this species are exceedingly allied to those of *E. vigintioctomaculata*, but different only in the following tendency of coloration :

1. The coloration is generally darker than that of *E. vigintioctomaculata*.
2. The basal dark part of the genital plates is rather black, and in general large, expanded to near the inner and apical margins.
3. Both the 6th sternite and the 8th tergite are generally black, the median

pale stripe of the 8th tergite being generally narrower than that of *E. vigintioctomaculata*, and sometimes absent as well as that of *E. vigintioctomaculata*.

E. sparsa

The female genitalia of this species are similar to those of *E. vigintioctomaculata*, but different in the following points:

The genitalia are small in general appearance, far paler in colour than those of *E. vigintioctomaculata*; 9th tergite uniformly brown; genital plates nearly uniformly brown, the inner margin of the plates being notched deeper than that of *E. vigintioctomaculata*, the edge of the notches tending to dark brown.

Each piece of the 6th sternite tinged with pale brown along the peripheral area, the middle portion tending to dark brown; 8th tergite brown, leaving the anterior pointed corners blackish brown, the posterior edges being somewhat angular in contrast with those of *E. vigintioctomaculata*, the ratio of the transverse length of the 8th tergite to the longitudinal length is smaller than that of *E. vigintioctomaculata*.

Male Internal Reproductive Organs

E. vigintioctomaculata

The male organs are composed of a pair of testes, of vasa deferentia, and of seminal vesicles, two pairs of accessory glands and a ejaculatory duct; each testis tuft-shaped, consisted of a number of separate thick testicular follicles, their bases being connected with a narrow and short duct[1] which leads to a blunt end of the large and oval seminal vesicle; the vas deferens arising from the sharp end of the vesicle, broad but narrowing gradually towards the junction of both the vasa deferentia; a slender long duct arising from the vas deferens near the sharp end of the seminal vesicle, narrower than the vas deferens; both the vasa deferentia united into a median ejaculatory duct, making a cordate junction; two pairs of the accessory glands attached to the junction of the vasa deferentia, blind, slender, exceedingly long, meandering, and

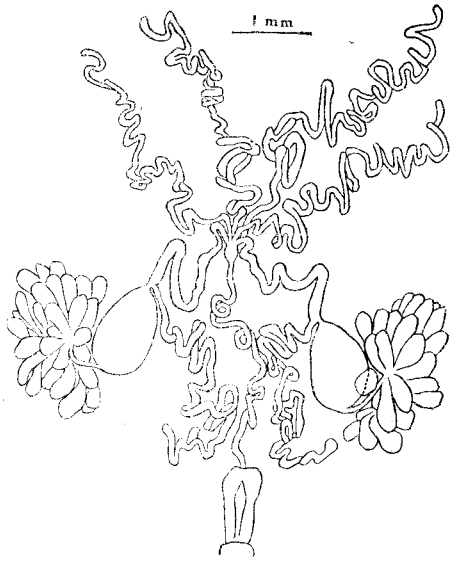

Fig. 3. Male internal reproductive organs of *E. vigintioctomaculata*.

1) Kurisaki (1926) identified the short and slender duct with the vas efferens (!)

unbranched, with a swollen apex; one pair of them somewhat more slender than the other, the slender and the broader one closely located on each side; the ejaculatory duct, more slender than the vasa deferentia, running posteriorly and connected with the genitalia, the base of which is surrounded by stout muscles possibly playing an important rôle in copulation.

E. pustulosa

As far as the author's observations go, there could be detected no difference of the male organs between this species and *E. vigintioctomaculata*.

E. sparsa

The male organs are, though similar on the whole to those of *E. vigintioctomaculata*, different in the narrowness of the vasa deferentia. Besides, the united portion of the vasa deferentia seems to be different in these species, though the specimens examined were few.

Female Internal Reproductive Organs

E. vigintioctomaculata

The female organs are consisted of a pair of ovaries, and of lateral oviduct, a common oviduct, a copulatory sac, a pair of accessory glands, and a spermatheca with a gland; each ovary fusiform, composed of a number of ovarioles, each of which contains some germ cells; the lateral oviduct arising from the lower end of each ovary, short and thick, running obliquely until united with its component on the opposite side, thus forming a median common oviduct; accessory glands obliquely attached to the common oviduct near the junction, with its apex directed towards the ovary, the glands being waxy-white in contrast with the remaining organs, which is white in colour, somewhat elongated ball-shaped, slightly

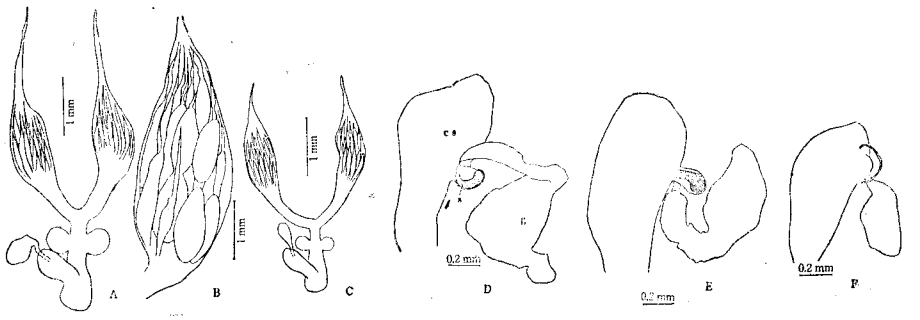

Fig. 4. Female internal reproductive organs. A. *E. vigintioctomaculata*. B. Ovary of *E. vigintioctomaculata*, with mature ova (June). C. *E. sparsa*. D-F. Spermatheca. D. *E. vigintioctomaculata*. (s. Spermatheca. g. Spermathecal gland. cs. Copulatory sac.) E. *E. pustulosa*. F. *E. sparsa*.

stouter than rest ; common oviduct thick, enlarged along the posterior part (the enlarged portion is called the vagina), entering into the ovipositor ; copulatory sac arising from the vagina, oblong and large, with swollen apical part ; spermatheca attached to the ventral side of the copulatory sac at about one-third from the apex of the sac, more or less chitinized, very small (about 0.23 mm long), white to brownish yellow, clavate and curved ; spermathecal gland composed of an apical swollen part and a short duct.

Table 1. The comparison of the spermatheca of *Epilachna*-species inhabiting Japan.

Species / Characters	*E. vigintioctomaculata*	*E. pustulosa*	*E. sparsa*
Shape	clavate	clavate, apex very swollen	slender, apex not swollen
Pigmentization (Coloration)	variable, from white to brownish yellow	generally brown	generally white

E. pustulosa

The female organs are very allied to those of *E. vigintioctomaculata*, but different from the latter in the pigmentization and the shape of the spermatheca : The coloration of the spermatheca is darker than that of *E. vigintioctomaculata*, in general rather brown and strongly chitinized ; the apex is rounded, though slightly, more swollen than that of the latter.

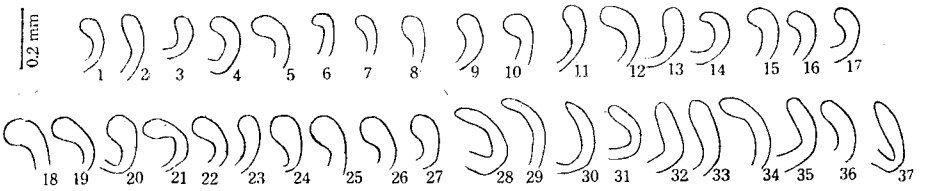

Fig. 5. Individual variation of spermatheca (camera-lucida drawing). 1-17. *E. vigintioctomaculata* (examined 17 specimens). 18-27. *E. pustulosa* (examined 10 specimens). 28-37. *E. sparsa* (examined 10 specimens).
The apex of spermathecae of Nos. 22 & 23 is not swollen in the figures, probably due to the fact that they were possibly drawn at the oblique position.

E. sparsa

The female organs are only distinguishable from those of *E. vigintioctomaculata* in the pigmentization and the shape of the spermatheca: The spermatheca is generally white and weakly chitinized; slender, the apex is rounded or somewhat pointed, not swollen.

Table 2. The comparison of the variation in the coloration of spermatheca between *E. vigintioctomaculata* and *E. pustulosa*.

Coloration \ Species	*E. vigintioctomaculata*	*E. pustulosa*
from white to yellowish white	27	0
apex and margin brownish yellow, other part yellowish white	2	0
brownish yellow	5	2
pale brown	0	2
dark brown	0	14
Total specimens examined	34	18

Discussion

As stated above, *E. vigintioctomaculata* and *E. sparsa* clearly differ from each other in the genitalia and the internal reproductive organs of both sexes, while *E. vigintioctomaculata* and *E. pustulosa* closely resemble each other in these organs. So far as the author's observations go, in the genitalia[1] the coloration is the only distinguishing character of *E. vigintioctomaculata* and *E. sparsa*; it has been generally perceived that the genitalia of both sexes of *E. pustulosa* are generally darker in colour than those of *E. vigintioctomaculata*. Dieke (1947) enumerated the shape of the median lobe as a very valuable character distinguishing the species in *Epilachna*. But the organ is closely allied in the two species here referred.

1) It must be accentuated here that the specimens of *E. pustulosa* employed in the present study were collected at Nopporo in the vicinity of Sapporo. For, it has been known that in the external characters *E. pustulosa* is considerably variable in different localities; *E. pustulosa* of Nopporo is generally deficient in the tubercle at the apex of elytron.

Dieke, who described the male and female genitalia of *E. vigintioctomaculata* and *E. sparsa*, did not refer to the shape of the basal piece and tegmen strut, and examine *E. pustulosa* at all. The male internal reproductive organs of the three species are nearly identical to each other, though the vasa deferentia of *E. sparsa* are slightly narrower than those of *E. vigintioctomaculata*. The spermatheca of the female organs seems to be very important, because it is distinct in shape and pigmentization or chitinization in different species as was summarized in the tables 1 & 2. There has been pointed out by some morphologists that the spermatheca is variable among different groups of insects; for instance, Snodgrass (1935) stated as follows: "The size, shape, and structure of the usual single spermatheca are highly variable in different insects." Besides, Dobzhansky (1921–'26) reported the similar case in the coccinellid-beetles.

Fig. 6. Photograph of a leaf of the rosette of the thistle, *Cirsium boreale* Kitamura, fed by *E. pustulosa*. Usually, in the vicinity of Sapporo, the leaves of *C. boreale* naturally wither in August. After the withering, the beetles feed mainly on the fresh rosettes of the plant during the autumn months before the hibernation.

Watanabe and Sakagami (1948) who examined the eggs, larvae, pupae, and adults, observed that the legs of *E. pustulosa* are black, while those of *E. vigintioctomaculata* reddish brown. They pointed out a remarkable difference of food-plants between these two species: *E. pustulosa* is mainly attracted to the thistle, *Cirsium boreale* Kitamura (Ezo-azami) belonging to the Asteraceae, while *E. vigintioctomaculata* feeds chiefly on potato belonging to the Solanaceae. The differences found in the present study between *E. pustulosa* and *E. vigintioctomaculata* lie in the coloration of the genitalia in both sexes, and the shape and coloration of the spermatheca. It should be recalled here that the spermatheca embry-

ologically originates from the ectoderm, as also the integument of legs, the male and female genitalia. The differences of the coloration or pigmentization may possibly be attributable to the difference of the food-plants. From the cytological viewpoint Yosida (1948) observed the close resemblance between the two problematical species. Yasutomi (1951) who studied on cross breeding between these two species, is of opinion that *E. pustulosa* is probably identical to *E. vigintioctomaculata*. At the present state the present author does not stand on the position which can give the definite conclusion on the identification of *E. pustulosa* and *E. vigintioctomaculata*.

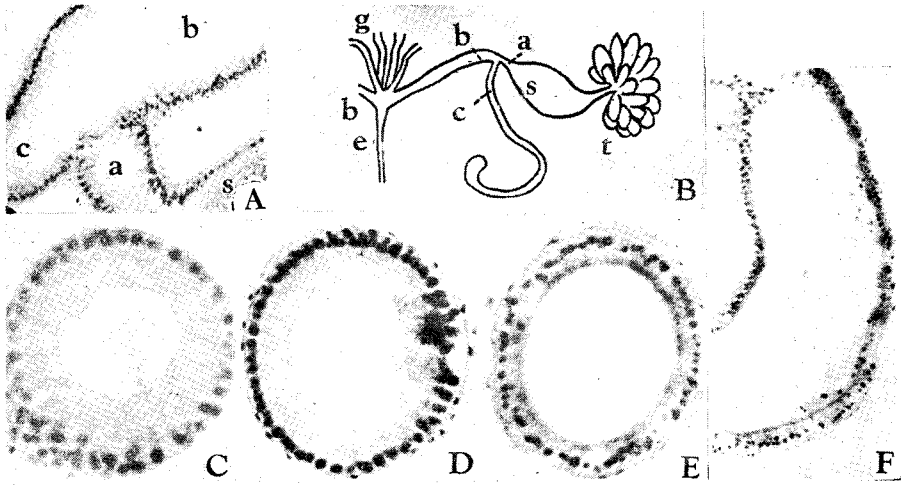

Fig. 7. Microphotographs of some parts of male internal reproductive organs of *E. vigintioctomaculata*. A. Longitudinal section of the united part of the vas deferens (a & b) and the slender long duct (c). ×130. B. Schema indicating each position of the sectioned parts shown in Fig. A. a & b. Vasa deferentia. c. The long slender duct. t. Testis. g. Accessory gland. s. Seminal vesicle. e. Ejaculatory duct. See Text-fig. 3. C. Transverse section of the slender long duct (c in Figs. 1 & 2). ×340. D. Transverse section of the vas deferens. ×250. E. Transverse section of the accessory gland. ×250. F. Longitudinal section of a part of the accessory gland. ×130.

Kurisaki (1926) investigated the internal reproductive organs of some coccinellid-beetles, containing *E. 28-maculata* (=*E. sparsa*). He unfortunately misidentified the spermathecal gland with the spermatheca and did not observe the true spermatheca, which is situated beneath the true spermathecal gland. Furthermore, in the male organs, he erroneously took the bundle of the stout muscles, which may possibly be concerned to copulation, surrounding the median lobe of the genitalia for the large fleshy sac (his "ejaculatory sac").

A slender duct connecting with the vas deferens near the sharp end of the seminal vesicle on either side was observed in the three species under study. Such the slender ducts seem to have been unknown in other families of the Coleoptera. Kurisaki thought these ducts as a part of the vasa deferentia, but the ducts seem not to be opened to the spermatozoa. On histological examinations of the ducts of *E. vigintioctomaculata* it revealed that the ducts are histologically rather attributable to a kind of glands, though doubtful on the function.

Summary

1. The comparative anatomy of the genitalia and the internal reproductive organs of both sexes of *Epilachna*-species inhabiting Japan (*E. vigintioctomaculata*, *E. pustulosa* and *E. sparsa*) were reported in the present paper.

2. *E. vigintioctomaculata* closely resembles *E. pustulosa* in all organs observed in the present study, while *E. sparsa* clearly differs from *E. vigintioctomaculata* in these organs. The differences between *E. vigintioctomaculata* and *E. pustulosa* are summarized as follows : 1) The male and female genitalia, and the spermatheca of *E. pustulosa* are darker in coloration than those of *E. vigintioctomaculata*. 2) The apex of the spermatheca of *E. pustulosa* is slightly more swollen than that of *E. vigintioctomaculata*.

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