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Sperm Storage Place in Adult Females of the Phytophagous Ladybirds Belonging to *Henosepilachna vigintioctomaculata* Complex (Coleoptera, Coccinellidae)*

Haruo Katakura

Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, Hokkaido 060, Japan

Synopsis The sperm storage place in adult females of *Henosepilachna viginti-octomaculata* complex was confirmed to be a node-like structure located at the common oviduct. The so-called spermatheca of this group is considerably reduced in comparison with that of some aphidophagous ladybirds, probably losing its primary function. The reduced spermatheca and the sperm storage node at the common oviduct may be common derived characters of the subfamily Epilachninae.

In our preliminary experiments on the crossing between Henosepilachna vigintioctomaculata (Motschulsky) and H. pustulosa (Kôno), two closely related phytophagous ladybirds belonging to H. vigintioctomaculata complex, it was noticed that the hatchability of eggs laid by the heterogamically mated females of both species was very low (KATAKURA & NAKANO, 1979). Since the larvae hatched from these eggs could grow normally under laboratory conditions, some kind of gametic isolation rather than the death of hybrid embryos was suspected to be responsible for the low hatchability. This idea required examination of the behavior of spermatozoa kept in females. In coccinellid beetles, a more or less chitinous capsule attached to the bursa copulatrix is called spermatheca or receptaculum seminis and regarded as the sperm storage place as well as in other Coleoptera. The spermatheca in the members of H. vigintioctomaculata complex is, however, very reduced and often difficult to detect as in other species of the subfamily Epilachninae (DIEKE, 1947; GORDON, 1975). Further, despite the fact that females of this complex can lay fertilized eggs successively even for one month after the last mating (NAKANO, unpubl.), only a few number of spermatozoa were rarely found in the spermatheca by inspection of post-mated females. From these facts the function of the spermatheca in H. vigintioctomaculata complex was questioned and a study was made to clarify the sperm storage place. This study fortunately succeeded in detecting the real sperm storage place of these beetles as described below.

Material and Method

There is no significant difference in the female reproductive system among the members of *H. vigintioctomaculata* complex, a series of closely related phytophagous

^{*} Contributions to the knowledge of Henosepilachna vigintioctomaculata complex. X.

ladybirds (cf. Katakura, 1981), except for the shape and color of spermatheca (Ehara, 1953; Katakura, op. cit.) and probably the average number of ovarioles per ovary. Therefore, this complex is represented by H. vigintioctomaculata (Motschulsky) (nominate form, form V-II of Katakura, 1981) in the following description. Specimens used were laboratory reared offspring whose parents were collected at Hiroshima-chô near Sapporo, northern Japan, in 1980. For comparison, the common aphidophagous species, Coccinella septempunctata brucki Mulsant, collected in the vicinity of Sapporo was also examined. Anatomical observation was made on living insects and on those fixed with Kahle's fluid. For histological observation, some individuals were fixed with Bouin's fluid, cut into 5 μ thick serial sections and stained with Delafield's hematoxylin and eosin.

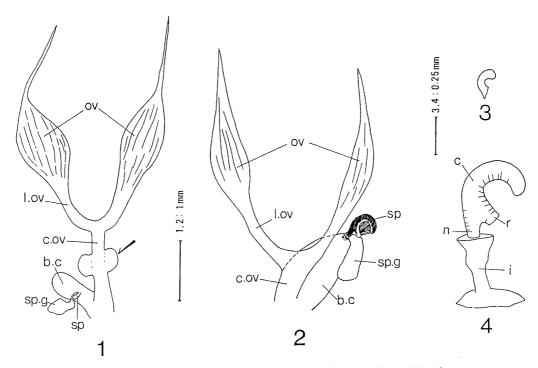
Sperm Storage Place

The female internal reproductive system of H. vigintioctomaculata was already described and illustrated by EHARA (1952), together with those of H. pustulosa (Kôno) (another member of the complex) and H. vigintioctopunctata (FABRICIUS) (Epilachna sparsa (HERBST) by EHARA). It consists of a pair of ovaries, a pair of lateral oviducts, a common oviduct, a bursa copulatrix, a spermatheca and a spermathecal gland (Fig. 1). The spermatheca (Fig. 3) is remarkably small and reduced compared with that of C. septempunctata brucki (Figs. 2, 4)*. The spermatheca of C. septempunctata brucki (Fig. 4) is a large and chitinous one with distinct ramus and nodulus, and with well developed muscle between the ramus and cornu. Further, a large infundibulum is present at the junction of the spermatheca and the bursa**. On the other hand, H. vigintioctomaculata has a small and less chitinous spermatheca whose ramus and nodulus are indistinct, and virtually lacks infundibulum and muscle around spermatheca. Judging from the above comparison and the fact that the spermatheca contains only rarely a few number of spermatozoa, it is almost certain that the spermatheca of H. vigintioctomaculata lost its primary role which is retained in Coccinella species.

On the other hand, the common oviduct of *H. vigintioctomaculata* is remarkably swollen laterally at about 3/5 from the union of lateral oviducts (Fig. 1). Although these swellings were considered as glands by EHARA (1952), the dissection of postmated living females clearly showed that the swellings and the part between them were, as a whole, real sperm storage place. The inner side of the node, namely the swellings and the part between them, is a shallow U-shaped chamber surrounded by a thick layer of columnar epithelium (Fig. 5) and is histologically different from

^{*} The specimens for Figs. 1~4 were fixed with Kahle's fluid. They had undeveloped ovaries and similar body size for each species (body length of figured specimens: *H. vigintiocto-maculata* ca. 7.7 mm; *C. septempunctata brucki* ca. 8.0 mm).

^{**} Shapes of spermatheca, infundibulum and bursa copulatrix are variable according to groups or species of ladybirds. For further detailed morphology and variation of the female internal reproductive system of Coccinellidae, see Dobzhansky (1924, 1926).



Figs. 1-4. Female internal reproductive system (1) and spermatheca (3) of *H. vigintiocto-maculata* and those of *C. septempunctata brucki* (2, 4) for comparison. Ventral view. Ov, Ovary; l.ov, lateral oviduct; c.ov, common oviduct; b.c, bursa copulatrix; sp, spermathecal gland; i, infundibulum. Note paired swellings forming a node of common oviduct in Fig. 1 (arrowed).

other portions of the oviduct. This chamber is roughly divided into two larger lateral parts (corresponding to the swellings) and a narrower part connecting them. As far as the present study is concerned, however, no significant difference was observed in the histology between the lateral and median parts, though the epithelium was thinner in the latter (Fig. 7). In mated females, this chamber is more or less expanded and filled with spermatozoa (Fig. 6). Externally, the node of virgin females is white in color like other portions of the common oviduct, while in postmated females it looks waxy or silver white by the contents, namely the spermatozoa.

Remarks

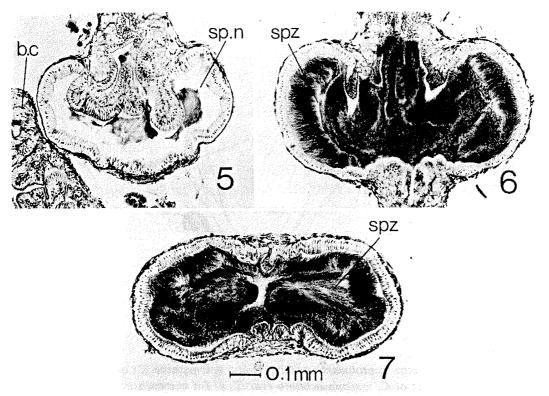
The very reduced spermatheca is commonly found in the members of the subfamily Epilachninae (DIEKE, 1947; GORDON, 1975). Further, in addition to *H. vigintioctomaculata* complex, at least the following six species belonging to two tribes of Epilachninae possess a pair of swellings or a node at the common oviduct:

Madaini: Subcoccinella vigintiquatuorpunctata L. (Dobzhansky, 1924, 1926;

Tanasijević, 1958; Ali, 1979)

Epilachnini: Epilachna varivestis (Mulsant) (Williams, 1945)

E. admirabilis Crotch (Katakura, unpubl.)



Figs. 5-7. Longitudinal (5, 6) and cross (7) sections of sperm node of *H. vigintioctomaculata*. 5, Virgin; 6, 7, inseminated. Sp.n, Sperm node; b.c, bursa copulatrix; spz, spermatozoa.

Henosepilachna pussilanima (Mulsant) (Dobzhansky, 1924, 1926) H. undecimvariolata (Boisduval) (Dobzhansky, 1924, 1926) H. vigintioctopunctata (Fabricius) (Ehara, 1952)

Probably the possession of paired swellings at common oviduct is characteristic to all the members of Epilachninae. Although some authors considered the swellings as colleterial glands, they are probably sperm storage places like in *H. vigintiocto-maculata*. Recently, ALI (1979) also described these swellings of *Subcoccinella viginti-quatuorpunctata* and, following SZELÉNYI*, called them "receptaculum seminis". But the term "receptaculum seminis" should not be used for these organs because this term usually means another strictly defined organ (cf. Figs. 1, 2). On the other hand, no such swellings have hitherto been known in coccinellids other than Epilachninae (Dobzhansky, 1924, 1926; Williams, 1945; Sasaji, pers. comm.; cf. Fig. 2). Based on the characters of female genital organs, Dobzhansky (1924) made a classification on the higher taxa of the family Coccinellidae. He separated Epilachninae** from other coccinellids by the possession of reduced spermatheca and paired swellings at the common oviduct. This treatment may be verified by

^{*} According to ALI (1979), already in 1944 SZELÉNYI briefly mentioned that the lateral swellings of the common oviduct in *S. vigintiquatuorpunctata* were the "receptaculum seminis" of this ladybird, but leaving one copy SZELÉNYI's original prints were lost by the World War II.

^{**} Originally it was referred to Epilachnini but included two tribes of Epilachninae, Madaini and Epilachnini (GORDON, 1975).

the present study in which these characters were proved to be apparently derived ones.

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References

- ALI, M., 1979. Ecological and Physiological Studies on the Alphalpha Ladybird. 200 pp. Akadémiai Kiadó, Budapest.
- DIEKE, G. H., 1947. Ladybeetles of the genus *Epilachna* (sens. lat.) in Asia, Europe, and Australia. *Smithsonian Misc. Coll.*, **106** (15): 1–187, 27 pls.
- DOBZHANSKY, Th., 1924. Die weiblichen Generationsorgane der Coccinelliden als Artmerkmal betrachtet (Col.). *Ent. Mitteil.*, 13: 18–27.
- EHARA, S., 1952. Comparative anatomy of the genitalia and the internal reproductive organs of ladybeetles belonging to *Epilachna* (Systematic studies of Coccinellidae, I). *J. Fac. Sci. Hokkaido Univ.*, (VI-Zool.), 11: 21-33.
- GORDON, R. D., 1975. A revision of the Epilachninae of the western hemisphere (Coleoptera: Coccinellidae). Tech. Bull. No. 1493, Agric. Res. Service, U. S. Dept. Agric.: 1-409.
- KATAKURA, H., 1981. Classification and evolution of the phytophagous ladybirds belonging to *Henosepilachna vigintioctomaculata* complex (Coleoptera, Coccinellidae). *J. Fac. Sci. Hokkaido Univ.*, (VI-Zool.), 22: 301–378.
- ——— & S. NAKANO, 1979. Preliminary experiments on the crossing between two puzzling phytophagous ladybirds, *Henosepilachna vigintioctomaculata* and *H. pustulosa* (Coleoptera: Coccinellidae). *Kontyû*, *Tokyo*, 47: 176–184.
- Sasaji, H., 1971. Fauna Japonica Coccinellidae (Insecta: Coleoptera). 340 pp+16 color pls. Keigaku Publishing Co. Ltd., Tokyo.
- Tanasijević, N., 1958. Zur Morphologie und Biologie des Luzernemarienkäfers Subcoccinella vigintiquatuorpunctata L. Beitr. Ent., 8: 23–78.
- WILLIAMS, J. L., 1945. The anatomy of the internal genitalia of some Coleoptera. *Proc. ent. Soc. Washington*, 47: 73–91.