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Sperm Transfer in the Potato Ladybird *Henosepilachna vigintioctomaculata* (Coleoptera, Coccinellidae, Epilachninae)

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Abstract The sperm transfer in the potato ladybird *Henosepilachna vigintioctomaculata* (MOTSCHULSKY) is described. In this species, a gelatinous substance, a putative homologue of spermatophore probably secreted from the male accessory gland, is formed in the bursa copulatrix of the female before the ejaculation. But it never encapsulates the sperm. The semen is ejected in mass in the proximal part of the bursa. Spermatozoa migrate along the common oviduct and are eventually preserved in the node-like sperm storage organ located at the middle part of the common oviduct. The gelatinous substance in the bursa copulatrix seems to be digested in the later period of sperm transfer. Approximately two days are needed to complete the whole process of sperm transfer in this species.

In a previous paper, I reported on the sperm storage organ in the female of the potato ladybird *Henosepilachna vigintioctomaculata* (MOTSCHULSKY) (KATAKURA, 1981 b). The spermathecae of many groups of coccinellid beetles are well developed and believed to be functional. However, the spermatheca of *H. vigintioctomaculata* is vestigial and apparently functionless as the sperm reservoir. Instead, the female of this species, and probably all the members of the phytophagous subfamily Epilachninae as well, stores spermatozoa in a unique node-like structure of the common oviduct that was previously misinterpreted as the colleterial glands. As the second report concerning the female sperm storage in *H. vigintioctomaculata*, the present paper deals with the process of sperm transfer in this phytophagous ladybird. Our knowledge on the method of sperm transfer in coccinellid beetles is yet scarce. As far as I know, there is only one report dealing with this subject (FISHER, 1959). Probably, this is the second report on the sperm transfer in the coccinellids and the first one dealing with the species having the node-like sperm storage organ.

Materials and Methods

The beetles used for the present study are the so-called Hokkaido form of *H. vigintioctomaculata* (Form V-II, *cf.* KATAKURA, 1974, 1981 a) collected as larvae or adults from potato fields at several localities in the vicinity of Sapporo, Hokkaido, northern Japan. They were reared with fresh potato leaves or the leaves of a herb,

Solanum megacarpum, until they are used for the experiment. Several to ten several individuals of adults including both sexes were placed in a transparent plastic case and were allowed to mate freely. Then, paired females were dissected at various intervals after the onset of copulation. For histological observations, the reproductive organs of each specimen were fixed with BOUIN's fluid immediately after the dissection. Then the parts excluding ovaries were cut into 5–8 μm serial sections and stained with DELAFIELD's hematoxylin and eosin. Anatomical observations were made on both living specimens and the fixed specimens.

Sperm Transfer

The internal reproductive system of the female of *H. vigintioctomaculata* consists of a pair of ovaries, a pair of lateral oviducts, a common oviduct with a node-like lateral swelling, a bursa copulatrix, a spermatheca and a spermathecal gland (Fig. 1) (KATAKURA, 1981 b). As mentioned before, the spermatheca is regarded as functionless. Spermatozoa are preserved in the node-like swelling of the common oviduct. It was revealed that in this species, spermatozoa are ejected by the male into the bursa copulatrix of the female and later transferred along the common oviduct to the sperm node. The detailed process of the sperm transfer is as follows (*cf.* Fig. 2): Prior to the ejaculation, a semitransparent substance appears in the bursa copulatrix. It gradually increases the size and distends the bursa (Fig. 2–1). The substance is gelatinous and eventually becomes spherical. Though not directly confirmed, this substance is probably of male accessory gland origin. Dissection

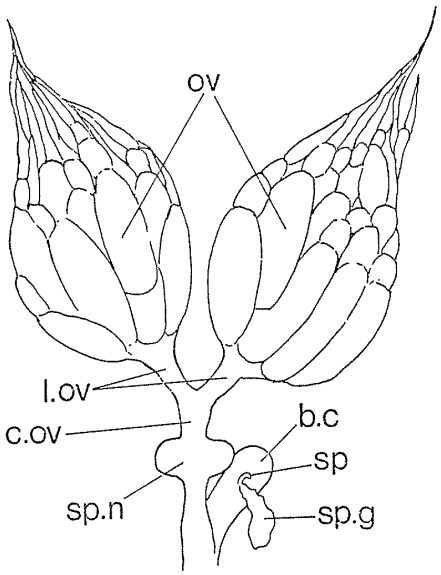

Fig. 1. The female internal reproductive system of *H. vigintioctomaculata*. Ventral view. Ov, ovary; l.ov, lateral oviduct; c.ov, common oviduct; sp.n, sperm node; b.c, bursa copulatrix; sp, spermatheca; sp.g, spermathecal gland.

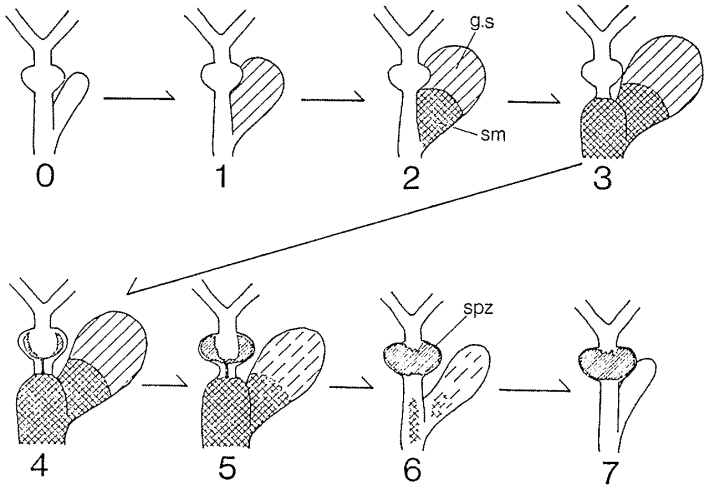

Fig. 2. Diagrammatic representation of the process of sperm transfer in *H. vigintioctomaculata*. Only the oviducts and the bursa copulatrix are shown. The numeral attached to each figure denotes the stage of sperm transfer defined in the text. G.s, gelatinous substance; sm, semen; spz, spermatozoa.

and sectioning of sexually mature individuals revealed that the male accessory gland contains large amount of secretion, whereas any gland capable of rapidly secreting such large amount of substance could not be detected in the bursa copulatrix and its vicinity of the female. After the gelatinous substance in the bursa has become fully large, ejaculation occurs. Ejected semen is found in mass at the proximal side of the bursa copulatrix (Fig. 2–2) but is not found in the gelatinous substance. Ejaculation may occur more than once, since two masses of semen are often discernible in the bursa. Semen moves toward the common oviduct. Mechanisms of this movement is unknown, but the presence of a large gelatinous substance in the bursa might be a necessary precondition for the movement. Semen enters the common oviduct in mass and distends the proximal part of the latter (Fig. 2–3). The mass of semen is blocked at the proximal side of the node. Spermatozoa enter the node through the narrow proximal neck of the node (probably by their own effort) (Figs. 2–4, 2–5). Spermatozoa first fill the lateral parts of the node, and then fill the part connecting the lateral parts. The gelatinous substance in the bursa begins to degenerate and the semen remained in the bursa and/or the common oviduct to decrease usually before the completion of the sperm transfer into the node (Figs. 2–5, 2–6). Then they disappear and both the bursa and the common oviduct recover to the ordinary states (Fig. 2–7). Thus, the gelatinous substance and excess semen seem to be digested within the body of female. Probably, the secretion from the spermathecal gland that opens to the bursa copulatrix through the spermatheca (Fig. 1), may be responsible for this digestion. As shown in Table 1, the spermathecal gland usually keeps secretion inside, but at the later period of the sperm transfer, the inner space is in most cases empty, suggesting the kept secretion is emitted into the bursa. In order to show the temporal sequence of sperm transfer,

Table 1. Change in the condition of spermathecal gland with respect to the process of sperm transfer in *H. vigintioctomaculata*. Explanations of the stages of sperm transfer in text (cf. Fig. 2). Conditions of spermathecal gland: −, inner space empty; ±, with small amount of secretion; +, with moderate amount of secretion; ++, with large amount of secretion.

Stage of sperm transfer	No. of individuals examined	Spermathecal gland			
		−	±	+	++
0	5				5
1	8		1		7
2	2				2
3	13		1	3	9
4	20	6	1		13
5	9	5	1	1	2
6	11	10			1
7	7	1	2	3	1
Total	75	22	6	7	40

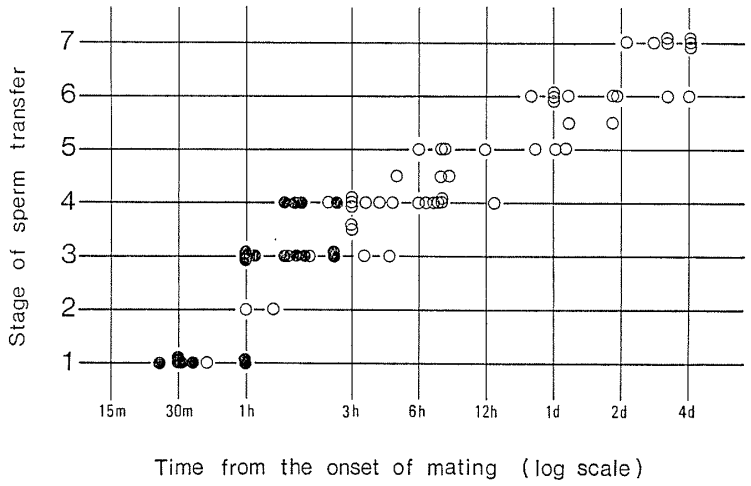

Fig. 3. Distribution of sectioned specimens arranged with respect to the stages of sperm transfer and the time from the onset of mating to the dissection. Solid circles, females in *copula* at the dissection; open circles, postcopulatory females.

states of reproductive system in the sectioned specimens were classified into eight successive stages given in Fig. 2, and are arranged with respect to the time from the onset of mating to the dissection (Fig. 3). Diagnoses of these stages are as follows:

Stage 0: State of premating females. Bursa and oviduct in normal states. Sperm node with or without spermatozoa.

Stage 1: Bursa expanding and semitransparent substance increasing in size. No sperm in bursa.

Stage 2: Bursa expanding. Gelatinous substance completed. Semen present

in bursa.

Stage 3: Size of bursa at the maximum, with both gelatinous substance and semen. Further, a mass of semen present in common oviduct, but entry of spermatozoa to sperm node not yet begin.

Stage 4: Sperm entering node. Size of bursa still at the maximum.

Stage 5: Gelatinous substance in degeneration. Entry of spermatozoa to node lasting.

Stage 6: Sperm transfer to sperm node ended. Gelatinous substance and remaining semen almost degenerated.

Stage 7 (=stage 0): Bursa and common oviduct recovered to ordinary states.

The formation of the gelatinous substance in the bursa requires up to an hour. The semen already begins to move toward the common oviduct, and in some cases even the spermatozoa begin to enter the node, when the female is still in copula (denoted by a solid circle in Fig. 3). Migration of sperm into the node takes place during approximately 1.5 hours to one day after the onset of copulation. And approximately two or more days are needed for completing all the process from the onset of copulation to the complete recovery of the bursa copulatrix and the common oviduct.

Remarks

In *H. vigintioctomaculata*, mating usually lasts more than an hour, and often continues for a few hours (Fig. 3). The present study revealed why this species remains in copula for such a long period of time. As mentioned above, the ejaculation occurs after the formation of gelatinous substance in the bursa that requires up to an hour. Therefore, at least approximately an hour seems necessary for the successful sperm transfer between the sexes in this species. Further, it is conceivable that males may ensure the entry of their sperms to the sperm nodes of the females by staying on the females after the ejaculation and guarding them from other conspecific males.

Another aspect worth noticing is the nature of the gelatinous substance found in the bursa copulatrix during the sperm transfer in *H. vigintioctomaculata*. As mentioned above, it never encapsulates the sperm or functions as an sperm plug to hold semen in the bursa copulatrix. Although it probably plays some important role in the sperm transfer of *H. vigintioctomaculata*, its exact role is yet unknown. Evidently, however, the gelatinous substance is homologous with the spermatophore of the coccinellid genus *Chilocorus* reported by FISHER (1959). He studied the spermatophore formation and sperm transfer in certain species of *Chilocorus*, especially in *C. discoideus*. In that species, a spermatophore is formed in the bursa copulatrix of the female prior to the ejaculation in a manner quite similar to *H. vigintioctomaculata*. Then, the sperm is ejected in the spermatophore. The spermatozoa migrate into the spermatheca that is in that genus functional and is derived

from the terminal end of the bursa copulatrix. Thereafter, the spermatophore is voided. Thus, *C. discoideus* and *H. vigintioctomaculata* are similar to each other in that both species ejaculate after formation of spermatophore or the gelatinous substance in the bursa copulatrix. On the other hand, they differ in the following three points: 1) the sperm is ejected in the spermatophore in *C. discoideus* whereas the sperm is not encapsulated in the gelatinous substance but ejected in the proximal side of the gelatinous substance in *H. vigintioctomaculata*, 2) spermatozoa are eventually preserved in the spermatheca in the former species, whereas in the sperm node of the common oviduct in the latter, and 3) the spermatophore is voided after the sperm transfer in the former but the gelatinous substance is probably digested in the latter. GERBER (1970) classified the methods of spermatophore formation in pterygotan insects into four types, namely, first male-determined, second male-determined, first female-determined and second female-determined methods. He assigned the method of spermatophore formation in *Chilocorus* to the first female-determined method, in that the male accessory gland secretion is ejected before or simultaneously with the semen in the body of the female to form spermatophore encapsulating the spermatozoa. The method of "spermatophore" formation in the present species may be also assignable to the first female-determined method in its general characteristics, but is distinctly different from the latter in that the "spermatophore" does not encapsulate spermatozoa.

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