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Functional Morphology of the Genitalia in *Epeorus ikanonis* (Ephemeroptera, Heptageniidae)*

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Abstract Morphology of the genitalia and other reproductive organs is described for both sexes of the heptageniid mayfly *Epeorus ikanonis* TAKAHASHI. Specimens fixed during copulation show that the male genitalia change in its shape when inserted in female genitalia. Morphological function of the male genitalia is examined in terms of sperm transfer into the seminal receptacle. And the sperm competition at successive copulation is discussed based on the location of the ejaculated sperm in female reproductive organs.

Introduction

HARKER (1986) pointed out a copulatory movement of penis in mayflies of the genus *Ecdyonurus*, and thereby he called attention to the taxonomic use of the morphology of genitalia. He showed that the dorsal side of each half of the penis lobes rotates inwards during copulation, though the function of this movement has never been explained. The morphological relations of genitalia between sexes should be examined using copulating pairs in order to clarify the function. It is difficult in general in mayflies, however, to obtain specimens of pairs connecting each other with genitalia, since they are apt to separate at sampling.

The imagines of *Epeorus ikanonis* copulate on the ground spending more than five minutes (TAKEMON, unpubl.), and thus it is rather easy to fix copulating pairs with their genitalia connecting each other. In this paper, the morphological function of the genitalia was investigated using these specimens. Females of this species show multiple copulation before oviposition (TAKEMON, unpubl.). The sperm competition at successive copulation was discussed based on the location of sperm in the reproductive organs of females.

Material and Methods

The heptageniid mayfly *Epeorus ikanonis* inhabits the upper to middle reaches of streams in Japanese low mountains (KANI, 1944), and has a univoltine life cycle, emerging in early spring (GOSE, 1970). Specimens of the mayfly were collected at Yuyagadani-deai (altitude 350 m) at the middle reaches of the Kibune Stream (35°0'N, 130°0'E), a tributary of the River Kamo running through Kyoto City, in

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April 1987 and 1988. Copulating pairs on the stream shore were picked up with fingers and were immediately dipped into cases with absolute alcohol so as to avoid separation at fixation. I collected the pair specimens at various timing during copulation: *i.e.*, 0'30", 1'00", 2'00", 3'00", and 5'00" after starting copulation, and just after copulation. I also sampled single males in the field and single females during or after oviposition. Virgin adults were obtained by rearing subimagines collected by sweeping during emergence flight or using emergence traps (TAKEMON, unpubl.).

Results

Morphology of female genitalia

The external form of the female sternum was characterized by the subgenital plate covering more than half of the eighth sternum (Fig. 1 a-b). There was a distinctive space inside the subgenital plate called a vestibule by BRINCK (1957) (Fig. 1 e). The oviducts opened separately into the vestibule from the sides of a chitinous plate (Fig. 1 d). There was a seminal receptacle and another flat pouch at the junction of the front wall of the oviducts (Fig. 1 e). The seminal receptacle opened with the slit under the flat pouch (Fig. 1 f). The seminal receptacle was egg-shaped and was made of soft tissue, while the flat pouch was very thin and was made of rather hard tissue. The latter, thus, may well be called "a plate" covering the vestibule. Morphology of reproductive organs was not different among female specimens examined, except that females before oviposition had full of eggs in the oviducts which reached the end of the 8th abdominal segment, whereas those after oviposition had only a few eggs in the posterior part of oviducts.

Morphology of male genitalia

Both virgin and single males had flat penis lobes outstretching postero-laterally (Fig. 2 a). The dorsal surface of penis lobes was made of thin chitinous membrane through which a posterior part of the ejaculatory duct was observed (Fig. 3 a), while their ventral side was rather strongly chitinous. A pair of spines was withdrawn in the depression at the base of penis lobes. The end of ejaculatory duct was closed at the brim of each penis lobe. A crevice opened longitudinally on the ventral side of the basal half of penis, which was closed with the white soft tissue.

Virgin males had a pair of deflated testes and swollen seminal vesicles (Fig. 4). Seminal vesicles connected with each other at the anterior part of the ejaculatory duct. This kind of connection has been known also in another mayfly, *Hexagenia limbata occulta* according to LEVY (1948). Specimens of swarming males showed various size of seminal vesicles irrespective with the body size represented by the fore wing length.

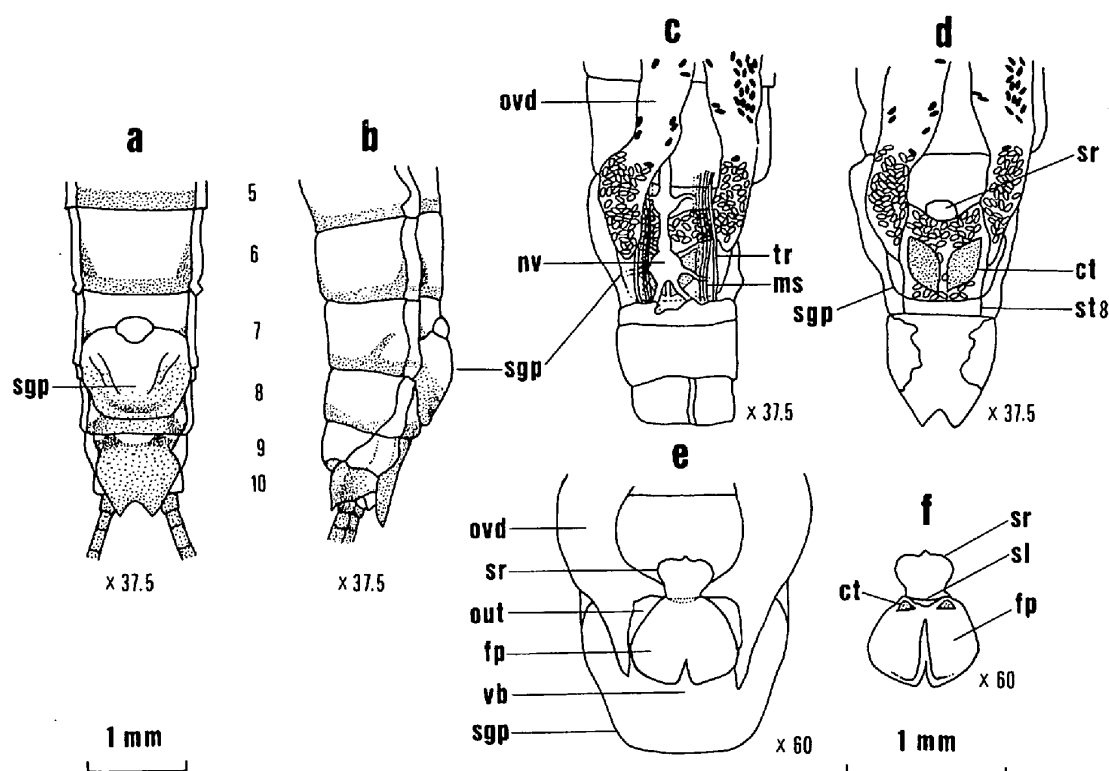


Fig. 1. Morphology of the female reproductive system in *Epeorus ikanonis*. The illustrated specimen was a female after oviposition preserved in alcohol. a) Ventral view of the female abdomen. b) Lateral view. c) Dorsal view after the dorsal tergite was removed. d) Dorsal view after tracheae, muscle and nerve system were removed. e) Dorsal view after the eighth sternum and the remaining eggs were removed. f) Ventral view of the seminal receptacle and the flat pouch. Abbreviations: ct=chitin, fp=flat pouch, ms=muscle, nv=nerve, out=outlet of oviduct, ovd=oviduct, sgp=subgenital plate, sl=slit, sr=seminal receptacle, st8=eighth sternum, tr=tracheae, vb=vestibule.

Change in morphology of male genitalia during copulation

Posture of abdomens during copulation is shown in Fig. 2 b. Male grasped female with a pair of forceps at the eighth abdominal segment and the penis was inserted into the vestibule with up side down. Dissection of the specimens revealed that the inserted penis reached in front of the seminal receptacle under the flat pouch.

The morphology of penis during copulation (copula-form: Figs. 2 c, 3 b) was different distinctly from that of single specimens (sole-form: Figs. 2 a, 3 a) in the following respects: 1) the penis bent up to the dorsal direction at the base of penis lobes, 2) each half of the penis lobes rotated inwards and was doubled up longitudinally, 3) the end of each ejaculatory duct opened with a small slit at the brim of penis lobe on the folding line, 4) a pair of spines projected laterally as a result of the bending of penis and the rotation of penis lobes, and 5) the crevice on

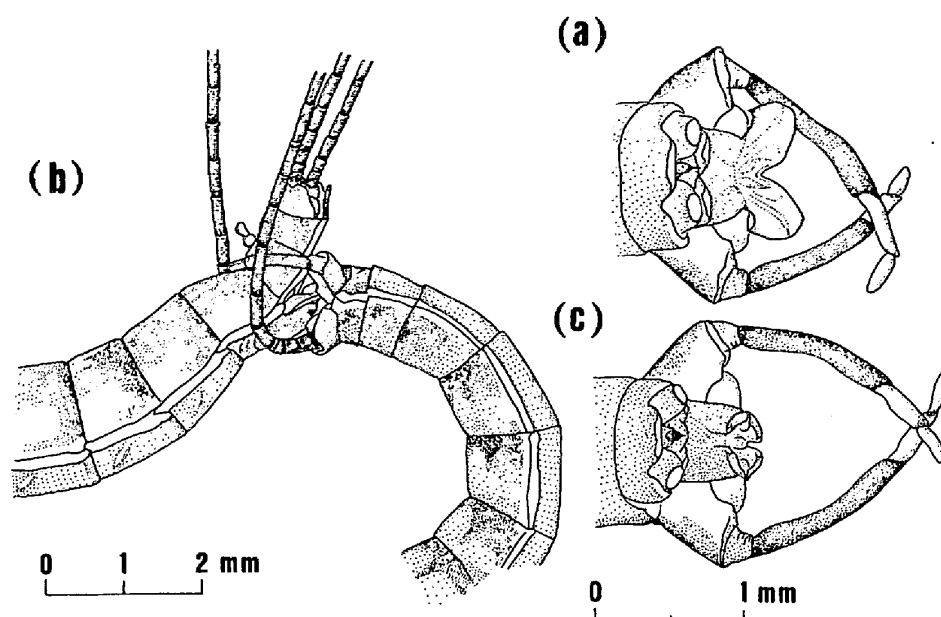


Fig. 2. External morphology of the male reproductive organs in *Epeorus ikanonis*. a) Dorsal view of the terminal abdomen of a solitary male. b) Posture of abdomens of a pair during copulation. c) Dorsal view of the terminal abdomen of a copulating male. Each figure was drawn from specimens in alcohol.

the ventral side widened and the ejaculatory duct could be seen through the thin white soft tissue.

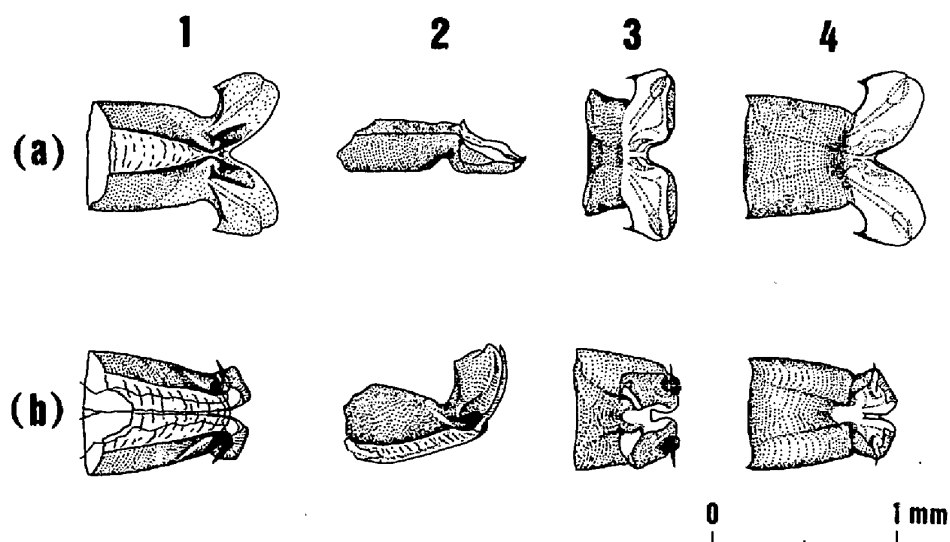


Fig. 3. Morphological comparison of penis between the sole-form (a) and the copula-form (b). 1; ventral view; 2, lateral view; 3, postero-dorsal view, and 4; dorsal view.

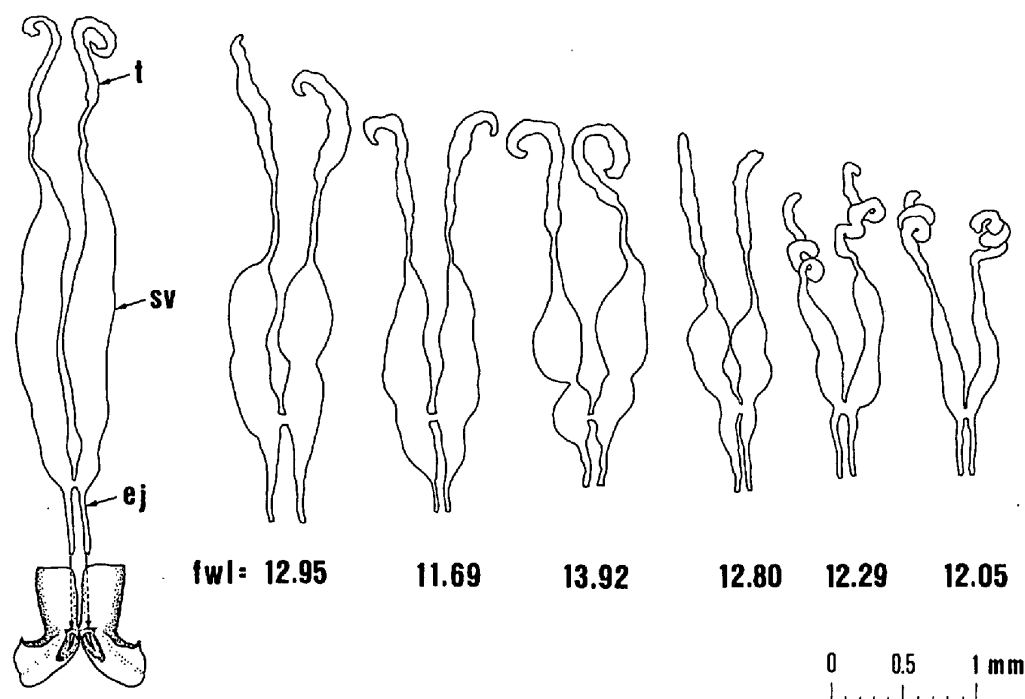


Fig. 4. Morphology of the male reproductive system of a virgin male in the ventral view (left figure) and the various stages of the sperm amount reserved in wild-caught males. The numerical value of fwf means the fore wing length in mm of each specimen. The fore wing length of the virgin male was 11.87 mm. Abbreviations: ej=ejaculatory duct, sv=seminal vesicle, t=testis.

Timing of morphological change during copulation

Table 1 shows the percentage of copula-form and sole-form in the male specimens at each timing of copulation. Males with inserting penis always showed the copula-form after 0'30" from the start of copulation. The presence of two males having a sole-form penis at 0'30" suggests that the penis of sole-form can get into the vestibule, and therefore the morphological change of the penis occurred after insertion.

When pairs separated during fixation, some males returned their penis into the sole-form, and moreover, males after copulation had the sole-form with a high percentage. These facts show that the morphological change is reversible and the copula-form is apt to occur only during insertion.

The percentage of inserted specimens was high at 1'00" and 2'00" and was low at 0'30" and 5'00". Thus coherency of the pair was high in the first half of copulation and was low at the beginning and the second half of copulation.

Sperm transfer into seminal receptacle

Females during and just after copulation carried sperm in the seminal receptacle and some of them carried it also in the vestibule (Table 2). The flat pouch was

Table 1. Timing of morphological change in the penis during copulation in *Epeorus ikanonis*. Numerals represent the number of males and those in parentheses percentage.

	after start of copulation					just after copulation
	0'30"	1'00"	2'00"	3'00"	5'00"	
Inserted*						
sole-form	2 (50.0)	0	0	0	0	—
copula-form	2 (50.0)	4 (100)	3 (100)	3 (100)	1 (100)	—
total***	4 (44.4)	4 (57.1)	3 (60.0)	3 (50.0)	1 (20.0)	—
Separated**						
sole-form	3 (60.0)	1 (33.3)	0	2 (66.7)	2 (50.0)	5 (83.3)
copula-form	2 (40.0)	2 (66.7)	2 (100)	1 (33.3)	2 (50.0)	1 (16.7)
total***	5 (55.6)	3 (42.9)	2 (40.0)	3 (50.0)	4 (80.0)	6 (100)
Total examined	9 (100)	7 (100)	5 (100)	6 (100)	5 (100)	6 (100)****

* Specimens of pairs being connected each other with genitalia.

** Specimens of pairs being separated each other during fixation.

*** Numbers in parentheses show the percentage of inserted- or separated-pairs in the specimens.

**** Copula duration of these pairs were 4'15", 4'48", 6'25", 7'10", 9'21", and 9'29".

vacant in all specimens. The sperm in the receptacle and in the vestibule was rather loose and was not bunched, and that in the vestibule was usually found under the flat pouch but in exceptional three cases it was found also on the flat pouch. Although I did not measure the amount of carried sperm, it looked like varied among females, some of which clearly carried more sperm in the vestibule than in the seminal receptacle.

When did the ejaculation occur? Since females of this species showed muti-

Table 2. Location of sperm in the reproductive organs of females during copulation in *Epeorus ikanonis*. Examined pairs are the same ones as in Table 1. Numerals represent the number of females and those in parentheses percentage. S.R.=seminal receptacle; V.=vestibule.

	after start of copulation					just after copulation	during or after oviposition
	0'30"	1'00"	2'00"	3'00"	5'00"		
Pre-oviposition*							
Stored in							
S.R.	7 (87.5)	6 (100)	5 (100)	6 (100)	5 (100)	6 (100)	—
V.	5 (62.5)	4 (66.7)	4 (80.0)	3 (50.0)	2 (40.0)	3 (50.0)	—
Vacant	1 (12.5)	0	0	0	0	0	—
Post-oviposition**							
Stored in							
S.R.	0	0	0	0	0	0	6 (75.0)
V.	0	1 (100)	0	0	0	0	0
Vacant	1 (100)	0	0	0	0	0	2 (25.0)
Total examined	9	7	5	6	5	6	8

* Female had full of eggs in the oviducts.

** Female had only a small number of eggs in the oviducts.

ple copulation (TAKEMON, unpubl.), the sperm in the female genitalia was not always derived from the copulating male of the specimen. But the following two facts suggest that the ejaculation occurs early in the copula duration. A female without sperm was found only at 0'30". On the other hand, a female carrying sperm in the vestibule was found at 1'00", though the female was after oviposition and spent all sperm in the seminal receptacle. The latter fact indicates that the sperm in the vestibule was derived from the pairing male.

Where was the sperm ejaculated? Considering the size of penis far bigger than the entrance of seminal receptacle, it seems to be impossible for males to ejaculate sperm directly into the seminal receptacle by inserting the penis lobes into it. Since there was a female carrying sperm only in the vestibule, males may ejaculate sperm in the vestibule. Then, why did almost all females carry sperm in the seminal receptacle in spite of only 40%–80% of females carrying it in the vestibule? The sperm may be transferred into the seminal receptacle by an unknown process and surplus sperm may remain in the vestibule.

Females during and after oviposition had sperm only in the seminal receptacle or had no sperm. Considering many females carrying sperm in the vestibule after copulation, sperm in the vestibule may be used or washed away during oviposition.

Discussion

Function of each reproductive organ in Epeorus ikanonis

THORNHILL and ALCOCK (1983) mentioned that females of mayflies lacked a spermatheca or other sperm storage organs and thus the sperm traveled directly to the eggs. They explained this was because mayflies are extremely short-lived in the adult stage and therefore derive no benefit from the ability to store sperm. However, BRINCK (1957) presented the morphological variation of the female reproductive organs in mayflies, ranging from the nonmodified simple gonopores (ex., Ephemeridae and Baetidae) to the strongly modified ones with the vestibule, a seminal receptacle, and copulatory pouches (ex., Heptageniidae, Siphonuridae and Ephemerellidae). Therefore the process of sperm reception and usage by females is expected to differ among species.

The morphology of male genitalia is also diverged in mayflies such as a simple membranous projection in Baetidae, a pair of separated chitinous penis in Ephemeridae, and a united chitinous penis in Heptageniidae, Leptophlebiidae and Ephemerellidae (MORGAN, 1911; 1913; MORISSON, 1919; NEEDHAM *et al.*, 1935; EDMUNDS *et al.*, 1976). The variation in the structure of male genitalia seems to correspond to that of female reproductive organs but further studies have never been done since BRINCK (1957). In order to discuss in future on the morphological variation in mayfly genitalia, the functional morphology of each variation should be examined.

The female of *E. ikanonis* has modified oviducts with a seminal receptacle and

a flat pouch. It is certain that the seminal receptacle functions to reserve the sperm until oviposition because all females after copulation had the sperm in this receptacle. Function of the flat pouch is uncertain. BRINCK (1957) confirmed the penetration of the penis into the copulatory pouch by examining mating pairs of *Parametetus chelifer*. PALMÉN (1884) also demonstrated the same function of the pouch in *Ecdyonurus*. Although the flat pouch of *E. ikanonis* seems to be homological to the copulatory pouch described in BRINCK (1957) and PALMÉN (1884), neither penetration of penis nor ejaculation of sperm in the pouch was observed in this species.

The male genitalia of this species is characterized by its reversible change from sole-form to copula-form. Morphological change of male genitalia at copulation has been also known in other mayflies such as *Baetis* of which male projects a membranous penis (EDMUNDS *et al.*, 1976), and *Ecdyonurus* of which male shows the movement of penis-lobes by rotation (HARKER, 1986). The latter case seems to have the similar function to that of *E. ikanonis*.

Why do males of this species bend their penis and rotate its lobes in the copula-form? Males seem to ejaculate sperm by the penis of copula-form. Outlets of ejaculatory ducts come together on the centre line and open toward dorsal direction as a result of the bending and the rotation. This posture will lead sperm to go downward at the centre of the vestibule at ejaculation, since the penis is inserted with up side down. Considering that the seminal receptacle is located middle at the front wall of oviducts and its entrance is under the flat pouch, the copula-form seems to be advantageous to transfer sperm into the seminal receptacle.

The penis of copula-form is also characterized by spines projecting laterally. How do the spines function? Coherency of a copulating pair was high in the first half of copulation, during which the ejaculation seems to occur. The projection of spines in the copula-form may be of use for fixing genitalia of each other during copulation.

Sperm competition in Epeorus ikanonis

The eggs of mayflies with non-modified simple gonopores are presumably fertilized by the sperm traveling into the oviducts. In contrast, the species with a seminal receptacle have various possibilities in terms of sperm precedence. Since females of this species conduct multiple copulation (TAKEMON, unpubl.), the sperm precedence at successive copulation becomes of importance for considering the mating system. In this section the mechanism of sperm utilization is inferred from the morphology of genitalia and the location of the ejaculated sperm in the female genitalia during and after copulation.

The penis of some odonates is modified so as to pull out the previous sperm (in case of Zygoptera) or to push out it (in case of Anisoptera) aiming at the displacement of sperm in the spermatheca (*e.g.*, WAAGE, 1984). The penis structure of this

species is unfit for such a kinematical sperm displacement. Absence of the sperm depletion is the seminal receptacle during copulation also suggests that males do not pull out the previous sperm in this species. Males of some dipteran species use a mating plug to prevent the sperm of successive mating from entering a spermatheca (NIELSEN, 1959; PARKER, 1970). The male of this species, however, lacks accessory glands for producing enough substance for a mating plug. Males of some lepidopteran species deposit a spermatophore at the outlet of a copulatory pouch and thus the sperm of the last copulation is transferred first into a spermatheca and is used for fertilization (DRUMMOND III, 1984). As the sperm of this species is held in a loose manner in the seminal receptacle and the vestibule, the "last-in first-out" mechanism seems to be improbable at least in a strict manner.

Although sperm was found in the vestibule in some females during and after copulation, females during and after oviposition did not carry it in the vestibule. Considering that some females had more sperm in the vestibule than in the seminal receptacle, the sperm in the vestibule must have been washed away during oviposition even if a part of it had been used for fertilization. In contrast, 75% of females retained sperm in the seminal receptacle. If the sperm in the vestibule had been pushed out with eggs early during oviposition, its fertilizing success may be lower than that of the sperm in the seminal receptacle. And vice versa if the sperm in the vestibule had been used through out oviposition. The former possibility seems more probable because the copula-form of male genitalia is aiming at ejaculation into the seminal receptacle. In case of the latter possibility, males do not have to ejaculate aiming at the seminal receptacle.

At last the sperm of successive copulation is expected to have some chance of fertilization, because the sperm of successive copulation seems to remain in the vestibule and it can fertilize eggs at least at the beginning of oviposition. The measurement of P2 ratio is wanted for the verification of this estimation.

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