SHORT COMMUNICATIONS

Studies on Eupyrene and Apyrene Spermatozoa in the Silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae) V. The Factor Related to the Separation of Eupyrene Sperm Bundles¹

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As reported in the previous paper (KATSUNO, 1977), the eupyrene sperm bundles were separated into spermatozoa in the copulatory pouch soon after ejaculation. The spermatozoa took a curved shape and migrated into the seminal receptacle. The present paper dealt with the factor of separation of the eupyrene sperm bundles.

The material used in the present study was a hybrid of J 131 × C 131. The four kinds of sample were prepared.

a. A very small amount of seminal fluid from the seminal vesicle of the unmated male adult. Seminal fluid contains seminal plasma, eupyrene sperm bundles and inactive apyrene spermatozoa.

b. Several eupyrene sperm bundles which were obtained from seminal fluid in the seminal vesicle of the unmated adult by a gradual dilution.

c. A very small amount of seminal plasma obtained from the copulatory pouch of the female adult which was mated with a male adult whose testis was removed on the first day of the fifth

instar. The seminal plasma contains the secretory substance in the ejaculatory duct.

d. A very small amount of SÖRENSEN's buffer solution of phosphatic acid (pH 6.5).

Each of four samples were mixed in three kinds of the combination as shown in Table 1 and the occurrence of separation of eupyrene spermatozoa was observed by the hanging-drop method. The mixed sample was placed on a cover slip, and the cover slip was inverted over a quartz depression slide and sealed with liquid paraffin. The preparation was observed with a phase contrast microscope at the 10- to 60-minute intervals up to 360 minutes after mixing of samples. Five preparations were prepared for each group and observed under a constant temperature (25 °C).

As shown in Table 2, when there were no apyrene spermatozoa at all (A group) or when the inactive apyrene spermatozoa were present (B group), eupyrene sperm bundles were hardly separated after mixing (Fig. 1 A and B). On the other hand, when the apyrene spermatozoa which activated by the secretory substance in the ejaculatory duct were present (C group) eupyrene sperm bundles began to separate at 30 minutes after mixing and were completely separated at 330 minutes (Fig. 1 C). It took 300 minutes for the separation of eupyrene sperm bundles in vitro, although eupyrene sperm bundles in the copulatory pouch were separated in about 70 minutes as reported in the previous paper (KATSUNO, 1977). From these results, it was clear that the active apyrene spermatozoa were necessary for the separation of the eupyrene sperm bundles, but the direct factor of the separation was not distinct.

RIEMANN (1970) stated that the eupyrene sperm bundles were separated into spermatozoa in the copulatory pouch in *Trichoplusia ni* (Lepidoptera)

Table 1. EXPERIMENTAL GROUP FOR THE TEST OF THE FACTOR WHICH SEPARATES THE EUPYRENE SPERM BUNDLES

Group	Sample	Existence of the secretory substance in the ejaculatory duct ^a	Existence and activity of the apyrene spermatozoa
A	b, c, d	+	Apyrene spermatozoa, absent
B	a, d	—	Inactive apyrene spermatozoa, present
C	a, c, d	+	Active apyrene spermatozoa, present

^a +: presence; —: absence.

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Fig. 1. 330 minutes after mixing. A: Eupyrene sperm bundle were not separated. Apyrene spermatozoa were absent; B: Eupyrene sperm bundles were not separated. Inactive apyrene spermatozoa were present; C: Eupyrene sperm bundles were separated. Active apyrene spermatozoa were present. eb, eupyrene sperm bundle; es, eupyrene spermatozoa; as, apyrene spermatozoa.

Table 2. STATE OF THE SEPARATION OF THE EUPYRENE SPERM BUNDLES^a

Time after mixing (minutes)	Group		
	A	B	C
20	—	—	—
30	—	—	+
40	—	—	+
60	—	—	+
70	—	—	++
80	—	—	++
90	—	—	++~##
120	—	—	++~##
150	—	—	++~##
160	—	—	++~##
210	—	—	++~##
270	—	—	++~##
300	—	—	++~##
330	—	—	##
360	—	—~±	##

^a State of the separation is shown by increased number of + symbols, ##; completely separated; —, not separated.

but the factor related to the separation were not described. The separation of the sperm bundles of insects other than the lepidopterous insect was noted by several investigators, although a distinction of the two types of sperm bundles were not described. PAYNE (1933) stated that the spermatozoa in the sperm bundles in *Chortophaga viridi-*

fasciata of Orthoptera were aggregated by a hyaline cap. The bundles were separated when the cap were dissolved by the function of the digestive enzyme in the seminal receptacle. Furthermore, NUR (1962) stated that the sperm bundles in *Pseudococcus obscurus* of Homoptera were stored in the ovarian junction between the two oviducts and the common oviduct. They were separated into spermatozoa shortly before oviposition by the function of an enzyme(s) which was produced by the tissue of the junction.

From above facts, it was suggested that the separation of the sperm bundles are closely related to the physiological function of the female reproductive organs. On the other hand, in the silkworm which shows two types of spermatozoa the active apyrene spermatozoa were related to the separation of the eupyrene sperm bundles.

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