Studies on Eupyrene and Apyrene Spermatozoa in the Silkworm,
Bombyx mori L. (Lepidoptera: Bombycidae)

IV. The Behaviour of the Spermatozoa in the Internal
Reproductive Organs of Female Adults

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The eupyrene sperm bundles and apyrene spermatozoa were ejaculated simultaneously into the copulatory pouch. The former were separated and took a curved shape at about 30 minutes after ejaculation and the latter shrunk immediately after ejaculation. The seminal receptacle consists of two chambers; one is termed the large lobe to which the accessory gland belongs and the other which protrudes from the large lobe is the small lobe. The eupyrene spermatozoa mainly migrated from the copulatory pouch into the large lobe, while the apyrene spermatozoa mainly migrated into the small lobe through the large lobe forming several masses. The masses of apyrene spermatozoa dispersed soon after. As the degeneration of the apyrene spermatozoa in the small lobe gradually proceeded, the eupyrene spermatozoa in the large lobe migrated into the small lobe. When a considerable number of the apyrene spermatozoa in the seminal receptacle degenerated, the adult females began oviposition. The present observation suggests that the apyrene spermatozoa plays a certain beneficial role to assist the eupyrene spermatozoa in the process of fertilization.

INTRODUCTION

The behaviour of the spermatozoa in the internal reproductive organs of lepidopterous female adults was reported by several investigators (ÔMURA, 1938; IRIKI, 1941; RIEMANN, 1970). However, no detailed reports are available on their behaviour during mating and after separation of the copulating adults, and especially on the relation between the behaviour of eupyrene and apyrene spermatozoa. The eupyrene sperm bundles and apyrene spermatozoa were ejaculated simultaneously from the seminal vesicle into the copulatory pouch through the ejaculatory duct (KATSUNO, 1977b). Those two types of spermatozoa then migrated from the copulatory pouch into the seminal receptacle. In the present study the behaviour of both types of the spermatozoa in the female reproductive organs was observed with the lapse of time from the onset of mating till the time of oviposition.

MATERIALS AND METHODS

The adult material used in the present study was a hybrid of J 131 × C 131. They were maintained under a constant temperature (25°C) with a humidity of R. H. 75% and were mated immediately after emergence. The female adults were divided into two groups. In one group the copulatory pouch and seminal receptacle of the female

adults in mating were removed at the 5 to 30 minute intervals up to 6 hours (Table 1) and in the other group these organs were taken out in the same manner from the female adults separated at 30 minutes after mating (Table 2). The sperm bundles and spermatozoa in those organs were observed by a phase contrast microscope as described in

Table 1. SEPARATION OF EUPYRENE SPERM BUNDLES AND AMOUNT OF EUPYRENE SPERM BUNDLES, EUPYRENE AND APYRENE SPERMATOZOA IN THE INTERNAL REPRODUCTIVE ORGANS OF FEMALE MATINNG FOR 6 HOURS^a

Time after commence- ment of mating (hour : minute)	Copulatory pouch				Seminal receptacle		Period (hour : minute)
	Eb	State of separation of Eb	Es	As	Es	As	
0:10	+ ^b	(- ^c)	- ^b	+ ^b	- ^b	- ^b	↑
0:15	+	(-)	-	+	-	-	The first ejaculatory period (0:20)
0:20	++	(-)	-	+	-	-	
0:25	++~+++	(-)	-	++	-	-	
0:30	+++	(-)	-	+++	-	-	
0:35	+++~++	(-~±)	-~±	+++	-	-	↓
0:50	+++~++	(±~+)	-~±	+++	-~±	-~±	↑
1:00	+++~++	(++~+++)	-~±	+++	-~±	-~±	The resting period (1:30)
1:20	+++~++	(++~+++)	-~±	+++	-~±	-~±	
1:30	++	(++~+++)	±~+	+++	-~±	-~±	
1:40	±	(+++)	++~+++	+++	±	±	
1:50	-		+++	+++	++~+++	++~+++ ^d	↓
2:00	-		+++	+++	+++	+++ ^d	↑
2:15	±	(-)	+++	+++	+++	+++	The second ejaculatory period (0:30)
2:30	+	(±)	+++	+++	+++	+++	
2:40	++	(±~+)	+++	+++	+++	+++	
2:45	+++	(+~++)	+++	+++	+++	+++	
2:50	+++~++	(++)	+++	+++	+++	+++	↓
3:00	+++~++	(++~+++)	+++	+++	+++	+++	↑
3:25	+++~+	(++~+++)	+++	+++	+++	+++	The resting period (2:45)
3:45	+~-	(+++)	+++	+++	+++	+++~++	
4:20	-		+++	+++	+++	++~+	
4:50	-		+++	+++	+++	++~+	
5:00	-		+++	+++	+++	++~+	↓
5:30	-		+++	+++	+++	++~+	The third ejaculatory period
5:35	±	(-)	+++	+++	+++	++~+++	
6:00	+	(-)	+++	+++	+++	+++	

^a Abbreviations are as follows: Eb, eupyrene sperm bundles; Es, eupyrene spermatozoa; As, apyrene spermatozoa.

^b Amounts of sperm bundles or spermatozoa are indicated by increased number of + symbols. -, absence; +, numerous in number.

^c States of separation are indicated by increased number of + symbols. -, not separated; +, completely separated.

^d Masses of apyrene spermatozoa.

the previous paper (KATSUNO, 1977b). Five female adults were used for each observation. In the result section of the present paper, the term of minutes was abbreviated as “,” for the convenience to the readers.

RESULTS

The sperm bundles or spermatozoa in the female reproductive organ during mating

Observation of the copulatory pouch. As shown in Table 1, the amount of eupyrene sperm bundles and apyrene spermatozoa in the copulatory pouch changed with the lapse of time during mating. They appeared in this organ at 10'. Thereafter they gradually increased in number, reaching the maximum amount at 30'. From the amount of eupyrene sperm bundles, it was ascertained that the first ejaculatory period continued for 20 minutes. All of the eupyrene sperm bundles separated into spermatozoa and took a curved shape during the period from 0:35' to 1:40'. Then a large number of eupyrene spermatozoa were invariably observed in the copulatory pouch till 6:00'. The apyrene spermatozoa began to take a shrunken shape immediately after ejaculation and a large number of them were observed from 0:30' to 6:00'. When the copulation reached 2:15', a small number of eupyrene sperm bundles were ejaculated again, and were seen mixed with two types of spermatozoa in the copulatory pouch. The eupyrene sperm bundles reached their maximum amount at 2:45'. They separated into spermatozoa during the period from 2:30' to 3:45'. Therefore, the second ejaculatory period seemed to continue for 30 minutes and took one hour and fifteen minutes for the separation of all the eupyrene sperm bundles. However, apyrene spermatozoa of the first ejaculation could not be distinguished from those spermatozoa of the second because the apyrene spermatozoa were ejaculated into the copulatory pouch not in the bundle state but as the separated spermatozoa. The observation of the eupyrene sperm bundles suggested that the third ejaculation began at 5:35'. The resting period corresponds to the period from the end of the previous ejaculation to the start of the next ejaculation. As shown in Table 1, the first resting period continued for one and a half hours and the second for two hours and forty-five minutes.

Based on the observation of the existence of the eupyrene sperm bundles in the copulatory pouch, the ejaculatory period or the resting period could be determined. The first ejaculation took place 10 minutes after commencement of mating and continued for 20 minutes, and then, the second ejaculation occurred after resting for one and a half hours and continued for 30 minutes. Thereafter, third ejaculation began after resting for two hours and forty-five minutes. Namely, the ejaculation occurred three times in 6 hours of mating. Furthermore, the ejaculatory, resting and separating periods were prolonged as the ejaculation was repeated.

Observation of the seminal receptacle. As shown in Table 1, a small number of eupyrene and apyrene spermatozoa began to migrate from the copulatory pouch into the seminal receptacle at 50' and the state continued up to 1:40'. A large number of eupyrene spermatozoa appeared at 1:50' and were observed till 6:00', while several masses of the apyrene spermatozoa were observed from 1:50' to 2:00' and then they were dispersed at 2:15'. These apyrene spermatozoa were began to decrease at 4:20'. During the period from 5:35' to 6:00' a large number of apyrene spermatozoa were observed again. It was rather hard to observe the masses of apyrene spermatozoa after the second ejaculation because the seminal receptacle was already filled and

packed with two types of spermatozoa of the first ejaculation.

The sperm bundles or spermatozoa in the reproductive organs of female adults separated at 30 minutes after mating

As described above, it was difficult to confirm the behaviour of the spermatozoa in detail in the copulatory pouch and the seminal receptacle after the second ejaculation. The behaviour of the spermatozoa by the first ejaculation was studied in the adults copulated for only 30 minutes. Furthermore, the seminal receptacle was divided into two parts in order to clarify the behaviour of the spermatozoa in these organs.

Observation of the copulatory pouch. As shown in Table 2, the copulatory pouch of the female adult immediately after separation was filled with eupyrene sperm bundles and apyrene spermatozoa (Fig. 1A). Eupyrene sperm bundles were separated into spermatozoa in the copulatory pouch during the period from 0:40' to 1:40' after onset of mating. In other words, the copulatory pouch was filled with eupyrene and apyrene spermatozoa at 1:40' (Fig. 1B). As to eupyrene spermatozoa such state lasted till 6:00'. On the other hand, a slight decreased of apyrene spermatozoa observed after 2:45'.

Table 2. SEPARATION OF EUPYRENE SPERM BUNDLES AND AMOUNT OF EUPYRENE SPERM BUNDLES, EUPYRENE AND APYRENE SPERMATOZOA IN THE INTERNAL REPRODUCTIVE ORGANS OF FEMALE SEPARATED AT 30 MINUTES AFTER MATING^a

Time after commence- ment of mating (hour: minute)	Copulatory pouch				Seminal receptacle				Note
	Eb	State of separation of Eb	Es	As	Large lobe		Small lobe		
					Es	As	Es	As	
0:30	≡ ^b	(- ^c)	- ^b	≡ ^b	- ^b	- ^b	- ^b	- ^b	
0:40	≡~≡	(-~±)	-~±	≡	-	-	-	-	
0:50	≡~≡	(±~+)	-~±	≡	-~±	-~±	-~±	-~±	
1:00	≡~≡	(≡~≡)	-~±	≡	-~±	-~±	-~±	-~±	
1:10	≡~≡	(≡~≡)	-~±	≡	-~±	-~±	-~±	-~±	
1:20	≡~≡	(≡~≡)	-~±	≡	-~±	-~±	-~±	-~±	
1:30	≡	(≡~≡)	±~+	≡	-~±	-~±	-~±	-~±	
1:40	±	(≡)	≡~≡	≡	±	±	±	±	
1:45	-		≡	≡	≡~≡	≡~≡ ^d	±~+	+	
1:50	-		≡	≡	≡~≡	≡~≡ ^d	±~+	≡~≡ ^d	
2:00	-		≡	≡	≡	+	±~+	≡ ^d	
2:20	-		≡	≡	≡	+	±~+	≡	
2:30	-		≡	≡	≡	+	±~+	≡	
2:45	-		≡	≡~≡	≡	+	±~+	≡	
3:00	-		≡	≡~≡	≡	+	±~+	≡	
3:30	-		≡	≡~≡	≡	+	±~+	≡	
3:45	-		≡	≡~≡	≡	+	+	≡~≡	
4:00	-		≡	≡~≡	≡	+	≡	≡~≡	
4:15	-		≡	≡~≡	≡	+	≡	≡~+	
4:30	-		≡	≡~≡	≡	+	≡	≡~+	
4:45	-		≡	≡~≡	≡	+	≡	≡~+	
5:00	-		≡	≡~≡	≡	+	≡	+	
5:30	-		≡	≡~≡	≡	+~±	≡	±	Beginning of oviposition
6:00	-		≡	≡~≡	≡	+~±	≡	±	

Explanation, see Table 1.

Fig. 1. Sperm bundles or spermatozoa in the copulatory pouch of the female adult separated at 30 minutes after mating. A: Immediately after separation. Eupyrene sperm bundles and apyrene spermatozoa were observed; B: 1:40' after commencement of mating. Eupyrene sperm bundles were separated into spermatozoa and were mixed with apyrene spermatozoa. eb, eupyrene sperm bundles; es, eupyrene spermatozoa; as, apyrene spermatozoa.

Fig. 2. The behaviour of eupyrene and apyrene spermatozoa in the seminal receptacle of the female adult separated at 30 minutes after mating. A: 1:45'~1:50' after commencement of mating. Masses of apyrene spermatozoa were observed in the large lobe; B: 1:50'~2:00'. Masses of apyrene spermatozoa were migrated from the large lobe into the small lobe; C: 2:20'~3:30'. Masses of apyrene spermatozoa in the small lobe were dispersed; D: 3:45'~5:00'. Eupyrene spermatozoa in the large lobe were migrated into the small lobe as the degeneration of apyrene spermatozoa in the small lobe proceeded; E: 5:30'~6:00'. A considerable number of apyrene spermatozoa in the large lobe and small lobe were degenerated. ag, accessory gland; ll, large lobe; sl, small lobe; mas, mass of apyrene spermatozoa.

Observation of the seminal receptacle. As shown in Fig. 2, the seminal receptacle consists of two chambers (ÔMURA, 1938). According to the description of WEIDNER's report of lepidopterous insects (1934) one to which the accessory gland belongs is termed the large lobe and the other which protrudes from the large lobe is the small lobe.

As shown in Table 2, a small number of eupyrene spermatozoa were observed in the large lobe and small lobe at 0:50' after the commencement of copulation. In the large lobe, a small number of them were invariably observed up to 1:40', and then

Fig. 3. Spermatozoa in the small lobe in the seminal receptacle of the female adult separated at 30 minutes after mating. A: 1 : 50' after commencement of mating. A mass of apyrene spermatozoa were observed; B: 3 : 30'. Eupyrene and apyrene spermatozoa were observed; C: 5 : 30'. A considerable number of apyrene spermatozoa were degenerated.

they gradually increased and a large number of them were observed from 2:00' to 6:00' (Fig. 2B, C, D and E). On the other hand, in the small lobe, only a small number of eupyrene spermatozoa were invariably observed up to 3:30' (Fig. 2A, B and C). As described later, as the degeneration of the apyrene spermatozoa in the small lobe gradually proceeded after 3:45', a considerable number of eupyrene spermatozoa in the large lobe migrated into the small lobe and they were continuously observed up to 6:00' (Fig. 2D and E).

A small number of apyrene spermatozoa were mixed with a small number of eupyrene spermatozoa in the large lobe and small lobe at 0:50' after onset of the copulation. Thereafter, in the large lobe, a small number of them continuously observed up to 1:40'. Then, a large number of apyrene spermatozoa appeared into the large lobe from the copulatory pouch forming several masses during the period from 1:45' to 1:50' (Fig. 2A). They migrated into the small lobe from 1:50' to 2:00' (Fig. 2B and Fig. 3A). Accordingly, only a small number of apyrene spermatozoa were observed in the large lobe during the period from 2:00' to 5:00' (Fig. 2B, C and D). In the small lobe, a small number of apyrene spermatozoa were continuously observed till 1:45'. Thereafter, as described above, several masses of apyrene spermatozoa migrated from the large lobe into the small lobe and were dispersed at 2:20'. This state continued up to 3:30' (Fig. 2C and Fig. 3B). The apyrene spermatozoa in the small lobe were then gradually disintegrated during the period from 3:45' to 5:00' (Fig. 2D). When a considerable number of apyrene spermatozoa in the large lobe and small lobe degenerated at 5:30' (Fig. 2E and Fig. 3C) the female adults began oviposition.

DISCUSSION

Separation of eupyrene sperm bundles

As reported in the previous paper on the silkworm (KATSUNO, 1977a), apyrene sperm bundles were separated into spermatozoa when they appeared from the testicular follicle

into the vas efferens through the basement membrane. On the other hand, the eupyrene sperm bundles began the separation into spermatozoa in the copulatory pouch at 30 minutes after ejaculation. The time length necessary for the separation of all eupyrene sperm bundles of the first ejaculation was about an hour. By the deduction of the time when the first sign of the separation of eupyrene sperm bundles was observed from the time when completely separated spermatozoa were first observed in the copulatory pouch, it was shown that the period needed for separation of the individual eupyrene sperm bundles was about 30 minutes.

In other lepidopterous insects, RIEMANN (1970) observed that the separation of the eupyrene sperm bundles of *Trichoplusia ni* were conducted in the copulatory pouch in the similar manner to the eupyrene spermatozoa of the silkworm observed in the present study. The separation of the sperm bundles of insects other than the lepidopterous insects was noted by several investigators, although the distinction of the two types of sperm bundles are not described. The sperm bundles in *Chortophaga viridifasciata* of Orthoptera were separated into spermatozoa in the seminal receptacle (PAYNE, 1933). NUR (1962) reported that sperm bundles in *Pseudococcus obscurus* of Homoptera were stored in ovarian junction between the two oviducts and the common oviduct and were separated shortly before oviposition.

The behaviour of the spermatozoa in the copulatory pouch and seminal receptacle

In the silkworm, the time when the spermatozoa were ejaculated in the copulatory pouch and the time when they arrived in the seminal receptacle after commencement of mating was reported by several investigators. MACHIDA and WATANABE (1927) stated that the ejaculation began at 9 minutes after commencement of mating and continued for 15 minutes, and the second ejaculation took place after resting from one hour to one and a half hours. YAMAZAKI et al. (1955) described that the ejaculation began at 15 minutes after commencement of mating. Measuring the time when eupyrene sperm bundles appeared into the copulatory pouch, the author obtained the similar results to MACHIDA and WATANABE (1927). According to ÔMURA (1938) the spermatozoa reached the seminal receptacle at 30 minutes after commencement of mating, while YAMAZAKI et al. (1955) described that the necessary time was one and a half hours. In the present observation, though it took 50 minutes for the spermatozoa to reach the seminal receptacle, only a small number of spermatozoa were observed up to one and a half hours.

The seminal receptacle of some lepidopterous insects consists of a large lobe and a small lobe (WEIDNER, 1934). CALLAHAN and CHAPIN (1960) also described the same observation in *Pseudaletia unipuncta* and *Peridroma margaritosa* (Lepidoptera). RIEMANN (1970) reported that in *Trichoplusia ni* the large lobe mainly contained eupyrene spermatozoa and the small lobe mainly contained apyrene spermatozoa. On the silkworm, the author obtained the same result. Before apyrene spermatozoa to degenerate, eupyrene spermatozoa mainly occupied the large lobe and apyrene spermatozoa were mainly seen in the small lobe.

On the silkworm, IRIKI (1941) reported that the migration of the eupyrene spermatozoa from the copulatory pouch into the seminal receptacle depended on the motility of the apyrene spermatozoa. In the present study, the apyrene spermatozoa showed a characteristic behaviour when they migrated from the copulatory pouch into the small lobe after passing through the large lobe. They formed several masses and dispersed later. Thereafter, as the disintegration of apyrene spermatozoa in the small

lobe proceeded, a considerable amount of eupyrene spermatozoa in the large lobe migrated into the small lobe. Adult females began oviposition when a large number of apyrene spermatozoa in the seminal receptacle degenerated. The above facts suggest that the apyrene spermatozoa play a certain beneficial role to assist the eupyrene spermatozoa in the process of fertilization.

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