



Title	The Relation of the Behavioral and Morphological Changes of the Spermatozoa in the Male Reproductive Organ to Fertilization of <i>Antheraea yamamai</i> : (Lepidoptera Saturniidae)
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**THE RELATION OF THE BEHAVIORAL  
AND MORPHOLOGICAL CHANGES OF THE  
SPERMATOOZOA IN THE MALE REPRODUCTIVE  
ORGAN TO FERTILIZATION OF *ANTHERAEA*  
*YAMAMAI* (LEPIDOPTERA: SATURNIIDAE)**

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**Introduction**

NISHIMURA<sup>9)</sup> reported that the percentage of copulation in the adult of *Antheraea yamamai* was only 30-50%. Furthermore, he reported that the percentage of fertilized eggs was low in a similar manner to the percentage of copulation of several adults selected at random. However, he stated that the percentage of fertilized eggs in the eggs which laid by the female adults was high, where copulation was ensured. From the above reports, it was suggested that the fall of the percentage of fertilized eggs was not caused by the existence of the abnormal eggs or spermatozoa but was caused by the fact that the copulation of the adults was not consummated. In the spermatozoa, in order to determine whether these observations were correct or not, the author carried out histological observations of the testis and the seminal vesicle. In other words, the author observed the behavioral and morphological changes of spermatozoa in these organs and compared them with the results of those of *Bombyx mori*<sup>1,2,9)</sup>.

**Materials and Methods**

The materials were kept under a constant temperature (28°C). The testes at various stages from the end of the 5th instar to the 7th day after emergence and the seminal vesicles at various stages from the 18th day after pupation to the 7th day after emergence were excised. These were fixed with Bouin's solution, sectioned in 5  $\mu$  thickness and stained with Delafield's haematoxylin and eosin. The terminology of various parts of the testis was

shown in Fig. 1.

### Results

The behavioral and morphological changes of the spermatozoa in the testicular follicle and the vas efferens of the testis and in the seminal vesicle were summarized as follows.

#### From the end of the 5th instar to the 1st day after spinning

The eupyrene sperm bundles were still not observed in the follicle and a small number of mitotic figures of the spermatocytes were observed in the lower part of the follicle (Fig. 2).

#### 2nd day after spinning

A small number of the eupyrene sperm bundles which began to elongate and a very small number of considerable elongated eupyrene sperm bundles were observed in the lower part of the follicle.

#### From the 3rd to 7th day after spinning (the day before pupation)

A small number of eupyrene sperm bundles undergoing development and a considerable number of elongated eupyrene sperm bundles were observed in the lower part of the follicle.

#### From the 1st to 8th day after pupation

On the 1st day after pupation, these eupyrene sperm bundles occupied the lower one fifth part of the follicle. As the pupal development proceeds, the space which was occupied by these bundles was spread toward the upper part of the follicle. On the 8th day after pupation, the follicle was mainly occupied by these bundles except for the part which was occupied by the spermatogonia and spermatocytes around the apical cell.

#### 9th day after pupation

Only a small number of eupyrene sperm bundles appeared around the apical cell for the first time (Fig. 3). A small number of abnormal eupyrene sperm bundles, in which the head part with nuclear like substance of irregular form was found swollen were observed in the follicle and the tail part was similar to normal bundles (Fig. 4). Furthermore, the fully formed eupyrene sperm bundles with small black granules in the tail part were observed in

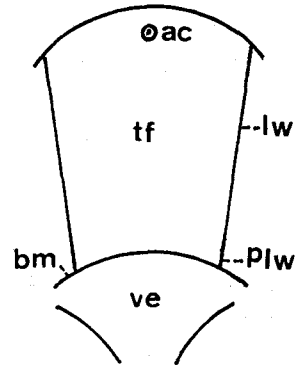


Fig. 1. Schematic figure of the testicular part.

ac, apical cell; bm, basement membrane; lw, lateral wall; plw, proximal part of the lateral wall; tf, testicular follicle; ve, vas efferens.

the follicle (Fig. 5).

#### **From the 10th to 14th day after pupation**

As the pupal development proceeded, the apyrene sperm bundles around the apical cell were observed at a high incidence. These bundles moved along the lateral wall toward the open end to surround the eupyrene sperm bundles which occupied the central part of the follicle. The abnormal eupyrene sperm bundles gradually increased and when they reached the 11th day after pupation, they were seen scattered over the whole part of the follicle.

#### **15th day after pupation**

The apyrene sperm bundles along the lateral wall cast off small granule-like nuclei in the follicle slightly above the proximal part (Fig. 6). These bundles began to congregate at the proximal part of the lateral wall and above the basement membrane. Whereas, the apyrene sperm bundles before ejecting small granule-like nuclei were observed around the apical cell.

#### **From the 16th to 17th day after pupation**

A part of the apyrene sperm bundles above the basement membrane appeared from the follicle into the vas efferens for the first time. They shed their sheaths and left them in the follicle and were separated into spermatozoa when they passed through the basement membrane.

#### **18th day after pupation**

The non-nucleated apyrene sperm bundles showed a remarkable congregation above the basement membrane (Fig. 7). A considerable number of apyrene spermatozoa appeared from the follicle into the vas efferens (Fig. 8) and continuously migrated into the seminal vesicle (Fig. 9). The eupyrene sperm bundles began to intrude into the basement membrane with their tails ahead (Fig. 10). A small number of the abnormal eupyrene sperm bundles began to degenerate.

#### **19th day after pupation**

A part of the eupyrene sperm bundles shed their sheaths and left them in the follicle and appeared from the follicle into the vas efferens through the basement membrane with their tails ahead, maintaining their bundle in an intact state. Therefore, a large number of apyrene spermatozoa and a small number of eupyrene sperm bundles were seen mixed together in the vas efferens and the seminal vesicle.

#### **From the 20th day after pupation to the 1st day after emergence**

A large number of eupyrene sperm bundles appeared from the follicle

into the vas efferens and continuously migrated into the seminal vesicle. Therefore, a large number of apyrene spermatozoa and eupyrene sperm bundles were seen mixed together in the vas efferens and the seminal vesicle (Figs. 11 and 12). Whereas, a considerable number of eupyrene sperm bundles and non-nucleated apyrene sperm bundles were still observed in the follicle. (Fig. 13). Most of the abnormal eupyrene sperm bundles were found degenerated.

#### **From the 2nd to 7th day after emergence**

On the 2nd day after emergence, a considerable number of eupyrene sperm bundles and non-nucleated apyrene sperm bundles were observed in the follicle similar to the 1st day after emergence but after reaching the 3rd and 4th day after emergence these bundles were found to be decreased. During the period from the 5th to 7th day after emergence only a few of them were observed in the follicle. During the period from the 2nd to 7th day similar to the 1st day after emergence, a large number of apyrene spermatozoa and eupyrene sperm bundles were observed in the seminal vesicle.

### **Discussion**

The principal behaviour of the spermatozoa in the male reproductive organ of *Antheraea yamamai* compared them with the results of those of *Bombyx mori*<sup>2,3)</sup>. The results were shown in Table 1. As shown in Table 1, the pupal period was different between both insects. Therefore, the stage of the pupa which was observed to have various behaviours of spermatozoa was different between both insects. However, the fundamental behaviour in which the appearance from the follicle into the vas efferens and continuous migration into the seminal vesicle of the apyrene sperm bundles was earlier than in the eupyrene sperm bundles was the same in both insects. Namely, in *Antheraea yamamai*, abnormal behaviour of the spermatozoa which were related to the fall of the percentage of the fertilized eggs was not observed. Furthermore, in *Bombyx mori*, the stage in which the eupyrene sperm bundles in the follicle showed a remarkable decrease was on the 1st day after emergence, whereas, in *Antheraea yamamai*, this stage corresponded to the period from the 5th to 7th day after emergence. The cause of the difference between insects seems to arise from the fact that *Bombyx mori* is domesticated but *Antheraea yamamai* is not.

In the morphological observations, in *Antheraea yamamai*, abnormal eupyrene sperm bundles in addition to the normal eupyrene sperm bundles were observed in the follicle of the normal individuals similar to *Bombyx*

TABLE 1. A comparison of *Antheraea yamamai* and *Bombyx mori* for the behavioral stages of the spermatozoa in the male reproductive organ

The behaviour of the spermatozoa in the male reproductive organ.	The stage of development	
	<i>Antheraea yamamai</i>	<i>Bombyx mori</i> *
The testicular follicle was mainly occupied by the eupyrene sperm bundles.	8th day after pupation	3rd day after spinning (The day before pupation)
The apyrene sperm bundles were observed in the follicle for the first time. Abnormal eupyrene sperm bundles were observed in the follicle.	9th day after pupation	1st day after pupation
The non-nucleated apyrene sperm bundles began to appear from the follicle into the vas efferens.	From the 16th to 17th day after pupation	6th day after pupation
The apyrene spermatozoa began to migrate from the vas efferens to the seminal vesicle.	18th day after pupation	6th day after pupation
The abnormal eupyrene sperm bundles began to degenerate.	18th day after pupation	5th day after pupation
The eupyrene sperm bundles appeared from the follicle into the vas efferens and continuously migrated into the seminal vesicle.	From the 19th to 20th day after pupation	8th day after pupation
A large number of eupyrene sperm bundles and apyrene spermatozoa were seen mixed in the vas efferens and the seminal vesicle. Most of the abnormal eupyrene sperm bundles were degenerated.	1st day after emergence (21th day after pupation)	1st day after emergence (11th day after pupation)
The eupyrene sperm bundles and non-nucleated apyrene sperm bundles in the follicle remarkably decreased.	From the 5th to 7th day after emergence	1st day after emergence

\* Strain; N 131×C 131, The protective temperature after commencement of spinning was 25°C.

*mori*. Though most of the abnormal bundles degenerated during the period from the 18th day after pupation to the 1st day after emergence, a large number of normal bundles were observed in these period. Therefore, it was thought that the existence of these abnormal bundles was not related to the fall of the percentage of the fertilized eggs. Furthermore, a small number of fully formed eupyrene sperm bundles with small granules in the tail part were observed. It was considered that this morphological characteristic did not represent the abnormality in the eupyrene sperm bundles as in the case of *Bombyx mori*<sup>1)</sup>. From the above observations, in *Antheraea yamamai*, the abnormal eupyrene sperm bundles which were related to the

fall of the percentage of fertilized eggs were not observed.

In *Bombyx mori*, the phenomenon in which the appearance of a large number of unfertilized eggs occurred owing to the abnormality of the spermatozoa were reported by following investigators. In the triploid male, TAKIZAWA *et al.*<sup>7)</sup> and in the tetraploid male, KATSUNO and TAMAZAWA<sup>8)</sup> reported that the abnormal eupyrene sperm bundles, in which numerous nucleus like-substance of irregular shape were abnormally arranged in the elongated bundle, and the bundles in which a roundish, elliptical and irregular shape were found, were observed in the follicle. They reported that the sterile or quite low fertility of the triploid and tetraploid may be caused by the existence of those abnormal bundles. Furthermore, SUZUKI and ÔMURA<sup>9)</sup> reported the inheritance of non-fertilization phenomena by oligospermy. The above results were obtained from the polyploid and special strain. However, these abnormal bundles and abnormal phenomena were not observed in *Antheraea yamamai* and the general strain of *Bombyx mori*.

### Summary

In *Antheraea yamamai*, the histological observations of the testes at various stages from the end of the 5th instar to the 7th day after emergence and of the seminal vesicles at various stages from the 18th day after pupation to the 7th day after emergence were conducted and compared with the results of those of *Bombyx mori*. As a result, the fundamental behaviour and morphological changes of the spermatozoa were found to be the same in both insects. Furthermore, in *Antheraea yamamai*, the abnormal behaviour and the morphological abnormality of the spermatozoa related to the fall of the percentage of the fertilized eggs were not observed.

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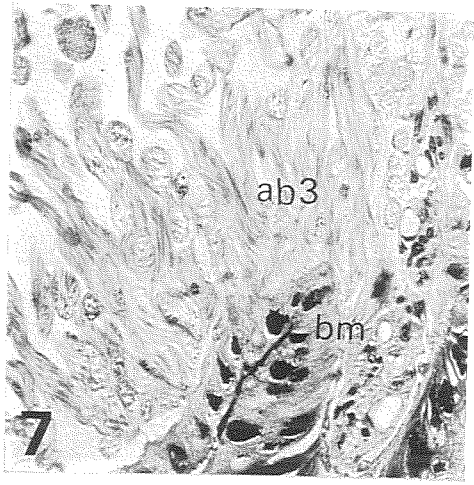
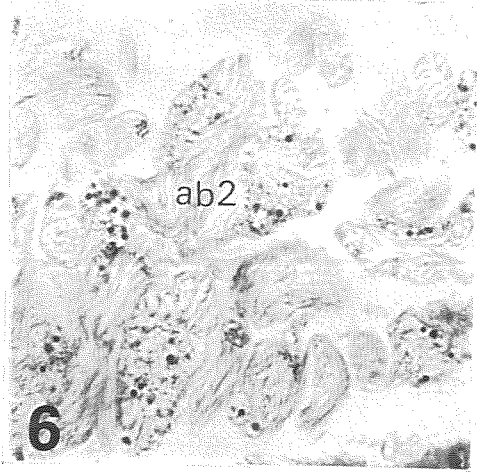
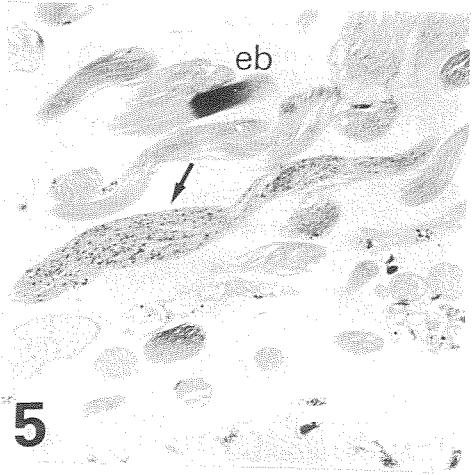
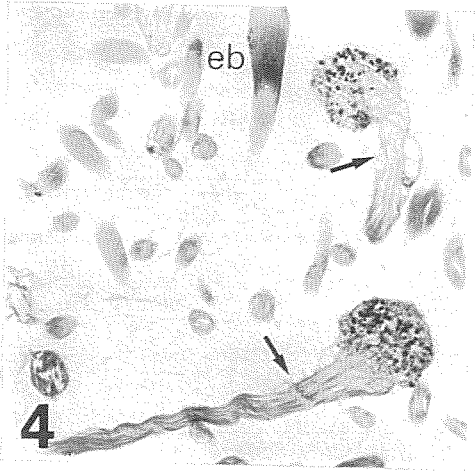
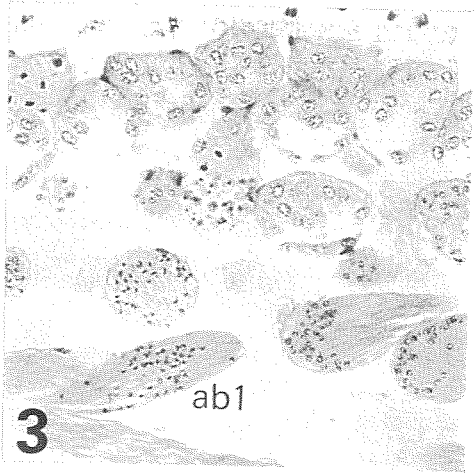
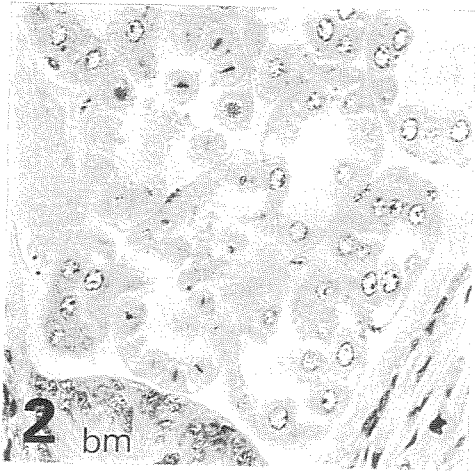
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### PLATE 1 EXPLANATION OF FIGURES

- Fig. 2. The first day after spinning. Mitotic figures of the spermatocytes in the lower part of the follicle.  $\times 290$ .
- Fig. 3. The 9th day after pupation. Apyrene sperm bundles before ejecting small granule-like nuclei (ab1) observed in the follicle for the first time.  $\times 280$ .
- Fig. 4. The 9th day after pupation. Abnormal eupyrene sperm bundles (arrows) in the follicle.  $\times 280$ .
- Fig. 5. The 9th day after pupation. Fully formed eupyrene sperm bundle (arrow) with small black granules in the tail part of the follicle.  $\times 280$ .
- Fig. 6. The 15th day after pupation. Apyrene sperm bundles ejecting small granule-like nuclei (ab2) are shown slightly above the proximal part of the lateral wall in the follicle.  $\times 520$ .
- Fig. 7. The 18th day after pupation. Apyrene sperm bundles after ejecting small granule-like nuclei (ab3) congregate above the basement membrane (bm) in the follicle.  $\times 290$ .





**PLATE 2**  
**EXPLANATION OF FIGURES**

- Fig. 8.** The 18th day after pupation. Apyrene spermatozoa (as) in the vas efferens.  $\times 280$ .
- Fig. 9.** The 18th day after pupation. Apyrene spermatozoa in the seminal vesicle.  $\times 280$ .
- Fig. 10.** The 18th day after pupation. Eupyrene sperm bundles (eb) intrude into the basement membrane of the follicle.  $\times 280$ .
- Fig. 11.** The 20th day after pupation. Eupyrene sperm bundles and apyrene spermatozoa in the vas efferens.  $\times 280$ .
- Fig. 12.** The 20th day after pupation. Eupyrene sperm bundles and apyrene spermatozoa in the seminal vesicle.  $\times 280$ .
- Fig. 13.** Immediately after emergence. A considerable number of eupyrene sperm bundles and non-nucleated apyrene sperm bundles above the basement membrane in the follicle.  $\times 280$ .

