



HOKKAIDO UNIVERSITY

Title	SPERMATOGENESIS AND THE ABNORMAL GERM CELLS IN BOMBYCIDAE AND SATURNIIDAE
Author(s)	KATSUNO, Sadaya
Citation	Journal of the Faculty of Agriculture, Hokkaido University, 64(1), 21-34
Issue Date	1989-03
Doc URL	https://hdl.handle.net/2115/13080
Type	departmental bulletin paper
File Information	64(1)_p21-34.pdf



SPERMATOGENESIS AND THE ABNORMAL GERM CELLS IN BOMBYCIDAE AND SATURNIIDAE

Sadaya KATSUNO

Laboratory of Sericology, Faculty of Agriculture
Hokkaido University, Sapporo 060, Japan

Received December 26, 1988

Introduction

In the *Bombyx mori* of Bombycidae, spermatogenesis was reported on by MACHIDA^{9,10}, SADO¹⁷, KATSUNO⁹ and other investigators. The behaviour of spermatozoa in the testicular follicle was reported on by KATSUNO⁹. Findings on abnormal sperm bundles in the testicular follicle were documented by SUGAI¹⁹ and KATSUNO⁹. In research using the Saturniidae, spermatogenesis was reported on in *Philosamia cynthia pryeri* by DEDERER¹, *Antheraea yamamai*, *Antheraea pernyi* by KAWAGUCHI⁷ and *Philosamia cynthia ricini* by SUMIMOTO²⁰. In another study, the behaviour of spermatozoa and abnormal sperm bundles in the testicular follicle was reported on in *Antheraea yamamai* by KATSUNO⁹. Comparative study of spermatogenesis and abnormal germ cells was performed on only some of the aforementioned materials by SUMIMOTO²⁰ and KATSUNO⁹. In this study, a histological comparison of spermatogenesis and abnormal germ cells was carried out between *Bombyx mori*, *Philosamia cynthia ricini* and *Antheraea yamamai* which do not undergo diapause in pupa, and *Antheraea pernyi* which does undergo diapause in pupa.

Materials and Methods

The materials used for the research were *Bombyx mori* in Bombycidae, *Philosamia cynthia ricini*, *Antheraea yamamai* and *Antheraea pernyi* in Saturniidae. The testis of each material was fixed with Bouin's solution, sectioned in 5 μ m thicknesses and stained with Delafield's haematoxylin and eosin. Developmental period and time of fixation for the testis of each material were as follows (Fig. 1):

Bombyx mori

The materials used for the research were J140 \times C140 that had been reared on mulberry leaves. The period of the 5th instar was 6 days, the period from spinning larva to prepupa was 4 days and the pupal period was 10 days under about 25°C. The testis was fixed each day during the period from the 5th instar to adult.

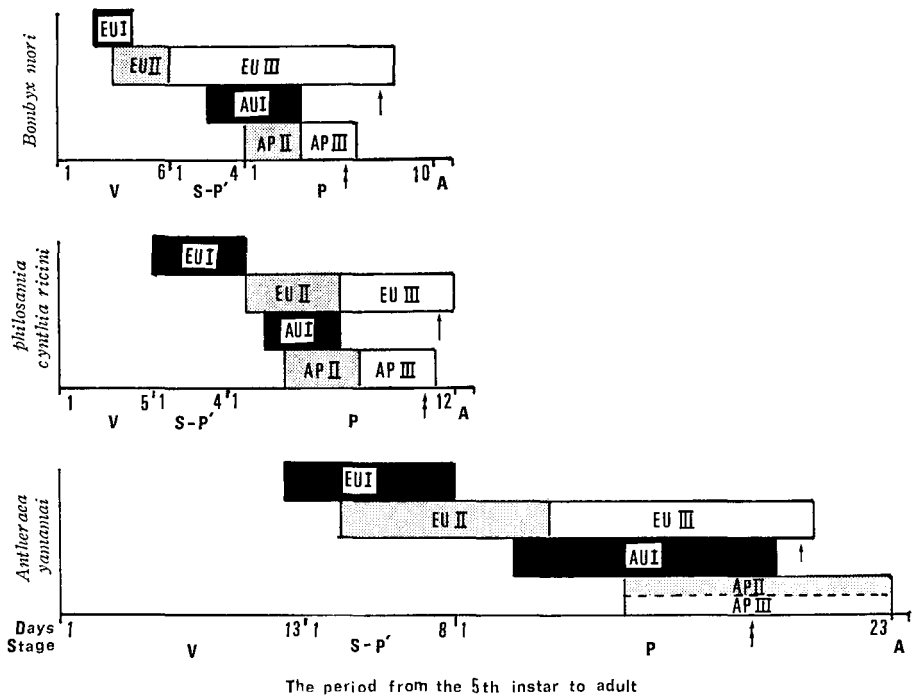


Fig. 1. Schematic illustration of spermatogenesis in relation to the developmental stages of *B. mori*, *P. cynthia ricini* and *A. yamamai*.

The period of meiotic division of eupyrene (EU I) and apyrene spermatocytes (AP I). The period of morphogenesis of eupyrene (EU II) and apyrene spermatocytes (AP II). The period of accumulation of eupyrene (EU III) and apyrene spermatozoa (AP III).

Single arrow: The time at which the eupyrene spermatozoa initially get out from the testicular follicle.

Double arrow: Indicates the same point for the apyrene spermatozoa.

Abbreviation: V, The 5th instar; S-P', Spinning larva and prepupa; P, Pupa; A, Adult.

Philosamia cynthia ricini

The materials used for the research were reared on *Ailanthus altissima* SWINGLE. The period of the 5th instar was 5 days, the period from spinning larva to prepupa was 4 days and the pupal period was 12 days under about 25°C. The testis was fixed each day during the period from the 5th instar to adult.

Antheraea yamamai

The materials used for the research were reared on *Quercus acutissima* CARRUTH at the faculty of Textile Science and Technology, Shinshu University. The period of the 5th instar was 13 days, the period from spinning larva to prepupa was 8 days and the pupal period was 23 days under about 28°C. The testis was fixed each day from the end of the 5th instar to adult.

Antheraea pernyi

The materials used for the research were the "Control" of KATSUNO and NAKAJIMA's report⁶⁾. Specifically, diapausing pupae were obtained from the first generation that were reared on *Quercus acutissima* CARRUTH and began to spinning during a period from early to late July at the Nagano Prefectural Sericultural Experimental Station, Ariake Branch in 1984. The pupae, after spinning, were kept at normal temperature in Sapporo. The range of temperature during the 131 days from Nov. 21, 1984 to Mar. 31, 1985 was $-5 \sim 10^{\circ}\text{C}$. Then the pupae were held for 2 days at 10°C from Apr. 1, and thereafter at 25°C until emergence. This period of incubation, from Apr. 1 to adult, was about 25 days. The testis of the pupa was fixed on Nov. 21, Dec. 17, Jan. 10, Feb. 4, Mar. 1 and 25, and the 5th, 10th, 15th, 20th and 25th day (adult) of incubation (Fig. 2).

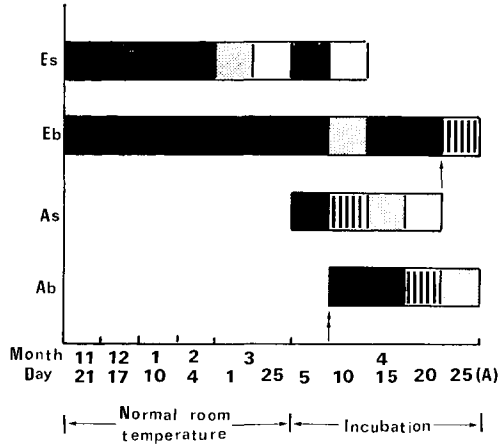


Fig. 2. Schematic illustration of quantitative changes of the spermatocytal cysts and sperm bundles of *A. pernyi*. Eupyrene (Es) and apyrene spermatocytal cysts (As) at the stage of meiotic division. Eupyrene (Eb) and apyrene sperm bundles (Ab). (A): Adult. Single and double arrow: For explanation, see Fig. 1. Amount: (a large number) $\blacksquare > \text{||||} > \text{stippled} > \square$ (a small number).

Results and Discussion

1. The relationship between spermatocytes at the leptotene stage and eupyrene, apyrene spermatocytes at the stage of meiotic division

In each material, the state of meiotic division of eupyrene spermatocytes was different from those of apyrene spermatocytes as has already been reported on in *Bombyx mori* by KATSUNO⁵⁾ (Fig. 3).

(1) *Bombyx mori*, *Philosamia cynthia ricini* and *Antheraea yamamai*

A large number of spermatocytes at the leptotene stage were observed in the 5th instar in each material. These spermatocytes subsequently developed into eupyrene spermatocytes at the stage of meiotic division. Thereafter, at the time when few or no spermatocytes at the leptotene stage were observed, namely, in the larva on the 3rd day after spinning or prepupa of *B. mori*, in the pupa on the 3rd day of *P. cynthia ricini* and in the pupa on the 6th day of *A. yamamai*, a large number of apyrene spermatocytes at the stage of meiotic division were observed in the upper part of the follicle.

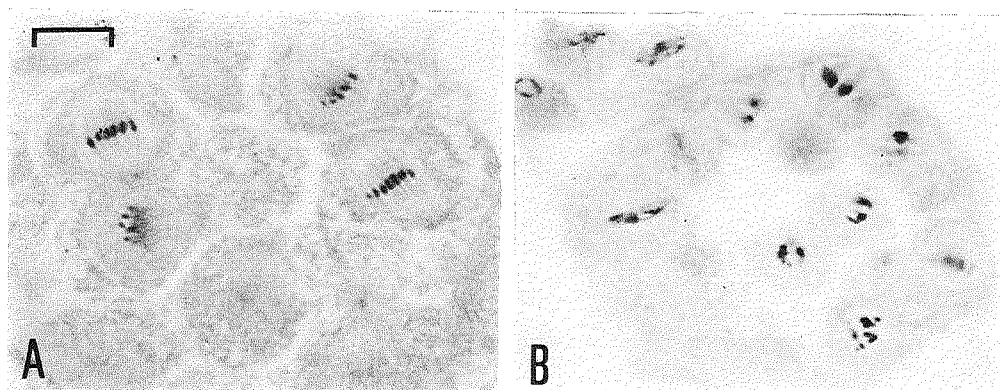


Fig. 3. The 1st meiotic division of the spermatocytes in *B. mori*.
 A: Eupyrene spermatocytes. B: Apyrene spermatocytes.
 Scale A, B: 10 μ m.

(2) *Antheraea pernyi*

QIAN and FENG¹⁰ reported that eupyrene spermatocytes at the stage of meiotic division were observed for the first time on the 10th day of the 5th instar in the second generation of diapause-type *A. pernyi*. In this study, a large number of eupyrene spermatocytes at the stage of meiotic division and eupyrene spermatozoa were observed in addition to the spermatocytes at the leptotene stage in the pupa on Nov. 21. From these facts, it seems that the spermatocytes at the leptotene stage had already existed before the 5th instar, and subsequently, continued to exist after pupation. These spermatocytes gradually developed into eupyrene spermatozoa passing over the eupyrene spermatocytes at the stage of meiotic division. Whereas a large number of apyrene spermatocytes at this stage were observed in the upper part of the follicle in the pupa on the 5th day of incubation, the spermatocytes at the leptotene stage were markedly decreased.

In viviparid snails, POLLISTER¹⁴, POLLISTER and POLLISTER¹⁵ reported that no synapsis occurs in the atypical spermatocytes. Furthermore, in the carbo moth *Ectomyelois ceratoniae* (Lepidoptera), LEVIATAN and FRIEDLÄNDER⁸ reported that the state of meiotic division is different between eupyrene and apyrene spermatocytes. In this study, in each material examined, it was found that the apyrene spermatocytes at the stage of meiotic division did not progress to the leptotene stage, in other words, the time of differentiation and determination of eupyrene and apyrene spermatozoa occurred between the spermatogonia and leptotene stages of the spermatocytes.

2. Spermatogenesis and the abnormal germ cells

(1) *Bombyx mori*, *Philosamia cynthia ricini* and *Antheraea yamamai*

1) Spermatogenesis

In the case of eupyrene spermatogenesis, the period from the time at which the meiotic division of the eupyrene spermatocytes was thriving to the time at which the eupyrene spermatozoa began to appear from the follicle to the vas efferens was divided into three divisions as follows (Fig. 1):

- (a) The period of meiotic division of eupyrene spermatocytes: The meiotic division was thriving
- (b) The period of morphogenesis of eupyrene spermatozoa: Spermatozoa were formed from the spermatocytes at the point of meiotic division
- (c) The period of accumulation of eupyrene spermatozoa: The period from a great number of spermatozoa were formed to began to appear from the follicle

Further, the period in the case of apyrene spermatogenesis, was also divided into three divisions in the same manner as described above (Fig. 1).

In this study, as mentioned above, it was proved that the state of meiotic division was different in the eupyrene and apyrene spermatocytes. Judging from these facts, the period of meiotic division of the eupyrene spermatocytes was from the 3rd to the 5th day of the 5th instar in *B. mori*, from the larva on the 1st day after spinning to the pupa on the 1st day in *P. cynthia ricini* and from the end of the 5th instar to the prepupa in *A. yamamai*. Therefore, it can be said that the eupyrene spermatozoa in *P. cynthia ricini* and *A. yamamai* were formed from the spermatocytes which perform meiotic division at different time that of *B. mori*. In *A. yamamai*, KAWAGUCHI⁷⁾ reported that meiotic division of the eupyrene spermatocytes occurred in the early pupa. Therefore, in *A. yamamai*, it is necessary to re-examine the time of meiotic division from the 5th instar. In contrast, the time of meiotic division of the apyrene spermatocytes was found to be after spinning in all of the cases examined. The period of accumulation of eupyrene spermatozoa in *P. cynthia ricini* was shorter than those of *B. mori* and *A. yamamai*. Furthermore, the period of accumulation of apyrene spermatozoa in *B. mori* and *P. cynthia ricini* was shorter than that of eupyrene spermatozoa. In the case of *A. yamamai*, as the period of morphogenesis and accumulation of apyrene spermatozoa overlapped each other, neither period could be clearly distinguished. However, apyrene spermatozoa appeared from the follicle and into the vas efferens earlier than the eupyrene spermatozoa in each material.

2) Abnormal germ cells

Abnormal germ cells in the follicle during the developmental process of the three materials were as follows:

- (a) Abnormal spermatogonia: Pycnotic nuclei existed, specifically, pycnotic spermatogonia
- (b) Abnormal spermatogonial cyst: Cysts containing pycnotic spermatogonia

- (c) Abnormal spermatocytal cyst I: Cysts containing pycnotic spermatocyte with pycnotic nuclei
- (d) Abnormal eupyrene spermatidal cyst I: Cysts containing spermatid with large granules
- (e) Abnormal eupyrene sperm bundle I: The head part was swollen with large granules
- (f) Abnormal eupyrene sperm bundle II: Large granules existed in the tail part

Bombyx mori

A large number of spermatocytal cysts were observed along the lateral wall of the follicle from the end of the 5th instar to the spinning stage and became abnormal spermatocytal cysts I at the prepupal stage (Plate I-1). Most of them became abnormal eupyrene sperm bundles I immediately after pupation, passing over the state of abnormal eupyrene spermatidal cysts I and moving towards the lower part of the follicle in the early pupal stage (Plate I-2). Such abnormal bundles I gradually degenerated, moving towards the upper part of the follicle as the pupae developed. These results were the same as those reported by KATSUNO²⁾ in *Bombyx mori*. Furthermore, a group of abnormal spermatogonia, abnormal spermatogonial and spermatocytal cysts I were observed in the upper part of the follicle during the period from the pupa on the 7th day to adult.

Philosamia cynthia ricini

A large number of abnormal spermatogonia and abnormal spermatogonial cysts were observed in the upper part of the follicle on the 2nd and 3rd day of the 5th instar (Plate I-3). Thereafter, they gradually degenerated, most degenerating in the early to middle pupal stage. Abnormal spermatocytal cysts I were observed in the whole of the follicle at the prepupal stage (Plate I-4). These cysts I broke and the spermatocytes degenerated into granules at an early pupal stage. A group of abnormal spermatogonial and spermatocytal cyst I were observed in the upper part of the follicle at the middle pupal stage, most of them degenerating at the end of the pupal stage. Furthermore, abnormal eupyrene sperm bundles I and II were observed in the lower part of the follicle at the end of the pupal stage. It seems that the bundles I became abnormal in the step of eupyrene spermatidal cyst, because formative process as observed in *B. mori* did not occur.

Antheraea yamamai

A small number of abnormal spermatogonial cysts were observed in the upper part of the follicle from the end of the 5th instar to the late pupal stage. A group of abnormal spermatocytal cysts I were observed in the upper part of the follicle at the middle pupal stage (Plate II-5) but soon after, these cysts I broke and the degenerated into granules. At the same time, a small number of

abnormal eupyrene spermatidal cysts I were observed in the middle part of the follicle. Furthermore, a small number of abnormal spermatogonia were observed in the upper part of the follicle at the late pupal stage. Abnormal eupyrene sperm bundles I were observed in the lower part of the follicle during the period from the pupa on the 9th day to adult, but the formative process of these bundles I was similar to the case of *P. cynthia ricini*.

The main abnormal germ cells in the three cases described above, except for those which seemed caused by the aging of the cell at the end of the pupal stage, of which only a few were observed, were as follows:

Bombyx mori: Abnormal spermatocytal cysts I were observed along the lateral wall of the follicle at the prepupal stage resulting in abnormal eupyrene sperm bundles I immediately after pupation (Plate I-1 and 2)

Philosamia cynthia ricini: Abnormal spermatogonia, abnormal spermatogonial and spermatocytal cysts I were observed in the upper or whole part of the follicle from the 2nd day of the 5th instar to the middle pupal stage (Plate I-3 and 4)

Antheraea yamamai: A group of abnormal spermatocytal cysts I were observed in the upper part of the follicle at the middle pupal stage (Plate II-b)

Abnormality of the apyrene sperm bundles was not observed in any of the materials.

Judging from the results of the observations described above, abnormal germ cells in *P. cynthia ricini* occurred in much larger quantity than those found in *B. mori* and *A. yamamai*. Although, in each case, the ratio of abnormal to normal germ cells was quite small and a large number of normal eupyrene and apyrene spermatozoa were observed in the vas efferens of the adult. Therefore, even if the abnormal germ cells play any role in spermatogenesis of the normal germ cells, it would seem to be of little significance.

(2) *Antheraea pernyi*

1) Spermatogenesis

Quantitative changes of the spermatocytal cysts containing spermatocytes at the stage of meiotic division and sperm bundles in the follicle during the period from the pupa on Nov. 21 to adult are shown in Fig. 2. A large number of spermatocytal cysts containing eupyrene spermatocytes at the stage of meiotic division had already been observed in the pupa on Nov. 21 and were continuously observed until Feb. 4. These results agree with KAWAGUCHI's report¹⁾ in that the eupyrene spermatocytes at the stage of meiotic division in the diapausing pupae on the second generation of *A. pernyi* were thriving during the period from Jan. to Feb. As described above, QIAN and FENG¹⁰⁾ reported that eupyrene spermatoc-

cytes at the stage of meiotic division were observed for the first time on the 10th day of the 5th instar of the second generation in diapause-type *A. pernyi*. Therefore, it is suggested that a large number of spermatocytal cysts containing eupyrene spermatocytes at the stage of meiotic division had existed during the period from the 5th instar to the pupa of Nov. 21. In this study, a decrease of such cysts was observed in the pupa, concurrent with the increased of temperature between Mar. 1 and 25. However, they increased again in the pupa on the 5th day of incubation, and thereafter, there was a decrease accompanied by a transfer to eupyrene sperm bundles. Therefore, the time of meiotic division of the eupyrene spermatocytes was distinguished by two steps. A large number of eupyrene sperm bundles had already been observed in the pupa on Nov. 21 as described above, and this state continued until the pupa on the 5th day of incubation. WAKU²¹⁾ observed a small number of eupyrene sperm bundles at the time of pupation in the first generation of diapause-type *A. pernyi*. For this reason it was concluded that eupyrene sperm bundles are formed at the time of pupation. However, in this study, a large number of eupyrene sperm bundles degenerated in the pupa on the 10th day of incubation. They were observed again in the pupa on the 15th day of incubation and a large number of them appeared from the follicle to the vas efferens during the period from the pupa on the 20th day of incubation to adult. Therefore, the period of morphogenesis of the eupyrene spermatozoa was also divided into two steps, similar to the period of meiotic division of the eupyrene spermatocytes. In contrast, the periods of meiotic division and morphogenesis of the apyrene spermatozoa did not have two distinct stages, differing markedly from the case of eupyrene spermatozoa. A large number of spermatocytal cysts containing apyrene spermatocytes at the stage of meiotic division were observed in the pupa on the 5th day of incubation, and thereafter, they decreased accompanied by a transfer to the apyrene sperm bundles. A large number of apyrene sperm bundles were observed in the pupa on the 10th day of incubation, and a small number of them began to appear from the follicle to the vas efferens during the period from the pupa on the 5th to 10th day of incubation. The apyrene sperm bundles in the follicle gradually decreased in the period from the pupa on the 20th to 25th day of incubation, a large number of them appearing from the follicle.

From the above results, it seems that many of the eupyrene spermatozoa formed originally degenerated until the pupa on the 10th day of incubation. But new eupyrene spermatozoa were formed from the eupyrene spermatocytes at the stage of meiotic division in the pupa on the 5th day of incubation. Therefore, many of new eupyrene spermatozoa were observed in the pupa on the 15th day of incubation. It can be said that the new eupyrene spermatozoa were formed from the spermatocytes which had undergone meiotic division during the pupal period. Apyrene spermatozoa formed later than eupyrene spermatozoa in *B. mori*²³⁾ but from the results, it can be concluded that apyrene spermatozoa were

formed earlier than reformed eupyrene spermatozoa in diapause-type *A. pernyi*.

2) Abnormal germ cell

In *Antheraea pernyi*, the following abnormal germ cells were observed in the follicle in addition to the ones in the case of *Bombyx mori*, *Philosamia cynthia ricini* and *Antheraea yamamai*.

- (g) Abnormal spermatocytal cyst II: Arrangement of cells in the cyst was indefinite, various abnormal substances or blank parts existed in the cyst
- (h) Abnormal eupyrene spermatidal cyst II: Consisted of irregularly formed cells
- (i) Abnormal eupyrene sperm bundle III: A thick fibriform substance existed in the tail part

QIAN and FENG¹⁰ reported that the germ cells in the diapause-type in the second generation of *A. pernyi* degenerated markedly, with abnormal spermatogonia and abnormal spermatocytes being observed during the period from the 2nd to the 5th instar. In this study, abnormal spermatogonia and abnormal spermatogonial cysts were observed in the upper part of the follicle during the period from pupa on Nov. 21 to adult but these were few except on the 4th day after incubation, when there was an increase observed. Abnormal spermatocytal cysts I were observed along the lateral wall of the follicle in the pupa from Nov. 21, an especially large number of them being observed in the pupa between Mar. 1 and 25, which rapidly degenerated in the pupa after incubation. A small number of abnormal spermatocytal cysts II were observed in the upper and middle part of the follicle interspersed among the normal spermatocytal cysts in the pupa from Nov. 21. These increased somewhat between Mar. 1 and 25 (Plate II-6) but rapidly degenerated in the pupa after incubation. QIAN and FENG¹⁰ reported that the degeneration of spermatocytes took place before meiotic division. In this study, the state of abnormal meiotic division was not confirmed, but a decrease of spermatocytal cysts containing eupyrene spermatocytes at the stage of meiotic division was observed in the pupa between Mar. 1 and 25, as described above. These results show that a part of spermatocytes before meiotic division in the pupa degenerated along with the increased temperature, though a further study is necessary to confirm whether the state observed was of abnormal meiotic division of spermatocytes or not. Abnormal eupyrene spermatidal cysts II were observed in the lower part of the follicle in the pupa from Nov. 21. These increased in the pupa in the period from Jan. 10 to Mar. 25 and rapidly degenerated in the pupa after incubation. A small number of abnormal eupyrene sperm bundles II were observed in the lower part of the follicle in the pupa on Mar. 25. Also a large number of abnormal eupyrene sperm bundles I, II and III were observed in the pupa on the 10th day of incubation (Plate II-7). As described above, this time corresponds to a period of degeneration of normal eupyrene sperm bundles. Furthermore,

the formative process of the bundles I was similar to the case of *P. cynthia ricini* and *A. yamamai*. Abnormality and degeneration of the germ cells, as described above, was supported in that many degenerate substances of the germ cells in the follicle were observed in the pupa during the period from Mar. 1 to the 10th day after incubation. On the other hand, abnormal apyrene sperm bundles were not recognized.

As mentioned above, a large number of abnormal germ cells were observed in the follicle of the diapausing male pupae at normal room temperature and the period from the pupa after incubation to adult. However, eupyrene spermatozoa re-formed as described above, therefore, a large number of new eupyrene spermatozoa were observed above the basement membrane of the testicular follicle and a large number of apyrene spermatozoa were observed in the vas efferens in the pupa on the 19th day of incubation (Plate II-8), and a large number of normal eupyrene and apyrene spermatozoa were observed in the vas efferens in the adult. From the above results, it seems that the presence of a large number of abnormal germ cells play an important role in the re-formation of eupyrene spermatogenesis. It was, however, unclear as to whether abnormal germ cells had the same effect on apyrene spermatogenesis as was noted on eupyrene spermatogenesis.

Abnormality and degeneration of the germ cells of lepidopterous insects which undergo diapause in pupa in addition to *A. pernyi* was reported by some investigators. According to SCHMIDT and WILLIAMS¹⁸⁾, a part of the germ cells of the diapausing male pupae in *Hyalophora cecropia* showed growth to spermatids in the prepupal period but later degenerated. Whereas later, in the diapause period, no spermatids were seen, spermatogonia and primary spermatocytes were observed and the meiotic division occurred rapidly with a shift to spermatozoa taking place after the diapause termination. NUMATA and HIDAOKA¹⁹⁾ reported that the spherical spermatid began to degenerate on the first day of the 5th instar in diapause-type of *Papilio xuthus* and the spermatogenesis stopped completely on the 4th day of the 5th instar. Degeneration occurred in all the spermatidal cysts and sperm bundles at the pupal stage. NISHITATSUJI-UWO¹¹⁾ tentatively ascribed the cause of degeneration of spermatocytes in diapause-type *P. xuthus* to the deficiency of the growth and differentiation hormone. It was not clearly evidenced by the implantation experiments of testis and endocrine organs¹²⁾. The abnormality and degeneration of the germ cells in *A. pernyi* of diapause-type in this study were similar to the case of *H. cecropia* and *P. xuthus*. From the above results and reports, it seems that abnormality and degeneration of the germ cells are closely related to the diapause of the pupa.

3. Time of appearance from the testicular follicle to the vas efferens of the eupyrene and apyrene sperm bundles

(1) *Bombyx mori*, *Philosamia cynthia ricini* and *Antheraea yamamai*

The order of appearance from the follicle to the vas efferens of the spermatozoa was reported in *B. mori*³⁾ and *A. yamamai*⁴⁾; specifically, the apyrene spermatozoa appeared from the follicle earlier than the eupyrene spermatozoa. This order was the same in the case of *P. cynthia ricini*. The time of commencement of appearance from the follicle of the apyrene and eupyrene spermatozoa compared with each material in Fig. 1. The results show that in *B. mori*, the time for the former was the pupa on the 6th day (the 5th day before emergence) and for the latter was the pupa on the 8th day (the 3rd day before emergence). In *P. cynthia ricini*, the time for the former was the pupa on the 11th day (the 2nd day before emergence) and for the latter was the pupa on the 12th day (the last day before emergence), *A. yamamai* showed the time for the former was the pupa on the 16th day (the 8th day before emergence) and for the latter was the pupa on the 19th day (the 5th day before emergence). From the above results, comparing each material for the time of commencement of appearance from the follicle of both spermatozoa, based on the time of emergence, it was seen that in the case of *P. cynthia ricini*, it was later than that of *B. mori* and *A. yamamai*. This appears to be because of the period of accumulation of the eupyrene spermatozoa in *P. cynthia ricini* being shorter than that of *B. mori* and *A. yamamai* as stated previously.

(2) *Antheraea pernyi*

The order of appearance from the follicle to the vas efferens of the spermatozoa was the same as in *B. mori*, *P. cynthia ricini* and *A. yamamai*, as described above. The time of commencement of appearance from the follicle of the spermatozoa was not definite with respect to the interval of fixed time of the testis was long. However, the period for the commencement of appearance was from the pupa on the 5th to 10th day of incubation (from the 10th to 15th day before emergence) in apyrene spermatozoa, and from the pupa on the 20th to 25th day of incubation (from the 5th day before emergence to the day of emergence) in eupyrene spermatozoa (Fig. 2). Therefore, it is clear that the time which apyrene spermatozoa begin to appear from the follicle in *A. pernyi* is earlier than *B. mori*, *P. cynthia ricini* and *A. yamamai* based on the time of emergence. This seems to be related to the fact that the apyrene spermatozoa were formed earlier than the reformed eupyrene spermatozoa, as previously stated.

From the results, it was clear that the difference in the developmental process of the germ cell between *B. mori*, *P. cynthia ricini*, and *A. yamamai* (which do not undergo diapause in pupa) and in *A. pernyi* (which does undergo diapause in pupa) also effects the time of the commencement of appearance from the follicle to the vas efferens of the spermatozoa.

Summary

The spermatogenesis and abnormal germ cells in *Bombyx mori*, *Philosamia cynthia ricini* and *Antheraea yamamai*, which do not undergo diapause in pupa, were studied histologically compared to those in *Antheraea pernyi* which does undergo diapause in pupa. The observation was carried out during the period from the 5th instar to adult on *Bombyx mori*, *Philosamia cynthia ricini* and *Antheraea yamamai*, and during the periods of the pupa from Nov. 21 to Mar. 31 at normal room temperature, and the pupa from Apr. 1 to adult under incubation on *Antheraea pernyi*.

1. In each case the apyrene spermatocytes at the time of meiotic division did not progress to the leptotene stage. The point of differentiation and determination of the eupyrene and apyrene spermatozoa occurred between the spermatogonia and leptotene stages of the spermatocytes.

2. The state of meiotic division was different in eupyrene and apyrene spermatocytes in each material observed.

3. In *Bombyx mori*, *Philosamia cynthia ricini* and *Antheraea yamamai*, the eupyrene and apyrene spermatozoa were formed normally and only a few abnormal germ cells were observed compared with *Antheraea pernyi*. Therefore, even if the abnormal germ cells do play any role in spermatogenesis, it seems that the significance of that role is small.

4. In the diapausing male pupae of *Antheraea pernyi*, a large number of abnormal germ cells and degenerated eupyrene spermatozoa were observed. However, new eupyrene spermatozoa were re-formed. Therefore, results indicate that abnormal germ cells play an important role in the re-formation of eupyrene spermatozoa. On the other hand, no resulting abnormalities of the apyrene spermatozoa could be detected.

Acknowledgement

The author wishes to thank Prof. K. TANAKA, Faculty of Textile Science and Technology, Shinshu University; Dr. H. FUGO, Faculty of Agriculture, Tokyo University of Agriculture and Technology; and Dr. F. NAKAJIMA, Nagano-ken Sericultural Experiment Station who kindly offered the materials for the present investigation.

This work was supported in part by Grant-in-Aid for Scientific Research [Co-operative Research (A) No. 62304016] (Rep. Dr. KATSUNO S.) from the Ministry of Education, Science and Culture, Japan.

Literature Cited

1. DEDERER, P. H.: Spermatogenesis in *Philosamia cynthia*. *Biol. Bull.* **13**: 94-106. 1907
2. KATSUNO, S.: Abnormal sperm bundles in the silkworm, *Bombyx mori* L. *J. Seric. Sci. Jpn.* **45**: 528-532. 1976 (in Japanese)
3. KATSUNO, S.: Studies on eupyrene and apyrene spermatozoa in the silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae) I. The intratesticular behaviour of the spermatozoa at various stages from the 5th instar to the adult. *Appl. Ent. Zool.* **12**: 142-153. 1977
4. KATSUNO, S.: The relation of the behavioral and morphological changes of the spermatozoa in the male reproductive organ to fertilization of *Antheraea yamamai* (Lepidoptera: Saturniidae). *J. Fac. Agr. Hokkaido Univ.* **61**: 272-278. 1983
5. KATSUNO, S.: Time of differentiation and determination of eupyrene and apyrene spermatozoa and resulting spermatogenesis in the silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae). *Appl. Ent. Zool.* **22**: 13-18. 1987
6. KATSUNO, S. and F. NAKAJIMA: Relation between the hibernation temperature and the fertility of male pupae of the chinese oak silkworm, *Antheraea pernyi* GUÉRIN (Lepidoptera: Saturniidae). *Appl. Ent. Zool.* **22**: 105-107. 1987
7. KAWAGUCHI, E.: Cytological study of the silkworm, *Bombyx mori* L. and their relatives II. Spermatogenesis of *Antheraea yamamai* GUÉRIN, *Antheraea pernyi* GUÉRIN and their hybrid. *Jap. J. Genet.* **10**: 135-151. 1934 (in Japanese)
8. LEVIATAN, R. and M. FRIEDLÄNDER: The eupyrene-apyrene dichotomous spermatogenesis of Lepidoptera. I. The relation with postembryonic development and the role of the decline in juvenile hormone titer toward pupation. *Dev. Biol.* **68**: 515-524. 1979
9. MACHIDA, J.: Fine experimentelle Untersuchung über die apyrene Spermatozoen des Seidenspinners *Bombyx mori* L. *Z. Zellforsch. mikro. Anat.* **9**: 466-510. 1929
10. MACHIDA, J.: Spermatogenesis of the silkworm, *Bombyx mori* L. with special concern to the cytoplasmic materials and spermatogenesis. *Zool. Mag.* **35**: 521-534. 1935 (in Japanese)
11. NISHITSUTSUJI-UWO, J.: Developmental features of the male germ cells in the prospective diapausing and non-diapausing swallowtails, *Papilio xuthus*. *Mem. Coll. Sci. Univ. Kyoto Ser. B.* **26**: 9-14. 1959
12. NISHITSUTSUJI-UWO, J.: Further studies on developmental features of the male germ cells in the prospective diapausing and non-diapausing swallowtails, *Papilio xuthus*. *Annot. Zool. Japon.* **34**: 30-37. 1961
13. NUMATA, H. and T. HIDAHA: Development of male sex cells in the swallowtail, *Papilio xuthus* L. (Lepidoptera: Papilionidae) in relation to pupal diapause. *Appl. Ent. Zool.* **15**: 151-158. 1980
14. POLLISTER, A. W.: Centrioles and chromosomes in the atypical spermatogenesis of *Vivipara*. *Proc. Nat. Acad. Sci. U. S. A.* **25**: 189-195. 1939
15. POLLISTER, A. W. and P. F. POLLISTER: The relation between centriole and centomere in atypical spermatogenesis of *Viviparid* snails. *Ann. N. Y. Acad. Sci.* **45**: 1-48. 1943
16. QIAN, H. and S. FENG: General text book of chinese tusser perny silk moth. Shengyang Agriculture College eds. Nōgyōshipanscha, Pekin pp. 251-257 1981 (in Chinese)
17. SADO, T.: Spermatogenesis of the silkworm and its bearing on radiation induced sterility. I. *J. Fac. Agric. Kyushu Univ.* **12**: 359-385. 1963
18. SCHMIDT, E. L. and C. M. WILLIAMS: Physiology of insect diapause. V. Assay of the

- growth and differentiation hormone of Lepidoptera by the method of tissue culture. *Biol. Bull.* **105**: 174-187. 1953
19. SUGAI, E.: The effect of starvation on silkworm, *Bombyx mori* L., Spermatogenesis. *Zool. Mag.* **75**: 34-39. 1966 (in Japanese)
 20. SUMIMOTO, K.: Development of the male sex cells in the eri-silkworms *Philosamia ricini* Bds. during their metamorphosis. *Mem. Fac. Text. Fib. Kyoto Univ. Indust. Arts Text. Fib.* **4**: 345-350. 1965 (in Japanese)
 21. WAKU, Y.: Seasonal change of the pupal respiratory metabolism in the chinese oak-silkworm, *Antheraea pernyi* GUÉRIN, with special regard to its hibernation and diapause. *Sci. Rep. Tōhoku Univ. Ser. 4. Biol.* **23**: 101-118. 1957

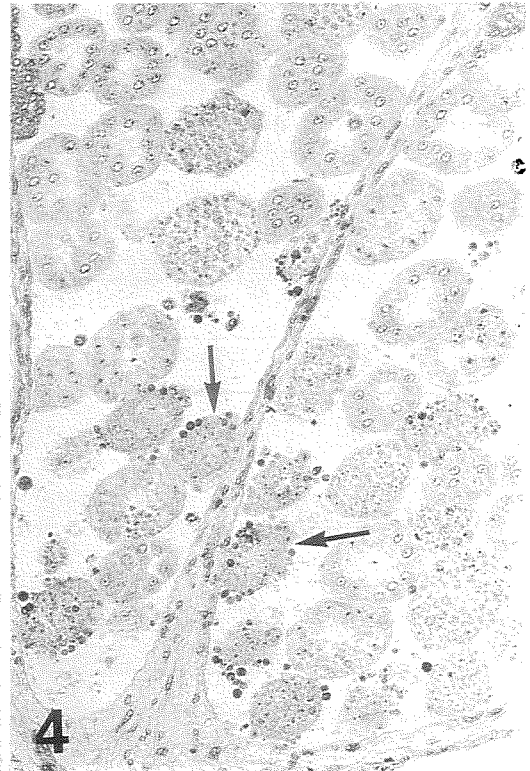
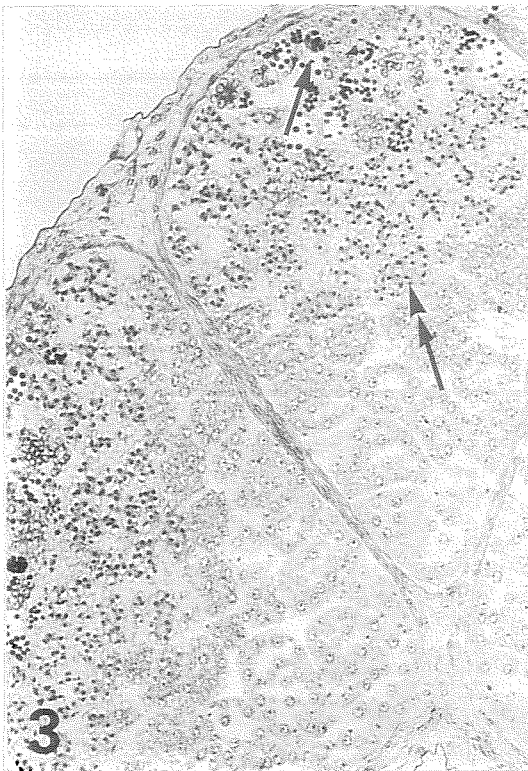
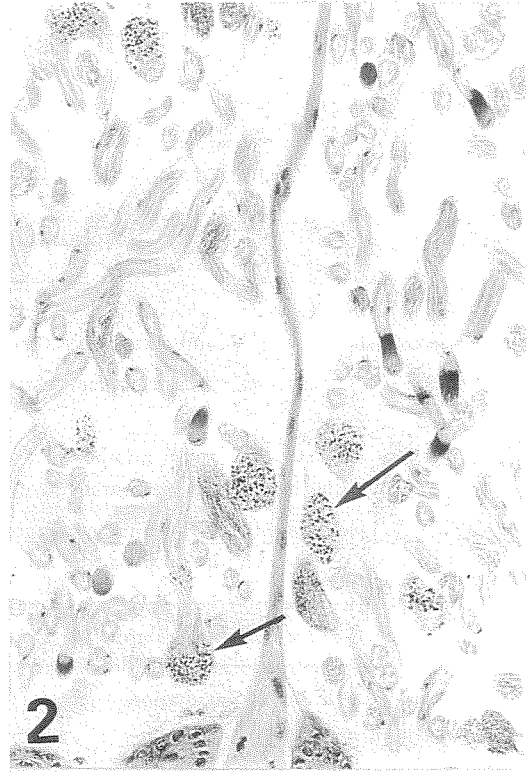
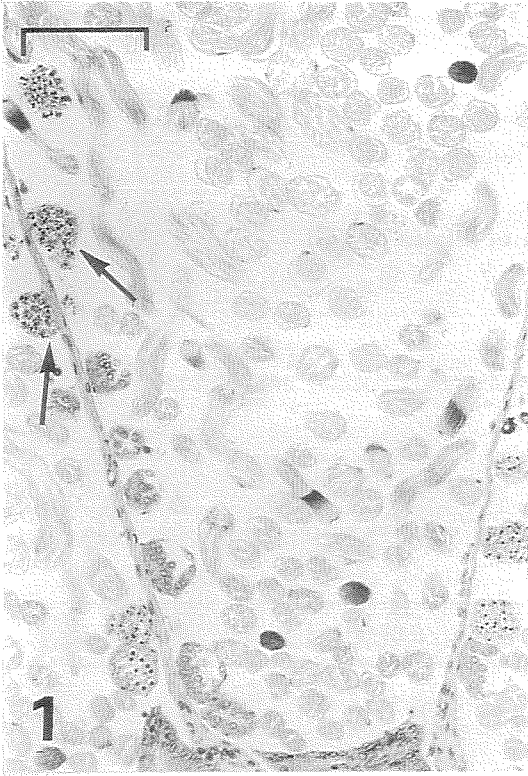
Explanation of Plates

Plate I

Abnormal germ cells

1. *B. mori*: Abnormal spermatocytal cysts I (arrows) at the prepupal stage.
2. *B. mori*: Abnormal eupyrene sperm bundles I (arrows) immediately after pupation.
3. *P. cynthia ricini*: Abnormal spermatogonia (single arrow) and abnormal spermatogonial cysts (double arrow) on the 3rd day of the 5th instar.
4. *P. cynthia ricini*: Abnormal spermatocytal cysts I (arrows) at the prepupal stage.

Scale (1-4): 100 μ m.



Explanation of Plates

Plate II

Abnormal germ cells and normal spermatozoa

5. *A. yamamai*: Abnormal spermatocytal cysts I (arrows) at the middle pupal stage.
6. *A. pernyi*: A small number of abnormal spermatocytal cysts II (arrows) mix with the normal spermatocytal cysts on Mar. 1.
7. *A. pernyi*: A large number of abnormal eupyrene sperm bundles (arrows) in the pupa on the 10th day of incubation.
8. *A. pernyi*: A large number of new eupyrene spermatozoa (single arrow) above the basement membrane of the testicular follicle and a large number of apyrene spermatozoa (double arrow) in the vas efferens in the pupa on the 19th day of incubation.

Scale (5-8): 100 μ m.

