Development of Male Sex Cells in the
Swallowtail, *Papilio xuthus* L.
(Lepidoptera : Papilionidae) after the
Termination of Diapause¹

Hideharu NUMATA and Toshitaka HIDAKA

Department of Zoology, Faculty of
Science, Kyoto University, Sakyo,
Kyoto 606, Japan.

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As reported in the previous paper (NUMATA and HIDAKA, 1980), spherical spermatidal cysts begin to degenerate in fifth-instar larvae of *Papilio xuthus* L. which are destined to enter pupal diapause. At the pupal stage, spermatogenesis stops completely and degeneration occurs in all spermatidal cysts and sperm bundles. In diapausing pupae, living sex cells in the testes are limited to spermatogonial cysts and spermatocytal cysts.

In the present paper, testicular development and spermatogenesis were studied in pupae which had resumed the adult development after the termination of diapause.

Eggs of *P. xuthus* were incubated at 25°C under a 16-hr photoperiod. The larvae hatching from them were reared on an artificial diet (KAMANO, 1965: slightly modified) at 25°C under a 12-hr photoperiod. Larvae reared under this condition invariably developed into diapause pupae (HIDAKA and HIRAI, 1970). The diapause pupae obtained were kept at 25°C under a 16-hr photoperiod for 15–20 days. Then the pupae were stored

at 5°C for 100 days. After chilling, the pupae were transferred to 25°C and a 16-hr photoperiod to stimulate adult development. The period from transfer to 25°C to adult emergence in male pupae was about 14 days.

Testicular development was examined at the following five stages: 0, 2, 5, and 9 days after the transfer from 5°C to 25°C, and 0–1 day before adult emergence (at this stage the adult color pattern is seen through the pupal cuticle).

The testes taken from each insect were placed on a depression slide containing 0.9% NaCl solution. The length and width of each testis were measured with an ocular micrometer, and the volume, V , of each testis was calculated by using the following formula, assuming that the testis is an ellipsoid (NISHITSUTSUJI-UWO, 1959): $V = \pi LW^2/6$ where L and W are length and width of the testis, respectively.

Each testis was split with fine forceps and the spermatocysts obtained were suspended in 0.9% NaCl solution. A drop of this suspension was placed on a grated slide glass (1 mm squares) and covered with a coverslip. One hundred spermatocysts from each testis were examined and classified into seven developmental groups: 1) spermatogonial cysts, 2) primary and secondary spermatocytal cysts, 3) spherical eupyrene spermatidal cysts, 4) elongated eupyrene spermatidal cysts, 5) completed eupyrene sperm bundles, 5') degenerated eupyrene sperm bundles, and 6) apyrene spermatidal cysts and apyrene sperm bundles (NUMATA and HIDAKA 1980).

When the pupae were transferred to 25°C and a 16-hr photoperiod, the volume of the testis began

TABLE 1. TESTIS SIZE AND FUSION OF TESTES IN PUPAE OF *P. xuthus*
AFTER TERMINATION OF DIAPAUSE^a

Days after transfer to 25°C	Testis size (mm ³) mean ± S.D.	Fusion of testes		
		None	Incomplete	Complete
0	0.758 ± 0.189	10	0	0
2	1.09 ± 0.21	8	2	0
5	1.48 ± 0.59	2	3	5 ^c
9	2.03 ± 0.48	0	1	9 ^c
BE ^b	1.82 ± 0.28	0	0	10 ^c

^a Number of animals: 10 for each stage group.

^b 0–1 day before adult emergence.

^c Half of the testis volume after fusion was taken as the testis size of these individuals.

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Table 2. DEVELOPMENTAL STAGES OF SPERMATOGENESIS IN PUPAE OF *P. xuthus* AFTER TERMINATION OF DIAPAUSE^a

Days after transfer to 25°C	Percentage of spermatocysts ^c						
	1	2	3	4	5	5'	6
0	50.7	49.0	0.3	0	0	0	0
2	41.2	52.7	3.4	2.6	0	0	0.1
5	25.3	55.6	2.7	5.8	5.1	0	5.5
9	15.7	38.3	2.0	4.2	13.5	4.7	21.6
BE ^b	14.6	24.5	0.7	1.6	11.1	8.7	38.8

^a Number of animals: 10 for each stage group.

^b 0-1 day before adult emergence.

^c 1: spermatogonial cysts

2: primary and secondary spermatocytal cysts

3: spherical eupyrene spermatidal cysts

4: elongated eupyrene spermatidal cysts

5: completed eupyrene sperm bundles

5': degenerated eupyrene sperm bundles

6: apyrene spermatidal cysts and apyrene sperm bundles

to increase and the pair of testes in each insect were fused to form a single body within nine days (Table 1). The right and left testes were reported to be still separated without fusion in 6- or 7-day-old adult of *Bombyx mori* (ÔMURA, 1936). However, the testes in each insect were fused at the prepupal stage in *Mamestra brassicae* (SANTA and OTUKA, 1955) and in *Hyphantria cunea* (SUGAI and TERAMINE, 1970), regardless of whether the individual was destined to become a diapause pupa or a non-diapause pupa. In *P. xuthus*, fusion of testes was observed on the third or fourth day of non-diapause pupae (NUMATA and HIDAKA, 1980), or after the termination of pupal diapause. It is certain that the fusion of testes occurs as the adult development proceeds in this insect.

After the transfer to 25°C the sex cells in each testis were promptly differentiated (Table 2). It is clear that sperms developed *de novo* from the spermatogonia and spermatocytes, which were saved from degeneration, in diapause-terminated pupae. Although a small number of degenerated eupyrene sperm bundles were observed in two later stages, no degeneration of spermatidal cysts was observed after the development was resumed.

Eupyrene spermatogenesis started earlier than apyrene spermatogenesis (Table 2). In a number of lepidopterans, eupyrene spermatogenesis proceeds during the last larval instar and slows down after pupation, while apyrene spermatogenesis proceeds at the pupal stage (e.g. MACHIDA, 1929,

in *Bombyx mori*; FRIEDLÄNDER et al., 1981, cation, in *Actias selene*). FRIEDLÄNDER et al. (1981) concluded that the switchover from eupyrene to apyrene spermatogenesis is related to an apyrene spermatogenesis-inducing factor (ASIF) becoming active toward pupation by the decline of JH titer in *A. selene*. Further study is needed to elucidate whether a similar mechanism is related to spermatogenesis in *P. xuthus*.

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