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# Developmental Features of the Male Germ Cells in the Prospective Diapausing and Non-Diapausing Swallowtails, *Papilio xuthus*

By

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The pupa of swallowtail, *Papilio xuthus* happens to become diapause or non-diapause according to the conditions in which the larva is experienced. Therefore, the establishment of the pupal diapause or non-diapause can be controlled by altering the breeding conditions in the larval stages (ISHIZAKI and KATO, 1956). In general, when the larva is constantly kept in darkness and in low temperature, it turns out the orange pupa and invariably enters the long diapause, while, when the larva is reared in the conditions of constant light and high temperature, the green pupa of non-diapause type appears. When the larva is reared in the normal laboratory conditions and then subjected to the constant darkness and low humidity at the time of pupation, it becomes brown pupa of non-diapause type. The correlation between the pupal colour and diapause, however, is not universal; even the pupae of green and brown types sometimes enter the diapause.

At any rate there are no visible differences in the larval stages. But that the outside conditions exert an influence upon the larvae or pre-pupae to become diapausing or non-diapausing implies that there occurs a serious change in the internal physiological condition of the worm at these stages.

The present work is aimed to examine this change of the physiological conditions with reference to the development of the testes of both types from the third instar to the late pupal stage.

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## Material and Method

Eggs and the first instar larvae were collected in the field from the end of September and were divided into two groups. In one group they were subjected to the constant illumination of fluorescent lamp (non-diapause series) and in the other group they were kept in the dark container (diapause series). In autumn these conditions are effective to produce 100 per cent of diapause or non-diapause pupae.

Larvae from the third instar were arbitrarily classified into 5 classes and pupae into 3.

The length of testis was measured with an ocular micrometer and the volume was estimated roughly by the following formula, on the assumption that the testis is ellipsoid;

$$V = \frac{\pi}{6} LW^2$$

where V indicates the volume of testis; L and W represent major and minor axes of the testis respectively.

The live testis was teared into sufficiently small pieces with the fine pincettes in 0.9% NaCl solution. Then, a drop of suspension of the spermatocysts thus made was transferred on to a graduated glass (15/15 mm.) and covered with a coverslip. More than 100 spermatocysts were examined in each testis and classified into six grades with respect to the advance of spermatogenesis; 1) spermatogonial cyst, 2) primary spermatocytal cyst, 3) secondary spermatocytal cyst, 4) spherical spermatidal cyst, 5) elongating spermatidal cyst and 6) elongated spermatidal cyst more than twice as long as wide.

## Experimental Results

1. *External change of the testis.*

A pair of testes of *Papilio xuthus* is located in the fifth abdominal segment at both sides of the dorsal vessel through the larval stage to the early pupal one. When the adult formation is ready to occur, a pair of testes conjugates each other to form a single body. This behaviour of the testes does not show any difference between the diapause and non-diapause series. A conspicuous difference, however, appears in the size of testis between the two series. The numerical values of the measurements are summarized in Table 1.

As is seen in the table, a difference in testicular size between the non-diapause and diapause series occurs first in the late stage of the third instar. And roughly speaking, the testis-volume of the former becomes twice as large as that of the latter and the same ratio is maintained throughout the whole larval stage to the time one or two days after pupation. Within four or five days after pupation, however, the testis of the non-diapause pupa grows rapidly and reaches to the maximal volume, 5.8 $mm^3$. This volume is about 2.4 times bigger than that of the diapause, even when the separate testes of the latter are added together. But, the testis of the non-diapause pupa becomes somewhat smaller till the time of emergence, while a pair of testes of the diapause pupa reaches to about 2 times bigger when observed 60–70 days later than those at the time of pupation.

Table 1. Size of the testes of larvae and pupae in the non-diapause and diapause series.

| Class | Type | No. of specimens | Minor axis | Major axis | Volume | $\frac{V_{non\text{-}diap.}}{V_{diap.}}$ |
|---|---|---|---|---|---|---|
| Late stage of 3rd instar | non-diap.<br>diap. | 8<br>6 | 0.48 mm<br>0.40 | 0.79 mm<br>0.63 | 0.09<br>0.05 | 1.8 |
| Early stage of 4th instar | non-diap.<br>diap. | 11<br>9 | 0.58<br>0.43 | 0.81<br>0.66 | 0.14<br>0.06 | 2.3 |
| Late stage of 4th instar | non-diap.<br>diap. | 7<br>10 | 0.83<br>0.69 | 1.11<br>1.02 | 0.40<br>0.25 | 1.6 |
| Early stage of 5th instar | non-diap.<br>diap. | 8<br>14 | 1.01<br>0.87 | 1.44<br>1.26 | 0.77<br>0.50 | 1.5 |
| Largest stage of 5th instar | non-diap.<br>diap. | 6<br>10 | 1.35<br>1.11 | 1.83<br>1.64 | 1.75<br>1.06 | 1.7 |
| 1–2 days after pupation | non-diap.<br>diap. | 10<br>6 | 1.38<br>1.23 | 2.24<br>1.61 | 2.23<br>1.28 | 1.7 |
| 4–5 days after pupation | non-diap.<br>diap. | 3<br>4 | 2.20<br>1.19 | 2.30<br>1.60 | 5.80*<br>1.19 | 2.4** |
| 1–2 days before emergence | non-diap. | 5 | 2.00 | 2.22 | 4.65* | / |
| 60–70 days after pupation | diap. | 7 | 1.49 | 2.43 | 2.82 | / |

* This value indicates the volume of the testis after conjugation.
** This value indicates V-non-diap./2V-diap.

## 2. *Internal differentiation of the testis.*

That the difference in the volume of the testis between the non-diapause and diapause forms is correlated with the degree of differentiating state of the cyst was already pointed out by SANTA and OTUKA with the cabbage armyworm, *Barathra brassicae* L. (1955). They drew the conclusion on the base of the histological observation. But, this method seems to be unsuitable for a statistical comparison between the diapause type and the non-diapause one because of the difficulty to get the random samplings. Here, the live cysts randomly sampled from the testis suspension prepared by the way described above was directly counted under the microscope. The results are summarized in Fig. 1, data of which were obtained from 10–20 specimens in every class.

In the early stage of the third instar, the germ cells do not seem to begin the differentiation in the diapause as well as in the non-diapause series. In the late stage of this instar of non-diapause series, however, spermatogenesis proceeds in general from the gonium to the cyte in a large number of the cysts, and in one specimen even the spherical spermatids appear in addition. On the other hand, in 3 out of 7 individuals of the diapause series, only a few spermatogonial cysts develop into the primary spermatocytal cysts,

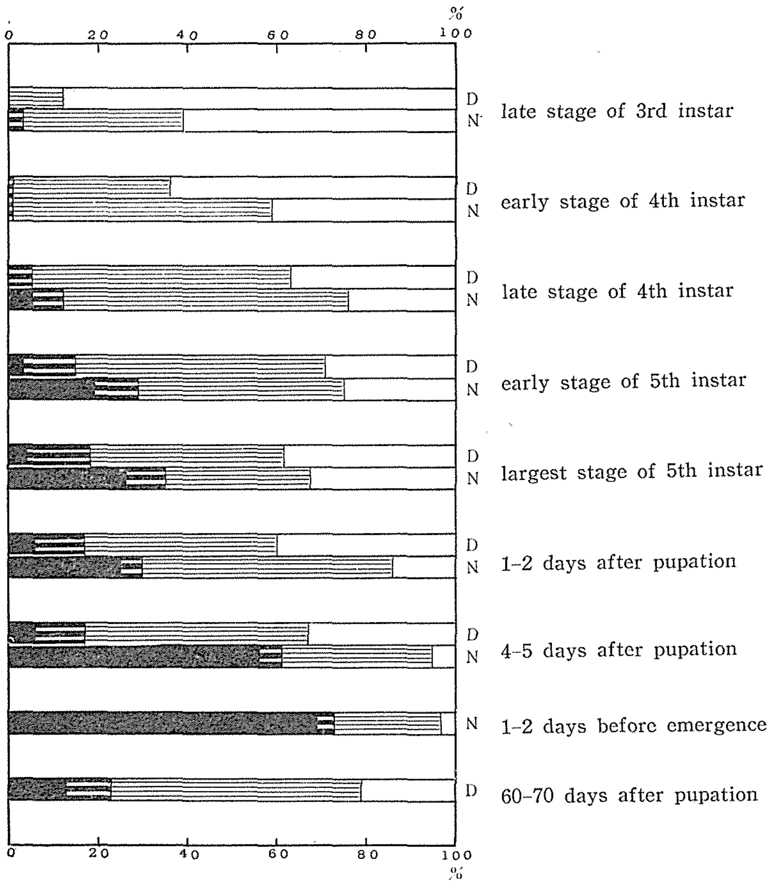


Fig. 1. Comparison of the developmental state of the spermatocysts found in larvae and pupae of diapause and non-diapause series.
Black: elongated spermatidal cyst; thick line: spherical spermatidal cyst; thin line: primary and secondary spermatocytal cyst; blank: gonial cyst.
D: diapause series; N: non-diapause series.

In the early stage of the fourth instar, a fairly large number of the secondary spermatocytal cysts appears and a few cysts develop into the spherical spermatidals in both series. But the spermatocytal cysts in diapause series are less in number than those in non-diapause series. In the late stage of this instar, there are found more spherical spermatidal cysts in both series, and in non-diapause series there occur some elongated spermatidal cysts. It is a special feature of the diapause series that the degeneration of the spermatocytes and spermatids takes place in many cysts from this stage on.

When the larva enters the fifth instar, there occur many cysts containing the elongated spermatids and even the sperms in the non-diapause series. In the diapause series, the spherical spermatidal cysts increase in number but the elongated spermatidals are yet very few.

Afterwards, in the non-diapause series the spermatogenesis proceeds gradually with the advance of the stage, while in diapause series the spermatogenesis stops almost completely and a tremendous degeneration occurs in almost of all the cysts of the spherical spermatids. The survivals in this catastrophe are confined to the cysts which contain the gonia, the cytes and the elongated tids.

The results so far obtained indicate that the spermatogenesis in the diapause series is delayed in comparison with that in the non-diapause series.

## Discussion

The present examination has revealed that the difference in the size of the testis is closely connected with the proceeding of the spermatogenesis. From the late stage of the third instar, the difference in the testicular size appears between the diapause and non-diapause series. The testis of the non-diapause series becomes twice as large as that of the diapause one, and this difference of the size is maintained throughout the whole larval stages. Internally, the difference in the testicular development begins also in the late stage of the third instar and becomes more marked with the progress of instar. In the largest stage of the fifth instar the occurrence rate of the elongated spermatidal cysts in the non-diapause series reaches to about 6.5 times higher than that in the diapause one.

A similar finding has also been reported with cabbage armyworm, *Barathra brassicae*, by SANTA and OTUKA (1955).

In the experiment of SCHMIDT and WILLIAMS (1953), it is clear that in the *Cecropia*-silkworm a factor to promote the development of the germ cells resides in the blood of the mature larva or the developing adult. They suggested that this factor is "growth and differentiation hormone" secreted from the prothoracic gland. In the Eri-silkworm, *Philosamia cynthia ricini* "growth and differentiation hormone" seems also to promote the development of the male and female germ cells (NISHIITSUTSUJI-UWO, 1959).

In *Papilio*, the spermatogonial cysts begin to differentiate earlier than in *Philosamia*, in the third instar, but the promotor of these precocious differentiation of the cysts would be also "growth and differentiation hormone" secreted at that time to induce the larval moulting in collaboration with the juvenile hormone coming from the corpora allata.

The facts that the spermatogenesis in the diapause series is delayed in comparison with that in non-diapause one, and that the stop of differentiation of the germ cells throughout the long pupal diapause may be due to the deficient amount of the hormone in question.

The characteristic feature of the testis in the diapause series is the severe degeneration of the cysts containing the spermatocytes and spherical spermatids at the time around pupation. A similar phenomenon has been reported in *Cecropia*-silkworm which has also a long pupal diapause (SCHMIDT and WILLIAMS, 1953). The cells which have undergone the precocious maturation by the pupal period appear to suffer terribly from the ill effect caused by the deficiency of the "growth and differentiation hormone". But the most advanced elongated spermatids can withstand the ill effect and can survive. The reason for these facts is unknown.

## Summary

1. Eggs and the first instar larvae of the swallowtail, *Papilio xuthus* were collected in the field in autumn and divided into two groups so as to rear under different conditions: in one they were subjected to the constant darkness (pupal diapause series) and in the other they were exposed to the constant light (non-diapause series). A comparison of the testicular development between both series was made from the early stage of the third instar to the late pupal stage.

2. The size of the testis did not show any difference between the diapause and the non-diapause series by the early stage of the third instar. But, considerable difference appeared first in the late stage of the same instar, and throughout the the whole larval stages the testis of the non-diapause series was twice as large as that of the diapause one when compared at a given stage.

3. The statistical analysis of the data obtained by the examination of the live cysts demonstrated following points: a) In the late stage of the third instar, spermatogenesis proceeds first from gonia to cytes in a large number of cysts and even the spermatidal cysts appear in some cysts of the non-diapause series, while in the diapause series only a small number of the spermatocytal cysts appears in this stage. b) In the late stage of the fourth instar the elongated spermatidal cysts occur in non-diapause series. In the diapause series, the spherical spermatidals at most begin to elongate in the next fifth instar. c) In the diapause series, there were many degenerating cells in both spermatocytal and spherical spermatidal cysts, especially in the latter, but in non-diapause series such phenomenon was not encountered.

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