

## Early Embryonic Development of the Parasitic Wasp, *Trichogramma chilonis* (Hymenoptera, Trichogrammatidae)

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### Synopsis

The early embryonic development, from fertilization to completion of the blastoderm, of *Trichogramma chilonis* in the eggs of *Papilio machaon hippocrates* and *Cephonodes hylas* was described. In *T. chilonis* the embryonic period lasted about a half day, the larval period about 2 days, and the prepupal and pupal ones about 5.5 days in early summer and autumn. At about 50 min. after oviposition there were found the female and the male pronucleus and the germ cell determinant in the egg. Fertilization finished at about 1 hr after oviposition, and then cleavage occurred. The 1st to the 4th cleavage were synchronous, and in the 5th the synchronism was lost and the formation of syncytial blastoderm began. The blastoderm was completed within 10 hr after oviposition, and about 6 primordial germ cells differentiated in its posterior end. Nucleolar granules were formed by multiplication of the remnant of energids left in the core of egg and the extrusion of daughter cells or nuclei were found in the syncytial and the cellular blastoderm.

### Introduction

Embryological studies on parasitic wasps have been done up to the present, but on the embryonic development of the genus *Trichogramma*, belonging to the superfamily of Chalcidoidea and of considerable economic importance as an egg parasite of insects, there are only few studies, so far as the author is aware.

Silvestri (1908) briefly described on the embryonic and post-embryonic development of *Oophthora semblidis* belonging to the subfamily Trichogrammatinae. Gatenby (1917) fully described on the embryonic development of *Trichogramma evanescence*, however the cleavage stage was not described by him. Krishnamurti (1938) showed photographs of some stages in the embryonic development of *T. minutum*, but he also did not describe them in detail.

The present paper deals with the description of the early stages, namely from fertilization to the completion of blastoderm, of *T. chilonis*.

## Materials and Methods

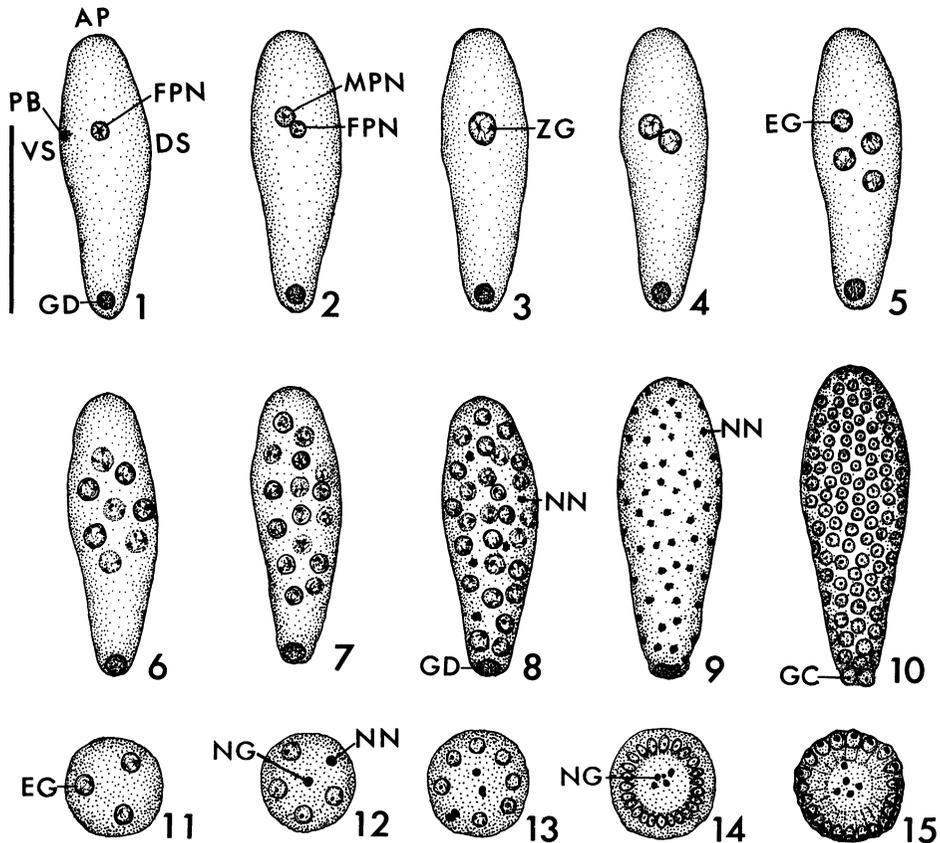
*Trichogramma chilonis* Ishii is an important egg-parasite of lepidopterous insects. It attacked the eggs of *Papilio machaon hippocrates* C. et R. Felder and *Cephonodes hylas* Linné, and many broods of the parasite appeared in early June to October in Gifu Pref., Central Japan.

All materials collected at several parts in Gifu Pref. were used for the present study. The parasitized eggs of *Papilio machaon hippocrates* and *Cephonodes hylas* obtained during June to September of 1981, 1982 and 1983 were kept at room temperature in the laboratory. The females of *T. chilonis* captured in the field during July to September of 1982 and 1983 laid their eggs in the host eggs of the both species mentioned above in the laboratory. These were also kept at room temperature. The host or parasitized eggs containing the parasite eggs at various stages were fixed with Carnoy's or alcoholic Bouin's fluid for 20 to 60 min. After fixing the chorion of host eggs was removed, then they were dehydrated and embedded in paraffin. They were sectioned 6 to 8  $\mu\text{m}$  in thickness and stained with Delafield's or Heidenhain's haematoxylin and eosin.

## Results

### 1. Host eggs and number of parasites within single host egg.

The eggs of *Papilio machaon hippocrates* were approximately spherical in shape, about 1.2 mm by 0.9 mm in size and those of *Cephonodes hylas* were spherical, about 1.2 mm by 1.1 mm. Eggs of these species have a thick chorion. In the normal or non-parasitized eggs of the former species the 1st instar larvae hatched out about 7 days after oviposition, and the latter about 3 days at early summer and autumn. In the infected host egg the size of the yolk globules changed in very tiny around the region where the parasite eggs were found. Most of the parasitized eggs were at the early embryonic stage, from fertilization to the germ band formation, however in rare cases the parasitized eggs or embryos developed to the stage of revolution of the embryo, in which the formation of alimentary canal had been completed. In some cases the parasite eggs were found in the developing parasite larva. The embryonic development of the host eggs was arrested by the parasitism and they were usually turned dark owing to the peculiar blackening of the vitelline membrane just before the parasites reached the prepupal stage. As many as 10 to 30 eggs of *T. chilonis*, average 19, developed in the single egg of *P. machaon hippocrates* and a large majority of adults emerged through the single hole of the chorion of a host egg, while 7 to 25 parasites, average 14, grew and flew out from each egg of *C. hylas*. The maximum number of parasites within a single host egg was 30. Generally 10 to 25 parasites were found as a group, but in rare cases there were only 2 or 3 parasites were seen, and sometimes 2 groups of developing parasites were observed in a single host egg.



Figs. 1-10. Eggs of *Trichogramma chilonis* at various stages of early embryonic development.

1. About a half hr after oviposition. AP anterior pole, DS dorsal side, FPN female pronucleus, GD germ cell determinant, PB polar body, VS ventral side. Scale: 50  $\mu$ m.
2. About 50 min after oviposition. MPN male pronucleus.
3. About 1 hr after oviposition. ZG zygote.
- 4-8. Cleavage stages. EG energid, NN naked nucleus.
9. Syncytial blastoderm stage, all nuclei show synchronous division.
10. Cellular blastoderm stage. GC germ cell.

Figs. 11-15. Cross sections through the middle portion of the egg of *T. chilonis* from cleavage to blastoderm stage.

11. About 16 energids stage. EG energid.
- 12-13. Syncytial blastoderm stage. NG nucleolar granule
- 14-15. Cellular blastoderm stage.

## 2. Duration of embryonic and post-embryonic development of *T. chilonis*

The duration of development, from oviposition to emergence, of *T. chilonis* was about 8 to 9 days at early summer and autumn. The parasite eggs began to develop immediately after oviposition.

The embryonic period was about a half day, the larval 2 days, and the prepupal and pupal period about 5.5 days. The adults emerged about 8 days after oviposition.

Table 1 shows the outline of embryogenesis of *T. chilonis* within the host egg.

Table 1. Time table of the embryonic development of *Trichogramma chilonis* at room temperature (in early summer)

Approximate age of egg	State of development
0 - 1 hr	Fertilization
1 - 4 hr	Cleavage
4 - 10 hr	Blastoderm formation
10 - 15 hr	Germ layer formation and inner layer formation
15 - 20 hr	Stomodaeum, proctodaeum and mid-gut formation
20 - 25 hr	Hatching

## 3. Egg structure of *T. chilonis* at about a half hour after oviposition

The author failed to observe the eggs just deposited. At about a half hour after oviposition the parasite eggs lay in or near the peripheral yolk of the host egg as a group or an egg-mass. The long axis of each parasite egg was directed to the center of the host one, and the anterior pole of the parasite eggs was located outside of the host egg.

Parasite eggs are light ivory in color and elongate with the one-third portion from the anterior pole distinctly expanded. Both poles of them are smoothly rounded and the anterior one is somewhat broader than the posterior, and they are convex dorsally. In fixed and sectioned materials the egg measured about 75  $\mu\text{m}$  by 23  $\mu\text{m}$  in major axes.

The eggs are covered with the thin membrane which is closely associated with the egg surface, but it is very difficult to distinguish the chorion from the vitelline membrane. The spherical egg nucleus or female pronucleus of 5.5  $\mu\text{m}$  in diameter is located at or near the center of the egg, about one-third from its anterior end (Fig. 1). A few chromatin granules are touching on the periphery of the nucleus. Degenerating polar bodies are found at the one-third from the anterior pole of the egg. The darkly stained large body lies near the posterior end of the egg (Fig. 1). This is so-called germ cell determinant and is oval or elliptic in shape, about 5.5  $\mu\text{m}$  by 7  $\mu\text{m}$ . The homogenous ooplasm stained with hematoxylin is observed except for in the posterior pole of the egg in which the germ cell determinant appears. Though the deutoplasmic substances or yolk granules of the host eggs are strongly stained with eosin, the heavily stained yolk granules are not found in the parasite eggs. In these a very thin layer of the periplasm continues with the internal protoplasmic reticulum and neither maturation of the egg nucleus nor formation of the polar body was observed.

#### 4. Fertilization

In the fertilized eggs at about 50 min. after oviposition, there were found three distinct structures, *i. e.*, the female and the male pronucleus and the germ cell determinant (Fig. 2), while in the unfertilized egg only the female pronucleus and the germ cell determinant were observed.

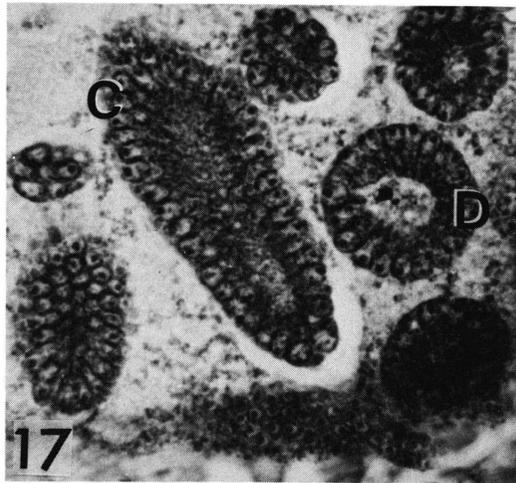
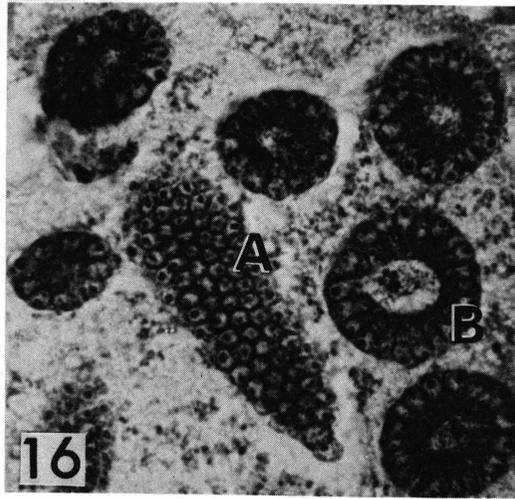
Soon after the female and the male pronucleus come to lie close together at the region about one-third from the anterior pole. At this time one nucleus is larger than the other, and it is thought that the former is a male pronucleus and the latter female one (Fig. 2). With the lapse of time they come to contact with each other, and finally fuse to form a single elliptic nucleus, *i. e.*, zygote, about  $9\ \mu\text{m}$  by  $7\ \mu\text{m}$  in diameter (Fig. 3). The zygote can be distinguished from the small female nucleus of the unfertilized egg. Thus the fertilization is completed at about 1 hr after oviposition.

#### 5. Cleavage

Cleavage begins between 1 hr and 1.5 hr after oviposition, and the course of development is the same in the fertilized and unfertilized eggs. Before beginning of the cleavage the zygote loses the nuclear membrane and becomes a naked nucleus or a chromatin mass. The first cleavage is observed at the site where the conjugation of nuclei have taken place, and two daughter nuclei of equal size are formed (Fig. 4). The direction of the 1st cleavage is less than the right angle to the longitudinal egg axis. In most of eggs consisting single brood the first cleavage begins at the same time, but in a few eggs start of the cleavage was delayed. The 2nd and the 3rd cleavage are completed within 2 hr after oviposition, and 4 to 8 energids appear (Fig. 5 - 6). The direction of the 2nd cleavage is vertical to the egg long-axis. These energids are spherical in shape, about same in size, approximately  $7\ \mu\text{m}$  in diameter. Sixteen energids, about  $5.5\ \mu\text{m}$  in diameter (Fig. 7), formed by the 3rd cleavage are smaller than those in previous cleavages. The spindle of the energid division not be observed at any time of the cleavage stage. The division was synchronous until the 4th cleavage, and it was lost in the 5th one. These 16 energids arrange in the central zone of the egg, and soon after they begin to migrate toward the anterior and the posterior pole of the egg (Fig. 7).

Number of the energids increased to about 30 by the time when they migrated toward the surface of the egg, and finally entered the periplasm (Fig. 8). It does not occur that all energids reach the egg periphery simultaneously. The first penetration of energid into the periplasm occurs at the middle portion of the egg, and then at the anterior and posterior poles. At this time there are a few energids remaining in the core of the egg.

The germ cell determinant retains its original size and position when the energids reach to the egg periphery. During the cleavage stage the eggs retain its original form and size.



Figs. 16-17. Longitudinal and cross sections of *T. chilonis* eggs at syncytial blastoderm stage. A. surface view of the blastoderm, B. cross section through the middle portion of the blastoderm, C, D. longitudinal and cross sections of the same embryos showed in Fig. 16.

## 6. Blastoderm formation

At about 4 hr after oviposition the syncytial blastoderm formation begins. At this time the number of nuclei in the egg periphery is about 30, and each nucleus is about 5 to 6  $\mu\text{m}$  in diameter. The nuclei at the egg surface are almost evenly distributed, while several energids or yolk nuclei remain in the core of the egg. Before the syncytial blastoderm completion the peripheral nuclei divide three times. Though the synchrony of division has been lost at the 5th cleavage, it occurs again in successive divisions, which produce altogether about 230 nuclei (Fig. 9). The syncytial blastoderm formed by the rapid multiplication of nuclei in the periplasm is uniform in thickness throughout the entire periphery of the egg, and its nuclei are spherical and about 5  $\mu\text{m}$  in diameter.

When the syncytial blastoderm is completed, the size of several nuclei locating at the posterior end of the egg increases gradually and the nuclei become to be distinguished from the other nuclei. These nuclei are the primordial germ cells, spherical in shape and 7  $\mu\text{m}$  in diameter. Though the germ cell determinant is maintaining its original structure by this stage, it changes into a crescent body when the germ cells are clearly formed, and then disappears gradually and finally it becomes impossible to trace visually. At this time about 10 to 20 nucleolar masses or granules are found in the core of the egg, and they are approximately same in size and shape. It was rarely observed that the division of blastoderm cells is vertical to the egg surface (Fig. 13). The inner nucleus produced by this division moves into the core of the egg and becomes the nucleolar granules. At the early blastoderm stage 20 to 30 of these nucleolar granules, about 1.5  $\mu\text{m}$  by 2  $\mu\text{m}$  in diameter, are found in the core of the egg.

Blastoderm formation occurs with the same way widely observed in other insect eggs, which is about 8  $\mu\text{m}$  in thickness when it has completed. The newly formed blastoderm cells with single, spherical or oval nucleus are columnar except those at the posterior region of the egg.

During the blastoderm formation the size of the egg increases gradually and now measures about 85  $\mu\text{m}$  by 35  $\mu\text{m}$  along the longitudinal egg axis. At this time the egg becomes mulberry-like in the external appearance (Fig. 16), because each of the blastoderm cells makes a slight bulge at the egg surface. The cellular blastoderm consists of about 350 cells, and henceforth the number of the cells remains nearly constant during the blastoderm stage. Thus the blastoderm is completed at 10 hr after oviposition.

Shortly after the completion of the blastoderm, the blastoderm cells begin to develop and the cell differentiation takes place. In this process remarkable changes occur in the whole blastoderm cells except for the germ cells at the posterior end of the egg; they are (1) nucleus changes its form from spherical to elliptic, (2) an nucleolus appears at the outer part of each nucleus facing the egg surface, and it grows larger and larger (Fig. 15). With the lapse of time the blastoderm at the ventral side of the egg becomes somewhat thicker than that at the dorsal side. At the same time the size of blastoderm cells is also different from one region of the egg to another, that is, the blastoderm cells in the anterior and mid-ventral regions become small and have a small and elliptic nucleus, while the cells in the rest of blastoderm become large, having a spherical large nucleus. The number of these blastoderm cells slightly decreases, and the egg becomes broad. By this time the germ cells have been retaining original state and position, but they gradually move toward the ventral edge of blastoderm with advance of the development.

When the blastoderm is completed, 40 to 50 nucleolar granules are found in the core of the egg. The total number and size of the nucleolar granules, however, vary according to the stage of development; *i. e.*, the number varies from 20 to 50 during the blastoderm stage, and it shows a tendency that these granules increase in number in the older blastoderm than in the younger one.

## Discussion

### 1. Host age and parasitism

Effect of the age of host eggs on the parasitism by *Trichogramma* has been studied in detail by Peterson (1930), Lewis and Redlinger (1939), Klomp and Teerink (1967), Marston and Ertle (1969), and Hiehata et al. (1976) etc. . It should be noted that the parasite eggs were rarely found in the old host eggs in which the embryos attained to the revolution stage and their alimentary canal had been formed. However the parasite eggs observed in the freshly infested host eggs collected in the field were at the earlier stage of embryogenesis ranging from fertilization to germ band formation. From these evidences the author believes that the age of host eggs suitable for the parasitism by *T. chilonis* is almost limited within one day after oviposition, and the host eggs older than one day are hardly parasitized by *T. chilonis*. This fact agrees with the conclusion by Hiehata et al. (1976) in three species of *Trichogramma*.

### 2. Developmental delay observed among some eggs in the same brood

Delay of beginning of the cleavage and the development in some of the parasite eggs among the same brood were found; in some instances several eggs were still at the early cleavage stage even though majority of the brood reach the blastoderm stage. The number of parasite which were behind the schedule of their development agreed with that of emerged male adults. Judging from the above facts it is probably true that the start of development of unfertilized eggs is later than that of fertilized one.

### 3. The nucleolar granules

In *T. evanescens*, Gatenby (1917) examined the formation of extruded nucleoli or the nucleolar granules, and he stated that a number of nucleolar granules were the extrusions from the blastoderm nuclei and no dividing nuclei were found in the sections. He said, moreover, that in the younger blastoderm there were found fewer extruded nucleoli, and that a shortening of the egg length always occurred with a decrease of the nuclear number in the growing blastoderm.

As to the origin of chromatin or nucleolar granules, however, the author obtained a different observations. In *T. chilonis*, when the energids reached to the egg periphery some of them remained in the center of the egg. The latter became chromatin or nucleolar granules. In the syncytial blastoderm stage the naked nuclei or two daughter nuclei just produced by the division were found between the nuclei in these blastoderm cells. Though it is not clear whether both daughter nuclei become the nucleolar granules or not, at least the inner one

was originated from the latter. In the early blastoderm stage the naked nuclei were observed between the blastoderm cells. In the present study, the author, however, can not determine whether these nuclei contribute to re-form the blastoderm or they are cast out into the center of the egg to form the nucleolar granules.

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