

## Early Embryogenesis of Fireflies, *Luciola cruciata*, *L. lateralis* and *Hotaria parvula* (Coleoptera, Lampyridae)

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### Synopsis

Early development of fireflies, *Luciola cruciata*, *L. lateralis* and *Hotaria parvula*, from the oviposition to the formation of germ band, is described. The egg of *L. cruciata* is laid after the completion of the second maturation division, and already at the oviposition the male pronucleus is found in the yolk. The germ rudiment develops as a solid ball-shaped body. It sinks into the yolk and differentiates into the embryonic and amniotic areas with the formation of amniotic cavity. The formation of the serosal cuticle and inner layer is also dealt with.

### Introduction

Our knowledge on the embryology of Lampyridae is limited to the following three studies: Williams' (1916) on American fireflies. *Photuris pennsylvanica* and *Photinus consanguineus*, which first found that the ball-shaped germ-rudiments are formed in these species, Hess' (1922) on the embryonic development of the photogenic organ of *P. pennsylvanica*, and Ando and Kobayashi's (1975) on the formation of blastoderm to the middle embryonic development of *Luciola cruciata* with brief descriptions which confirmed that the ball-shaped germ rudiment also appears in a Japanese firefly. These studies are rather fragmentary and do not cover the whole process of the embryonic development of fireflies. Accordingly we set about the second embryological study on the Japanese fireflies, and follow the early development of three lampyrids, *Luciola cruciata*, *L. lateralis* and *Hotaria parvula*.

## Materials and Methods

The imaginal and larval insects of *Luciola cruciata* Motschulsky, *L. lateralis* Motschulsky and *Hotaria parvula* Kiesenwetter collected in Tatsuno, Nagano Pref., Central Japan, were reared. Eggs were obtained by the procedure mentioned in the previous paper (1975) and were incubated at room temperature. They were fixed with alcoholic Bouin's fluid. The intervals of sampling eggs were 5 min. during first 2 hr, 20 min. from 2 to 4 hr after oviposition (a. o.), 60 min. from 4 to 10 hr a. o., 3 hr from 10 to 36 hr a. o., 6 hr from 36 to 72 hr a. o. and 1 day from 72 hr a. o. to hatching. For the satisfactory fixation of eggs after a few days a. o. of which the chorion hardens, the hot fixative (60°C for 20 min.) was employed.

For sectioning, the normal paraffin method was utilized. Sections, 7  $\mu\text{m}$ -thick, were stained with Delafield's hematoxylin and eosin. For observation of whole embryo, after dechoriation the embryos were removed from the yolk mass, and stained with Borax-Carmine. A scanning electron microscope was also used to observe micropyles.

## Observations

### (1) Early development

#### a. Structure of egg just after oviposition and fertilization

As previously described (Ando and Kobayashi, 1975), the newly laid eggs of *Luciola cruciata* are whitish yellow, nearly spherical and 0.5-0.6 mm in diameter. It is difficult to distinguish the anterior and posterior poles, and to determine the polarity of the egg. The surface of egg is coated with a thin gelatinous membrane secreted from the mother insect. The chorion bears numerous depressions on its surface. A thickened micropylar area is reddish brown in color, and about 20 micropylar canals radially arranged are found in the area (Fig. 1-1). The chorion of newly laid egg is very thin and elastic. The general structure of eggs in *Luciola lateralis* resembles that of *L. cruciata*. Also the newly laid eggs of *L. lateralis* are elastic and whitish yellow. It is 0.6-0.7 mm in diameter and covered with a thin gelatinous membrane and chorion. The general structure of eggs in *Hotaria parvula* is in good agreement with those of the former two species, and the micropylar structure is also similar to that of *L. cruciata*. The egg size is 0.6-0.7 mm in *H. parvula*.

The eggs of these three species have common features in the inner construction of egg. At the surface of egg proper, a fine vitelline membrane is observed. Beneath the vitelline membrane is the periplasm which is 3-4  $\mu\text{m}$  in thickness in *L. cruciata* (Fig. 2-1) and 8-13  $\mu\text{m}$  in *L. lateralis* and *H. parvula*. Thickness of the periplasm varies regionally in each egg. In the case of *L. cruciata* remarkable periplasmic protrusions (Fig. 2-2) are often found in the yolk, and disappear before the germ disc formation. The inside of egg is filled with yolk spherules ca. 2.5-5  $\mu\text{m}$  in diameter (Fig. 2-1). They are smaller at the egg surface, and increase their size towards innerly. Between yolk spherules a small amount of the cytoplasmic reticulum is observed. Spherical vacuoles found through the yolk show the trace of leached fatty materials, and polar granules are not found. The egg nucleus (ca. 10  $\mu\text{m}$  in diameter)

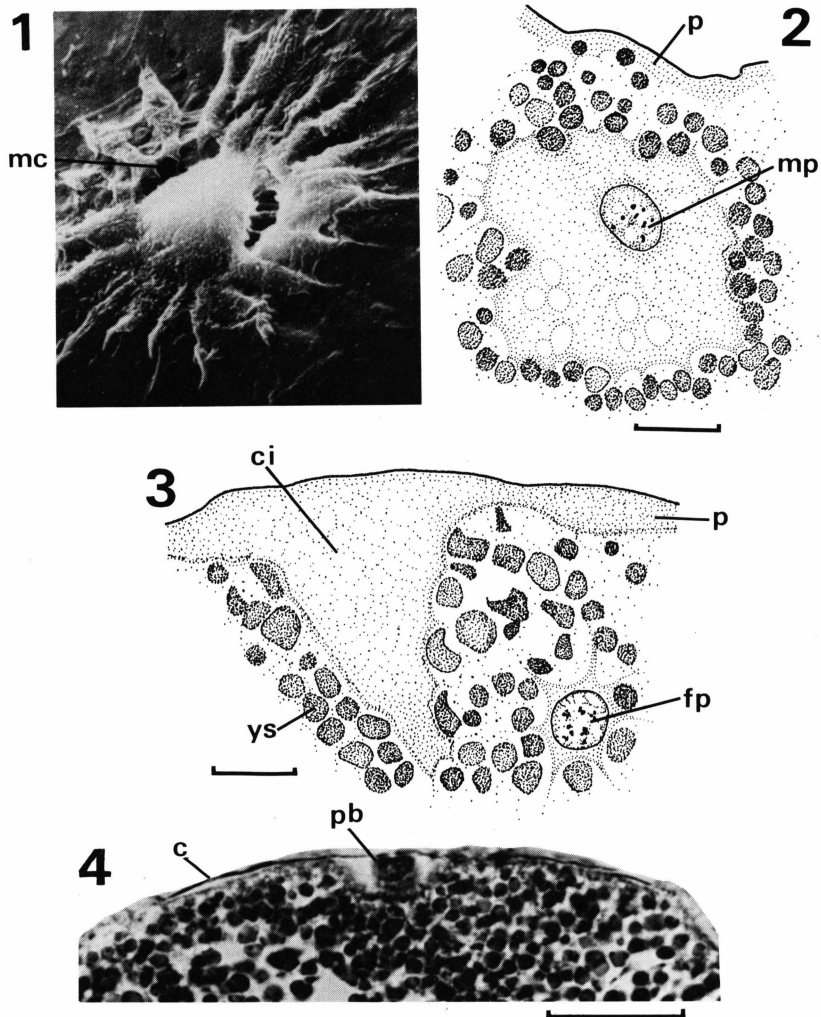


Fig. 1. Micropyle, pronuclei and polar bodies of *Luciola cruciata*  
 1. micropylar area, 2. male pronucleus; 5 min. after oviposition, 3. female pronucleus and cytoplasmic island; 5 min a. o., 4. polar bodies; just after oviposition 2, 3. Scale: 10  $\mu\text{m}$ , 4. Scale: 30  $\mu\text{m}$ .  
 c chorion, ci cytoplasmic island, fp female pronucleus, mc micropyle, mp male pronucleus, p periplasm, ys yolk spherule.

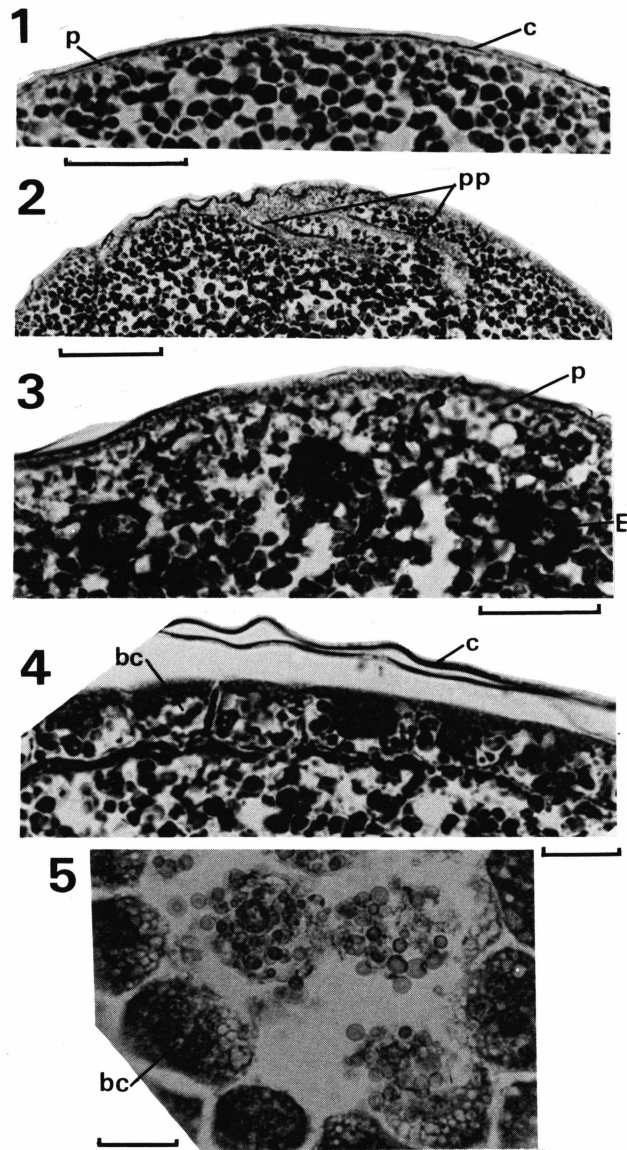


Fig. 2. Early developed eggs of *Luciola cruciata*  
 1, 2. peripheral region of egg just after oviposition. 3. energids at 7th cleavage: 10hr a. o.,  
 4, 5. blastoderm, 4. sectioned view, 5. surface view, 4-nucleated blastoderm cell.  
 1, 2, 3, Scale: 30  $\mu\text{m}$ , 4, 5. Scale: 20  $\mu\text{m}$ .  
 bc blastoderm cell, c chorion, e energid, p periplasm, pp periplasmic protrusion.



and two or three polar bodies are situated near the protoplasmic island or thickened area of the periplasm in which the maturation divisions carry out (Fig. 1-4). In the egg of *L. cruciata*, just under the periplasm a pronucleus is observed, which is thought the male one (Fig. 1-2) although in only one example. The female pronucleus leaves from the protoplasmic island and migrates into the core of the egg (Fig. 1-3). The female and male pronuclei are surrounded with the scanty cytoplasm radially spread around themselves. At 3 to 4 hr a. o., they fuse usually at the center of egg and the fertilization completes. Figure 3 shows the approachment of the male and female pronuclei. The process of fertilization in *L. lateralis* and *H. parvula* are similar to that in *L. cruciata*. We failed to detect spermatozoa invaded in the eggs.

#### b. Cleavage

At 4 to 5 hr. a. o., the first cleavage occurs at the site of syngamy located (Fig. 3-1). Meanwhile the polar bodies degenerate. This case is also true in *L. lateralis* and *H. parvula*. Cleavage follows successively, and their timings in *L. cruciata* are as follows: the 2nd cleavage about 6 hr a. o., the 3rd about 7 hr a. o., the 4th about 8 hr. a. o., the 5th about 9 hr. a. o., and the 6th about 9 to 10 hr a. o. The energids in *Luciola* are rather large in size. They then commence to migrate to the periphery of the egg (Figs. 3-2, 3, 4, 5). In *L. cruciata* and *L. lateralis* the migration of energids begins at about the time of the 7th cleavage, and cytoplasmic masses around the nuclei are now clearly observed (Fig. 2-3). The beginning of migration in *H. parvula* takes place earlier than that in the former two species. In *L. cruciata* and *L. lateralis*, the first entry of energids into the periplasm takes

Table 1. Time table of early embryonic development in *Luciola cruciata* (a. o. after ovposition).

Stage	State of development
0 - 60 min a. o.	Female, male pronuclei and polar bodies
3 - 4 hr a. o.	Fertilization.
4 - 5 hr a. o.	1st cleavage.
6 - 7 hr a. o.	2nd to 3rd cleavage.
8 - 10 hr a. o.	4th to 6th cleavage.
10 - 14 hr a. o.	7th to 8th cleavage, migration of energids to egg periphery.
15 - 20 hr a. o.	Formation and completion of blastoderm.
ca. 21 - 24 hr a. o.	Formation of germ disc.
ca. 30 hr a. o.	Solid ball-shaped germ rudiment sinks into the yolk and amniotic cavity is formed in the germ rudiment.
ca. 48 hr a. o.	Beginning of regional differentiation of germ rudiment.
ca. 70 hr a. o.	Germ band with protocephalic lobe and protocorm, appearance of primitive groove and beginning of inner layer formation.

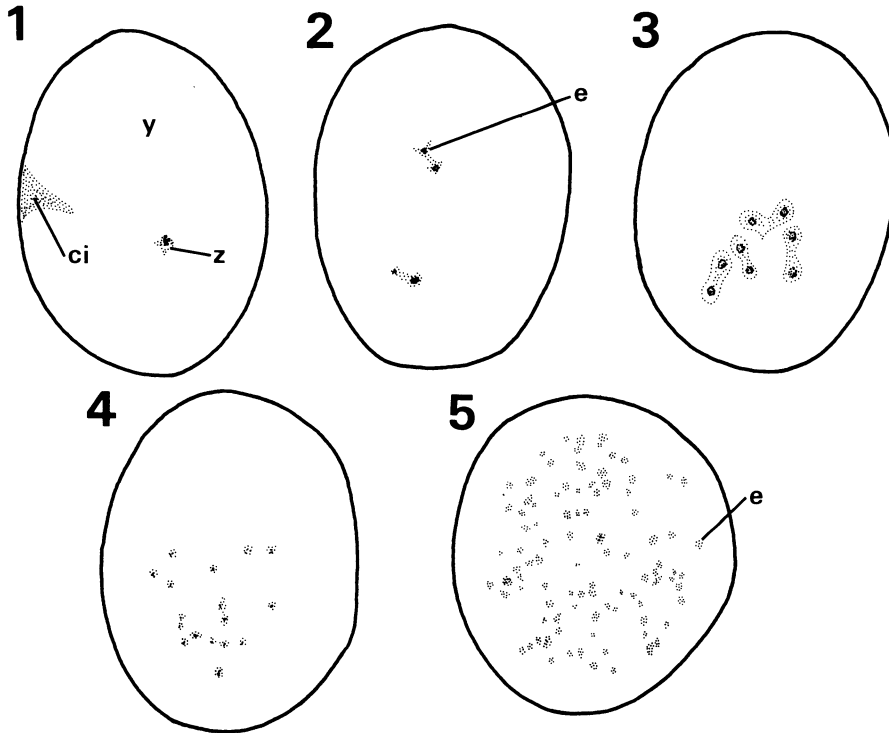


Fig. 3. Cleavage of *Luciola cruciata*, diagrammatical

1. 3 hr after oviposition, 2. 2-4 energids; 4 hr 20 min a. o., 3. 8 energids; 5 hr 20 min. a. o., 4. 6 hr 20 min a. o., 5. about 128 energids; 7 hr 20 min a. o.  
 ci cytoplasmic island, e energid, y yolk, z zygote.

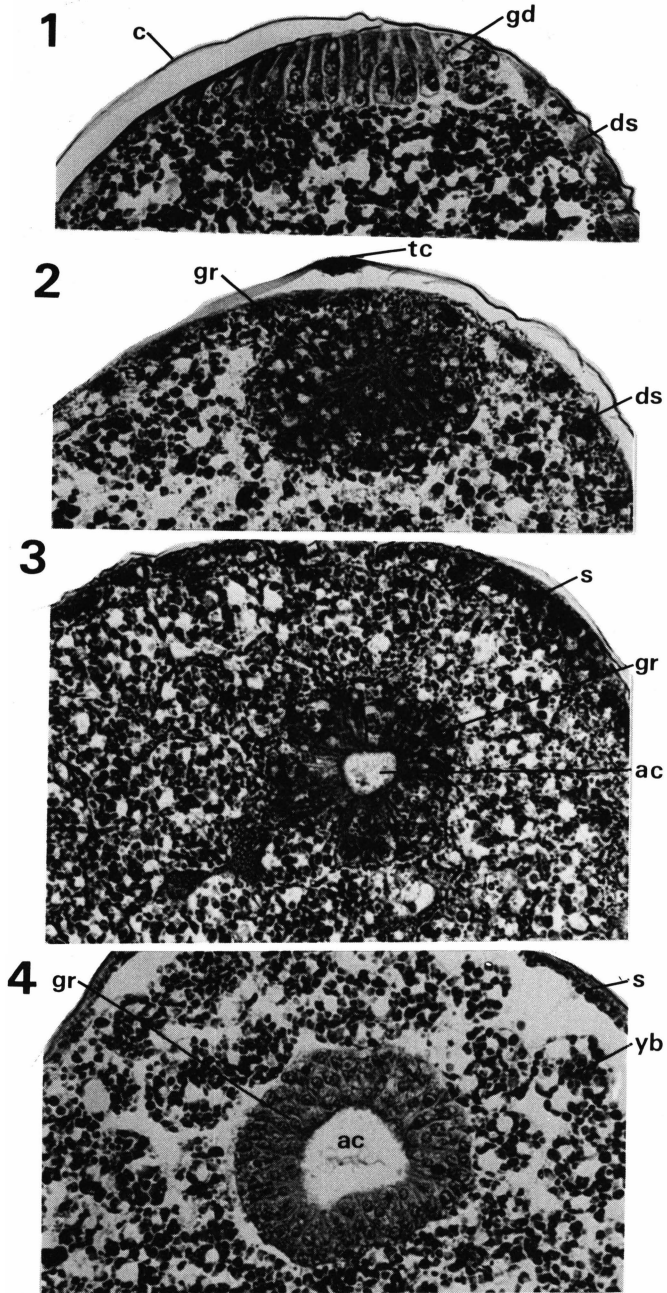


Fig. 4. Formation of germ disc and germ rudiment of *Luciola cruciata*, cross section  
 1. gem disc; 21 hr after oviposition, 2. solid ball-shaped germ rudiment; 24 hr a. o.,  
 3. ball-shaped germ rudiment with developing amniotic cavity; 1 day 12 hr a. o., 4.  
 beginning of regional differentiation of germ rudiment, 2 days a. o. Scale: 50  $\mu$ m  
 ac amniotic cavity, c chorion, ds developing serosa, gd germ disc, gr germ rudiment,  
 s serosa, tc thickening part of chorion, yb yolk block.

place about 10 to 14 hr a. o., prior to the 8th cleavage. When the energids reach the egg periphery, the cytoplasm around them fused with the periplasm. The settlement of energids in the periplasm does not take place synchronously. Not all energids migrate into the egg periphery, but some of them remains in the yolk to form the primary yolk nuclei. The developmental time table until the arrival of energids to the periphery and the completion of blastoderm in *L. cruciata* is presented in Table 1.

### c. Formation of blastoderm, germ disc and germ rudiment

Just after the settlement of energids on the egg surface, the periplasm containing energid nuclei thickens to *ca.* 20  $\mu\text{m}$  in *L. cruciata* and *L. lateralis*, and to 20-25  $\mu\text{m}$  in *H. parvula*. These nuclei soon undergo mitoses twice, then the formation of cell membrane follows the multiplication of nuclei, and the blastoderm completes. We often found polynucleated blastoderm cells with two- or four-grouped nuclei (Figs. 2-4, 5). A small amount of yolk spherules is found incorporated in the blastoderm cells, but they disappear within a relatively short time. A similar phenomenon is observed in *L. lateralis* and *H. parvula*, although the amount of yolk materials is smaller than in *L. cruciata*. The diameter of blastoderm cell and its nucleus in *L. cruciata* are 15-25  $\mu\text{m}$  and 6-8  $\mu\text{m}$  respectively.

After the completion of blastoderm (about 20 hr a. o.), several of the blastoderm cells proliferate to form the small germ disc comprising the columnar cells, *ca.* 170  $\mu\text{m}$  in width and 50  $\mu\text{m}$  in height (Fig. 4-1). With the proliferation of cells, the germ disc which now became a round body begins to sink into the yolk, and its cell arrangement alters into nearly radial from vertical to the surface (Fig. 4-2). At about 30hr. a. o., the ball-shaped germ rudiment increases in size, whereas its connecting or necked part with the blastoderm becomes narrower. Soon the germ rudiment with a central small cavity, *ca.* 10  $\mu\text{m}$  in diameter, which is the future amniotic cavity, leaves the blastoderm or rudimental serosa and migrates into the yolk (Figs. 4-3, 4; 5-1, 2). The germ rudiment which changed from ball-shaped to airship-one is composed of columnar cells, and its inner side later develops into the embryo and the outer one is the future amnion (Fig. 5-3). The germ rudiment develops in a similar manner in three species excepting the case of *H. parvula*, in which a small invagination appears at the center of sinking germ disc in the germ rudiment formation (Figs. 6-1, 2). The remarkable serosal cuticle is formed during the stage of germ rudiment accompanied with atrophy of serosal cells (Figs. 5-4, 5).

## (2) Further development of embryo

### a. Germ band formation

The germ rudiment of 36 hr. a. o. is situated in the yolk at about the one-third of egg axis. The diameter of germ rudiment and its cavity are *ca.* 100  $\mu\text{m}$  and 20  $\mu\text{m}$  respectively. With the progression of development, the cells of germ rudiment continue to increase in number, and they are arranged more densely; consequently the extension of the future amniotic cavity occurs. At 54 hr a. o., the cavity is 50-60  $\mu\text{m}$  in diameter, and its wall is 25-30  $\mu\text{m}$ -thick.

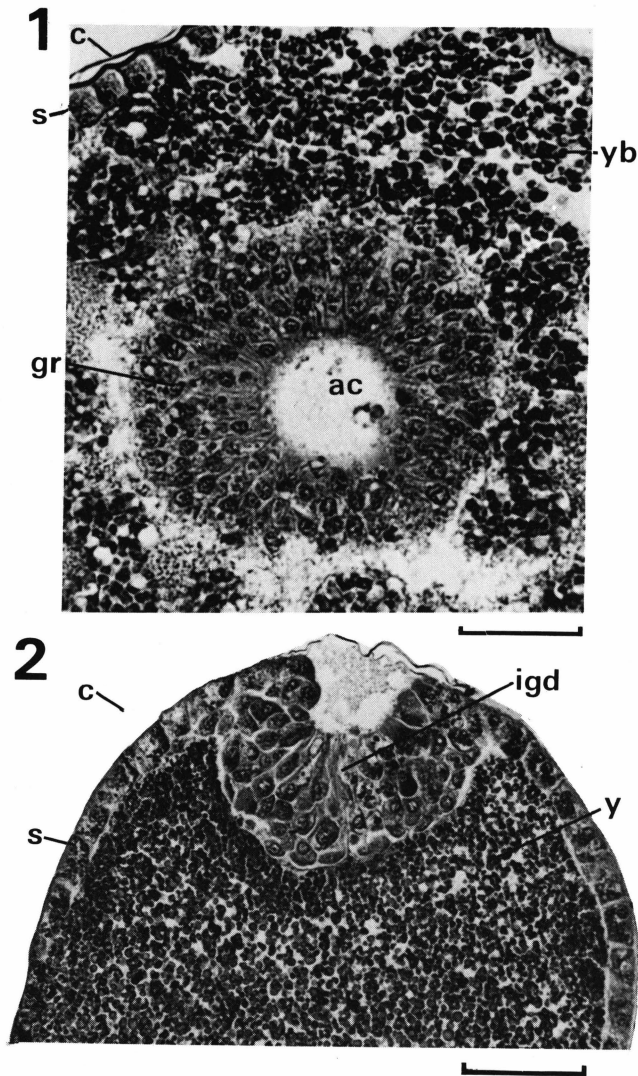


Fig. 5. Formation of germ rudiment of *Luciola lateralis* and *Hotaria parvula*  
 1. ball-shaped germ rudiment with developing amniotic cavity of *L. lateralis*; 1 day 12 hr after oviposition, cross section. Scale: 50  $\mu\text{m}$ , 2. invaginating germ disc of *H. parvula*; 21 hr a. o., longitudinal section. Scale: 50  $\mu\text{m}$   
 ac amniotic cavity, c chorion, igd invagination germ disc, s serosa, y yolk, yb yolk block.

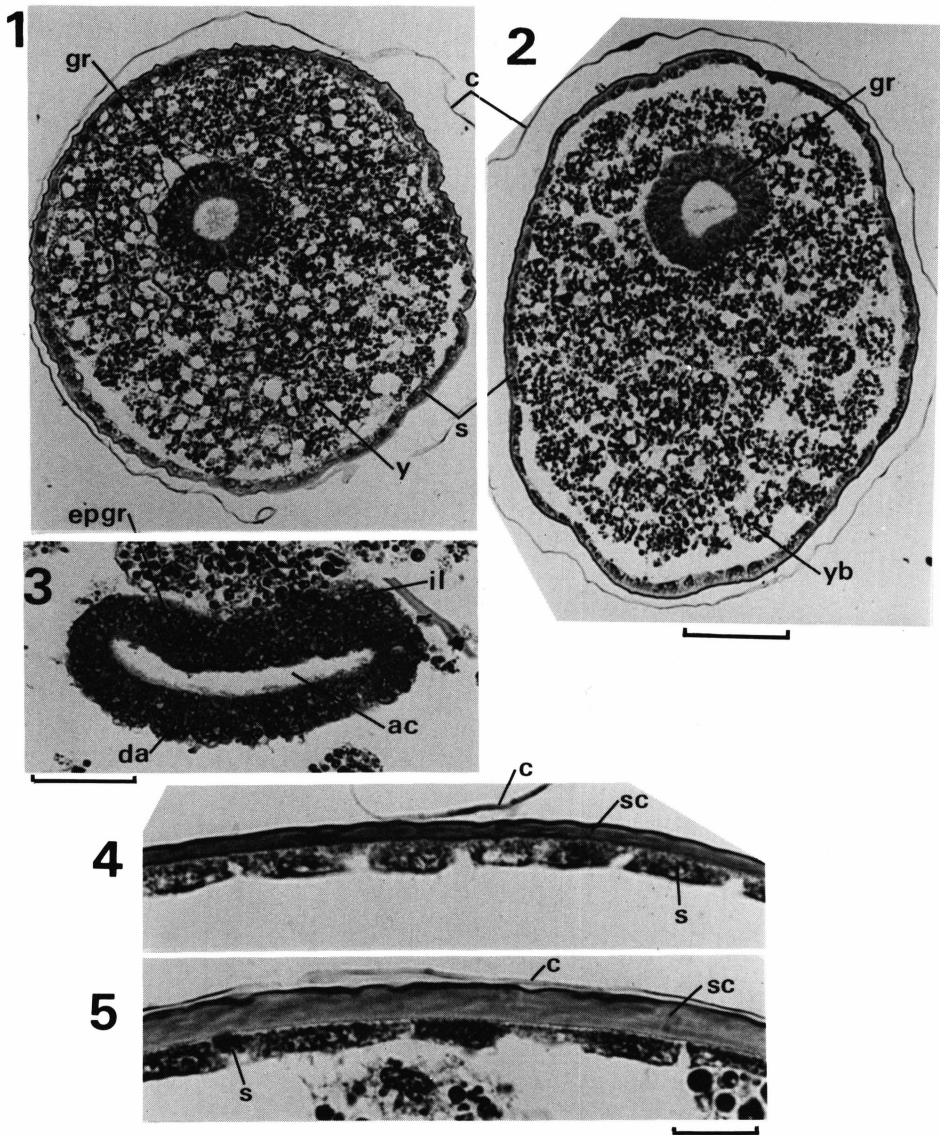


Fig. 6. Germ rudiment and serosal cuticle of *Lucila cruciata*  
 1. egg with ball-shaped germ band; 1 day 18 hr after oviposition, 2. regional differentiated germ rudiment, same section of Fig. 4-4; 2 days a. o., 3. flattened and elongated germ rudiment with inner layer formation; 2 days 18 hr a. o., 3. serosal cuticle; 3 days 6 hr a. o., 5. 4 days 18 hr a. o. 1, 2. Scale: 100  $\mu$ m. 3. Scale: 50  $\mu$ m. 4, 5. Scale: 20  $\mu$ m  
 ac. amniotic cavity, c chorion, da developing amnion, epgr ectodermal part of germ rudiment, gr germ rudiment, sc serosal cuticle, y yolk, yb yolk block.

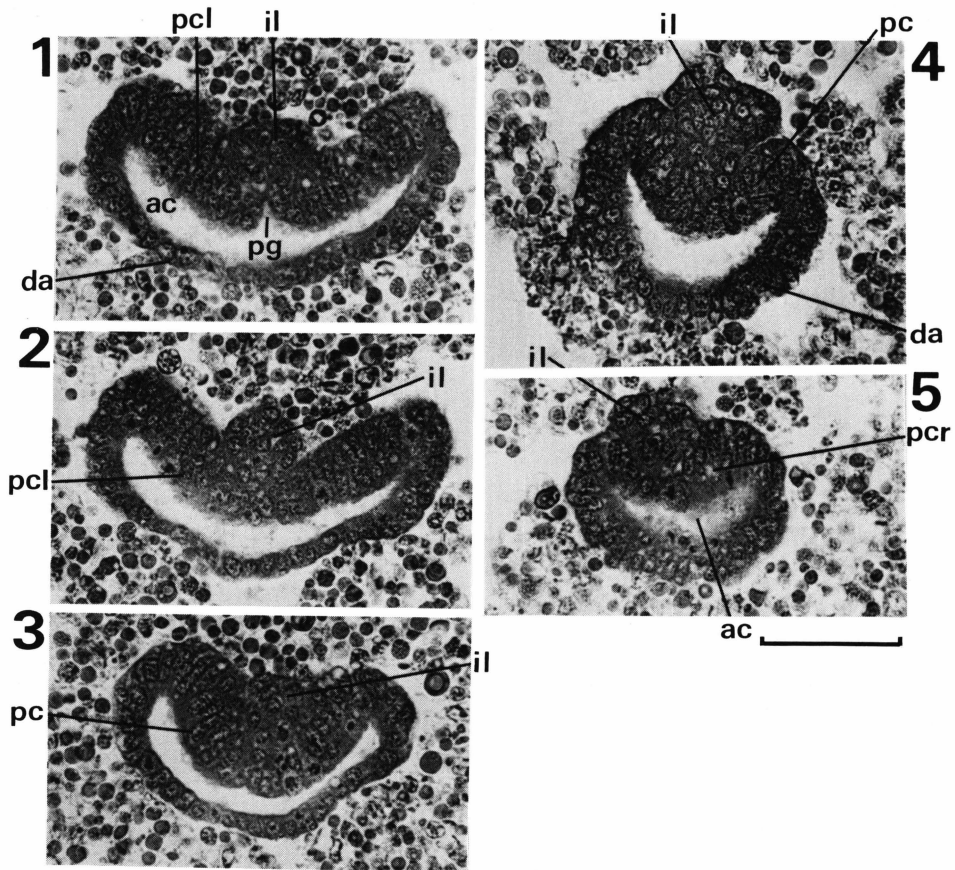


Fig. 7. Inner layer formation of *Luciola cruciata*, successive sections; about 3 days after oviposition

1. anterior part of middle level of protocephalic lobe, 2. middle level of protocephalic lobe, 3. anterior part of protocorm, 4. posterior part of protocorm, 5. caudal part of protocorm. Scale: 30  $\mu\text{m}$

ac amniotic cavity, da developing amnion, il inner layer, pc protocorm, pcl protocephalic lobe, pcr posterior end of protocorm, pg primitive groove.

At about 60 hr a. o., the germ rudiment differentiates into the germ band and the developing amnion. The germ band loses the uniformity in thickness owing to the beginning of inner layer formation. In parallel with this change, the spherical amniotic cavity begins to flatten (Figs. 6-1,2,3). At this time, the germ band is *ca.*  $50\ \mu\text{m} \times 120\ \mu\text{m} \times 70\ \mu\text{m}$  (length, width and thickness) in size and the developing amnion is *ca.*  $25\ \mu\text{m}$  in thickness. In *L. lateralis* and *H. parvula* the differentiation of germ band and amnion takes place in the similar manner to in *L. cruciata*.

At about 36 hr a. o., the yolk is divided into spherical yolk blocks and the amount of whole yolk material begins to reduce. Each block, 10-15  $\mu\text{m}$  in diameter, is surrounded with a fine membrane, and contains a single nucleus.

#### b. Inner layer formation

As previously mentioned, the inner layer formation commences at about 60 hr a. o. Now the germ band elongates, and the differentiation of protocephalon and protocorm occurs. Along the median line of shoe-shaped germ band, the primitive groove begins to appear, and its formation proceeds cephalads from the posterior region of protocorm. The cells situated on the bottom of the primitive groove proliferate actively and their daughter cells form the inner layer or mesoderm. In the embryo of 70 hr a. o., the inner layer formation is the most progressive in the posterior half of protocorm. Fig. 7 illustrates the regional differences in the progression of inner layer formation in the embryo of *L. cruciata*.

## Discussion

### 1. Phase of egg at the oviposition

In the newly laid egg of *L. cruciata* the female pronucleus found in the yolk has already escaped from the cytoplasmic island, in which the maturation divisions took place. This shows that the egg of *L. cruciata* on the oviposition has already undergone the secondary maturation division. In contrast with the common trend of insects in which germinal vesicle shows the metaphase of the first maturation division at the time of oviposition, the egg in *L. cruciata* is laid in a more progressing stage of development.

### 2. Germ rudiment

In all of the lampyrid species so far studied, *Photuris pennsylvanica*, *Photinus consanguines* (Williams, 1916), *L. cruciata*, *L. lateralis* and *H. parvula*, the germ rudiment is formed as a solid ball-shaped body sunk into the yolk, which is composed of the embryonic and extraembryonic (amniotic) areas, and the amniotic cavity later develops as an inner cavity occurred in the germ rudiment. The formation of the embryo of fireflies is unique and probably specialized among the coleopterans whose germ band generally develops on the surface of egg (the superficial type). The small invagination appears in the formation of germ rudiment in *H. parvula* may suggest a possible derivation of the ball-shaped germ



rudiment of fireflies from the germ rudiment formation of the invagination type which is less common in the Coleoptera.

The superfamily Cantharoidea are generally regarded as a rather primitive group of the Coleoptera in spite of their specialization for luminosity, and among the superfamily the Lampyridae are accepted as a more primitive group and the Cantharidae as the most advanced (Crawson, 1955, 1960). According to unpublished studies by Kobayashi and Fujiwara a cantharid *Athemus suturellus* has a typical germ rudiment of superficial type. To sum up, *A. suturellus* which has been regarded as the most advanced group shows a typical or plesiomorphic type in the formation of germ rudiment, and the Lampyridae regarded as more primitive shows a specialized or apomorphic one. Moreover, the formation of embryonic membrane of these two families is essentially different in manner. The phylogenetic significance of the ball-shaped germ rudiment in the Lampyridae is unknown. Further considerations are expected.

### 3. Serosal cuticle

In *L. cruciata* and the other two species, the secretion of serosal cuticle commences at about the time when the germ rudiment sinks into the yolk to become ball-shaped. The formation of serosal cuticle in the corresponding stage of development is also reported in a mecopteran *Panorpodes paradoxa* (Suzuki, 1983). The formation in the relatively earlier stage might be related to a thin and fragile chorion of these insect eggs.

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