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Early Embryonic Development of *Amata fortunei* (Lepidoptera, Amatidae)

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Synopsis

The egg structure, the early embryonic development from oviposition to serosa formation and the formation of primordial germ cells of *Amata fortunei* are described in detail. In a newly laid egg the egg surface just beneath the micropylar region is covered with a thin membrane. The whole egg surface is uniformly covered with numerous microvilli. The periplasm is divided into a large light-stained area encircling almost the entire quatorial region of the egg and a dark-stained dumbell-shaped area. The cytoplasm in the anterior pole of the egg is very thick and protrudes into the yolk forming a cone. The yolk mass consists of three regions, the central core, the Auflösungszone and the outer zone, and the yolk granules are grouped into four classes according to their form and affinity for dyes. Three polar bodies resulted from maturation division soon fuse and change into a single chromatin group, and form a row perpendicular to the surface of egg. This chromatin group persists until the energids penetrating into the periplasm. With the lapse of time, within one hour after oviposition the microvilli shrink and become irregularly winding microprojections. Synchronous divisions at the cleavage stage are usually maintained until the seventh cleavage and each energid lacks a long cytoplasmic tail. The blastoderm cells are formed by the protrusion of energids beyond the initial level of the periplasm and by the lateral elongation of the plasma membrane. The partition of embryonic and extra-embryonic areas agrees precisely with that of the surface of the egg in which a difference of stainability for Delafield's haematoxylin is found just after oviposition. The primordial germ cells are formed by mitosis near the center of the germ band shortly after the germ band formation, but before the inner layer formation. The number of them is 13 to 18.

Introduction

The moth, *Amata fortunei* belongs to the family Amatidae. Though the embryogenesis of the order Lepidoptera has been widely studied to compare with those of other insect orders, the family Amatidae, so far as the author is aware, has never been studied from the em-

bryological standpoint. The author therefore intends to study the embryogenesis of this insect in detail and to compare with those in the other families of the Lepidoptera.

In the present paper, the author describes the early embryonic development which covers the egg structure, maturation, fertilization, cleavage, blastoderm formation, ventral plate and serosa formation and formation of primordial germ cells of *A. fortunei*.

Materials and Methods

Pregnant females of *Amata fortunei* de L'Orza were captured at Gifu Prefecture, in June or September of 1982 and 1983. The female moths laid their eggs on the leaves of various plants in the laboratory. The eggs were kept at room temperature, and fixed with Carnoy's or alcoholic Bouin's fluids at 50 – 60°C. They were cut into 7 to 10 μm thichness and stained with Delafield's or Heidenhain's haematoxylin and eosin. For observation of the whole egg, Delafield's haematoxylin was used to stain the eggs after removing the chorion.

Results

Duration of embryonic development

The duration of development from oviposition to hatching of *A. fortunei* was about 6.5 days at room temperature in June in Gifu.

Table 1 shows the outline of early embryonic development of *A. fortunei* at room temperature.

Table 1. Time table of the early embryonic development of *Amata fortunei* at room temperature (in June).

Approximate age of egg	State of development
0 – 40 min	1st maturation division.
1 – 1.5 hr	2nd maturation division.
2 – 2.5 hr	Union of the male and female pronuclei.
3 – 3.5 hr	1st cleavage.
4 – 5 hr	2nd and 3rd cleavages.
9 – 10 hr	Migrating energids enter the periplasm.
12 – 13 hr	Blastoderm formation.
15 – 16 hr	Ventral plate formation.
18 – 21 hr	Serosa covers the ventral plate completely. Differentiation of primordial germ cells.

Structure of newly laid egg

About 100 to 150 eggs are laid as a mass in a neat pattern of diagonal rows in a single brood, and each egg touches neighboring ones. The newly laid eggs are oval in shape, about 0.75 mm by 0.7 mm, and milky white in colour. The lower end of the egg contacting with the surface of a leaf is the posterior pole and somewhat flattened.

The eggs are covered with a thin and nearly transparent chorion with a characteristic polygonal network. The micropylar area is located exactly in the center of the upper side (anterior pole) of the egg and surrounded by a rosette with 14 – 16 petal-like reticulations (Fig. 1). Five to seven micropyles are found inside a rosette. An aeropylar opening is present at each junction of the networked chorionic ribs (Fig. 2).

The egg surface just beneath the chorion is covered with a very thin membrane dark-stained with Heidenhain's iron alum haematoxylin, which does not adhere closely to the egg surface, but separates by numerous microvilli on the periplasm (Fig. 5). The microvilli average 5 μm long and are longer in the anterior pole than in other parts of the egg.

The periplasm averages 20 μm in depth, except for the anterior region of the egg, and is divided into the outer and inner layers. The former, about 5 μm in depth through the egg surface, has higher affinity for eosin and lower for Delafield's haematoxylin than in the latter. The inner layer is 15 μm in average depth, but slightly thin at the anterior and posterior regions, and it contains a lot of small dot-like yolk granules dark-stained with Heidenhain's iron alum haematoxylin. The polar cytoplasm under the micropylar region is very thick, does not contain any yolk granules, and protrudes into the yolk forming a cone (Fig. 6). The innermost periplasm is continuous with cytoplasmic reticulum in the yolk.

In the outer layer of the periplasm two areas are distinguished from the stainability: the one is a light large area encircling almost the entire surface of the egg except at the anterior and posterior poles, and the other is the dark dumbell-shaped one covering a narrow dorsal surface and both poles of the egg (Figs, 20, 21). This difference of stainability is maintained until the migrating energids reach the periplasm.

The egg nucleus is in metaphase or anaphase of the first maturation division and embedded in a cytoplasmic island separating or expanding inward from the periplasm near the anterior pole (Figs. 11, 22).

The yolk consists of three regions, the central core, the "Auflösungszone" termed by Christensen (1942), and the outer zone (Figs. 3, 4). The central core consists of a mass of irregularly shaped yolk spherules which are composed of light-stained small yolk granules and a few dark-stained dot-like granules. This region connects with the anterior polar cytoplasm. The Auflösungszone encircling the central core except at the anterior pole of the egg is tumbler-shaped, and contains more yolk substance than in the central core. This region is composed of large yolk spherules, about 25 μm in maximum diameter.

The outer zone is a region between the Auflösungszone and the periplasm. This zone is divided into the outer and inner layers; the former contains only a lot of dot-like granules, and the latter consists of two kinds of yolk granules supported in a cytoplasmic reticulum; the one is dark-stained dot-like granules and the other dark-stained yolk granules with a small light-stained part inside them (Fig. 7).

A sperm showing a straight rod without a tail, about 12 μm in length and about 1.5 μm in width, lies in the polar plasm or cytoplasmic island just under the micropyle (Figs. 8, 22).

More than one sperm are not found within an egg.

Maturation and fertilization

Immediately after oviposition the egg nucleus is in metaphase or early anaphase of the first maturation division. The direction of the spindle is perpendicular or parallel to the egg surface. In the anaphase of the division a dark-stained equatorial disk is clearly seen, and it seems to coincide with the deformed synaptonemal complex observed in *Bombyx* (Rasmussen, 1977). The first maturation division is completed within 40 min. after oviposition.

The division of the first polar body is synchronized to the second maturation division (Figs. 12, 23). The direction of both spindles is usually perpendicular to the egg surface, but sometimes is parallel. At this time a rod-like sperm nucleus lies in a small cytoplasmic island with a penetrating trail at about 80 μm from the anterior pole (Figs. 9, 23).

Within 1.5 hr after oviposition three polar bodies are formed in the cytoplasmic island (Figs. 13, 25). No difference in size is observed among them. While the egg nucleus migrates interiorly and becomes swollen gradually to form the female pronucleus (Figs. 14, 25), the sperm also becomes swollen to transform into the male pronucleus with cytoplasmic coat. The female pronucleus is about 15 μm in diameter and is as large as the male pronucleus.

The union of the male and female pronuclei takes place in the yolk at the region about one-fifth of the egg length from the anterior pole, within 2.5 hr after oviposition (Figs. 15, 16). During this process, many rod-like chromosomes are found in each pronucles, but the synkaryon could not be observed.

During the syngamy three polar bodies gradually increase in size and the maximum size reaches about 18 μm in diameter (Figs. 17, 26). Though the whole surface of egg is covered with numerous microvilli, 5 μm in average length, until 30 min. after oviposition, the microvilli shrink and become irregularly winding microprojections by 1 hr. During the cleavage stage, no significant morphological changes occur in the surface of egg.

Cleavage

Up to 3 – 3.5 hr after oviposition the mitotic figure of the first cleavage is seen at the region of fertilization (Figs. 16, 27). Within 4 – 5 hr the second and third cleavage occur and the cleavage nuclei, or energids are observed near the center of anterior half of the egg. The mitotic spindles do not show any definite direction.

As the number of energids increases, they are arranged in a sphere near the center of the egg and some of them destined to become the primary yolk cells or vitellophags remain within this sphere, and then the centrifugal migration of the energids begins. At the 6th and the 7th cleavage the number of remaining energids is about 8 and 20 – 30 respectively. Energids migrating or remaining in the sphere are about 16 – 18 μm in diameter and surrounded by the amoeboid cytoplasm, but the migrating energids lack a long cytoplasmic tail usually observed in other lepidopteran eggs. Synchrony of divisions is maintained until the 6th or 7th cleavage. It may continue till the 8th division. After the 8th cleavage the migrating energids first enter the periplasm in the anterior half of the egg almost simultaneously.

Soon after the formation of zygote, the nuclear envelope of polar bodies disappears and

their chromatin assembles to form a spherical mass. Subsequently this mass changes into a rod-like structure being perpendicular to the egg surface (Figs. 19, 28). This figure persists until the penetration of migrating energids into the periplasm at the anterior half of the egg.

Blastoderm formation

Up to 9 hr after oviposition the number of energids increases to about 150 and they enter the periplasm in the anterior half of the egg (Fig. 30). At this stage the numerous, irregular microprojections are reformed on the surface of periplasm. When the energids arrive near the periplasm, their associated cytoplasm fuses with the inner layer of periplasm. As the energids penetrate into the periplasm, they push up the surface of the periplasm and protrude beyond its initial level to form the hillocks (Fig. 31). Consequently, the surface of the egg becomes mulberry-like in the external appearance. In the sections through the hillocks the associated cytoplasm surrounding each nucleus of energids is enclosed with the outer layer of periplasm with abundant microprojections (Fig. 31). The penetration of energids into the periplasm occurs not simultaneously at the whole egg surface, but at the anterior pole of the egg at first, and then proceeds posteriorly.

At about 10 hr after oviposition the entire periphery of the egg is covered with about 350 protruded nuclei or pre-blastoderm cells being scattered apart from each other. At this stage the polar bodies disappear and about 80 energids remain in the yolk to become the primary vitellophags or yolk cells.

Development from the pre-blastoderm to the blastoderm is performed not by the formation of the clevage furrows, but by the lateral elongation of the plasma membranes between the adjacent pre-blastoderm cells, and the round blastoderm cells with the yolk granules are formed (Fig. 32). However, another type of the blastoderm formation by the appearance of cleavage furrows is often observed in the equatorial zone of the egg. At about 13 hr after oviposition the egg periphery is covered with a single layer of the blastoderm cells (Fig. 33). Each blastoderm cell with a single nucleus at its outer part and a considerable amount of yolk granules, Yg2 and Ygd, at its inner part is segregated from the inner yolk mass, resulting in formation of a thin yolk membrane or the secondary vitelline membrane just under the blastoderm.

The large majority of blastoderm cells again undergo synchronous division parallel to the surface of egg, (Fig. 36). Consequently, the blastoderm consists of about 1500 cells and forms a compact cell layer, about 30 μm in thickness. In the yolk some mitotic figures also are seen among the vitellophags. Immediately before the formation of the germ rudiment, the nucleus of each blastoderm cell migrates to its center and two or three large nucleoli appear in each nucleus.

Ventral plate formation and serosa formation

At the next stage the cells of the equatorial region of the egg divide actively to form the columnar cells, while those in the rest of the blastoderm decrease in thickness and increase in width without cell division. The broad equatorial zone is the embryonic area or ventral plate and the rest is the extra-embryonic area or presumptive serosa. It is noticeable that the embryonic area coincides with the light stained region of the periplasm observed in the newly laid eggs.

Soon after formation of the ventral plate, the presumptive serosal cells lying along the margin of the ventral plate begin to grow over the ventral plate to form a complete covering, while the ventral plate sinks slightly into the yolk. At beginning of the serosa formation the presumptive serosal cells undergo one or two amitotic divisions without the formation of the cell membrane, and the cells of the ventral plate or germ band continue to divide tangentally. Consequently, the serosal cells are large in size and are two- or four-nucleate, while the cells of the ventral plate are rectangular in shape and mono-nucleate. At this stage aminion is not yet formed.

Primordial germ cells

In *A. fortunei* there is neither the polar granules at the posterior pole in the newly laid egg, nor the pole cells among the blastoderm cells. Further, during the formation of the serosa, no specialized cells can be observed among the cells of the ventral plate.

After the completion of the serosa several mitotic figures are seen at the mid-line near the center of the germ band (Fig. 38). They show the first appearance of primordial germ cells. These mitotic figures are clearly distinguishable from those of somatic cells by their position and the direction of spindles. The mitotic figures of somatic cells are always parallel to the surface of the germ band and locate at its outer part (Fig. 37). The primordial germ cells formed through this process are remarkably different from the somatic cells in their shape and size (Fig. 39). As the development progresses, the primordial germ cells aggregate to make a cluster near the center of the germ band (Fig. 40). At this time they are polygonal, and their nucleus is round, about 10 μm in diameter and has several nucleoli. Then the cluster of primordial germ cells is pushed out from the germ band into the yolk in which they scattered in the limited area (Fig. 41). Now, the number of primordial germ cells is 13 to 18.

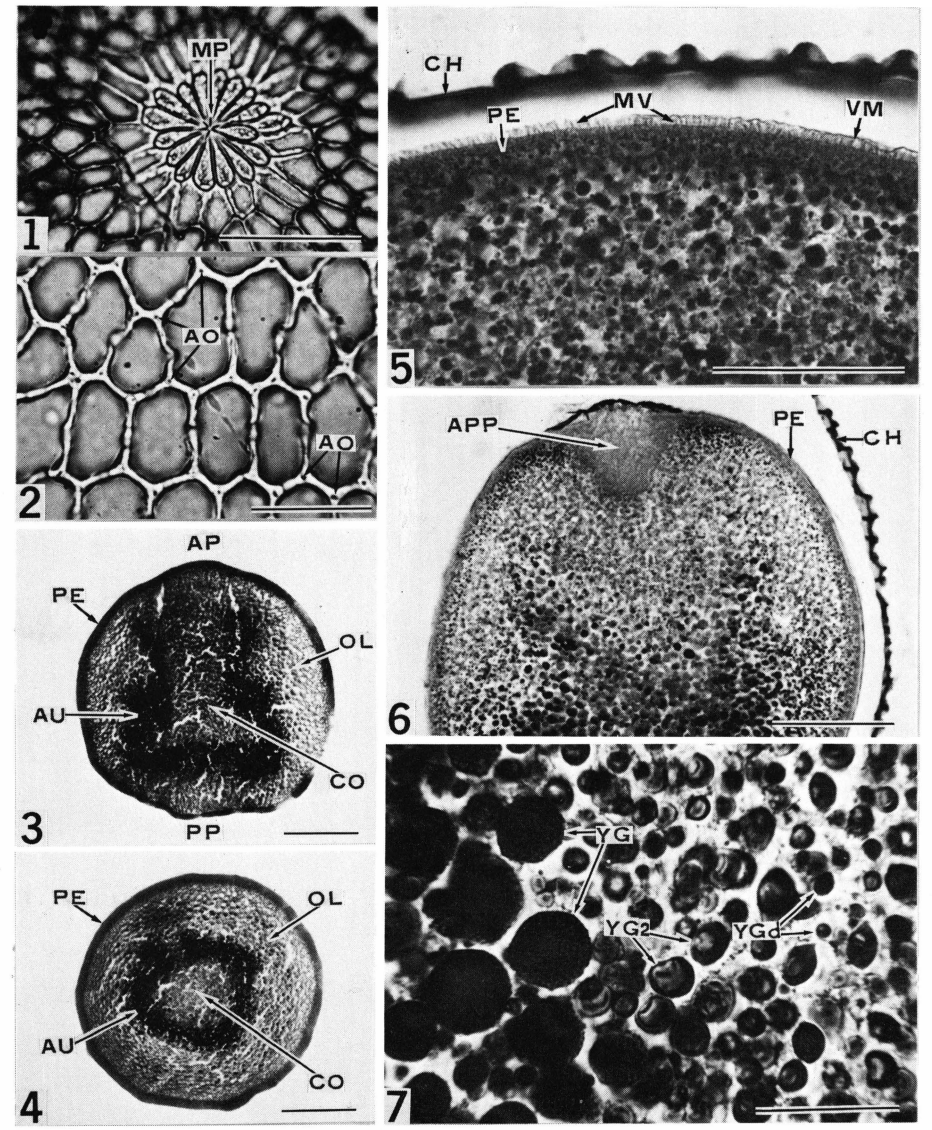
Shortly before the differentiation of protocephalon and protocorm the primordial germ cells reenter separately among the somatic cells near the original area, and representing the similar characteristic feature to that in other species of the Lepidoptera. At this time they are spherical in shape, about 20 μm in diameter and have a round, large nucleus with one or two large nucleoli and light-stained cytoplasm (Fig. 42).

Discussion

Yolk

In the *A. fortunei* eggs the yolk consists of three regions, namely the central core, the Auflösungszone, and the outer zone. Similar structures were also described by Gross and Howland (1940) in *Prodenia eridania* and Presser and Rutschky (1957) in *Heliothis zea.*

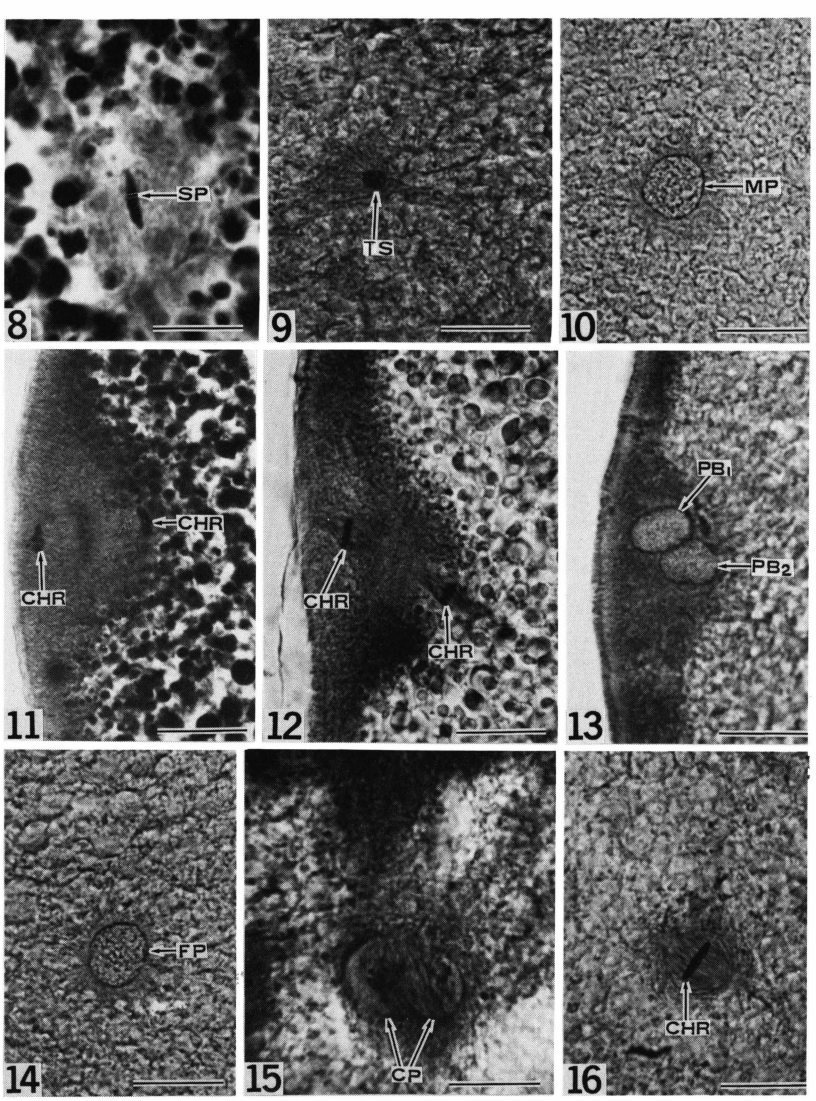
In regard of the yolk granules Takesue *et al.* (1971, 1976, 1982) described that the newly laid eggs of *Bombyx mori* have three kinds of yolk granules, *i. e.*, Yg1, Yg2 and Yg3 or Ygd. In the *A. fortunei* eggs the yolk granules were grouped into four classes according to their form and affinity for dyes. The large yolk spherules located in the Auflösungszone, the light-stained yolk granules with a small dark-stained granule located in the outer zone, and the dark-stained dot-like granules located in the outer zone and the periplasm are similar to



Figs. 1 – 7. Structure of newly laid egg.

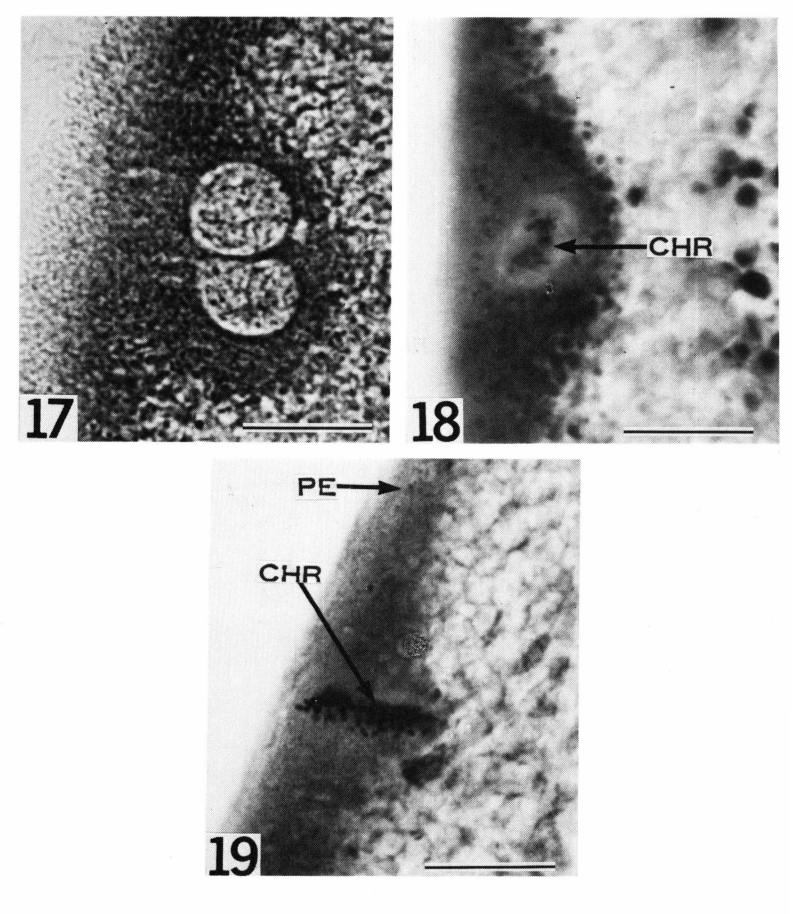
1. Micropylar region of anterior pole of egg. Scale: 100 μm. 2. Surface structure of chorion. Scale: 50 μm. 3. Longitudinal section of egg. Scale: 200 μm. 4. Cross section of egg. Scale: 200 μm. 5. Longitudinal section through ventral side of egg, showing vitelline membrane, microvilli and periplasm. Scale: 100 μm. 6. Longitudinal section of anterior pole plasm. Scale: 200 μm. 7. Longitudinal section through anterior region of egg, showing variant yolk granules. Scale: 30 μm.

AO aeropylar opening, AP anterior pole, APP anterior polar plasm, AU Auflösungszone of yolk, CH chorion, CO central core of yolk, MP micropyle, MV microvilli, OL outer layer of yolk, PE periplasm, PP posterior pole, VM vitelline membrane, YG, YG_2, YGd 3 types of yolk granules.

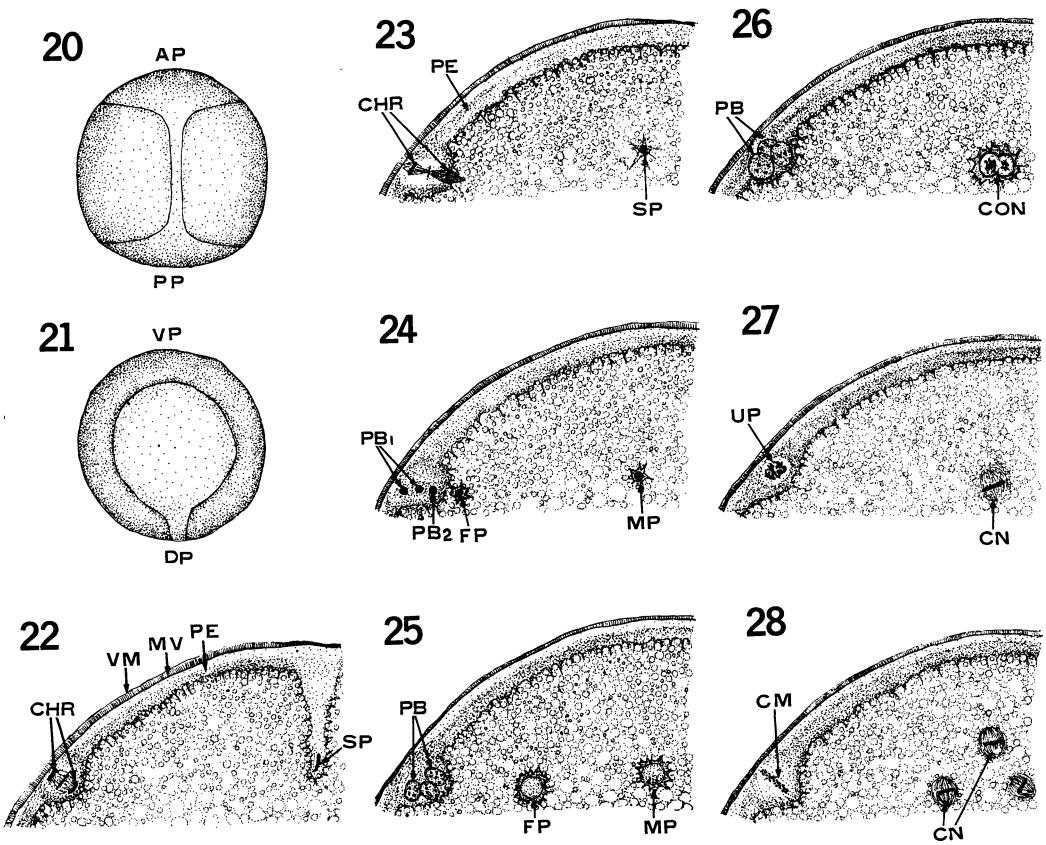


Figs. 8 – 16. Maturation, fertilization and early cleavage. Scales: 20μm. 8. Sperm in cytoplasmic island, about 5 min after oviposition. 9. Transformed sperm surrounded with radial cytoplasm, about 1 hr after oviposition. 10. Male pronucleus, about 2 hr after oviposition. 11. Anaphase of 1st maturation division, about 10 min after oviposition. 12. Metaphase of 2nd maturation division, about 1 hr after oviposition. 13. 1st and 2nd polar bodies in cytoplasmic island, about 2 hr after oviposition. 14. Female pronucleus, about 2 hr after oviposition. 15. Conjugated nuclei, about 2.5 hr after oviposition. 16. Metaphase of 1st cleavage, about 3 hr after oviposition.

CHR chromosome, CP conjugated nuclei, FP female pronucleus, MP male pronucleus, PB_1, PB_2 1st and 2nd polar bodies, SP sperm, TS transformed sperm.



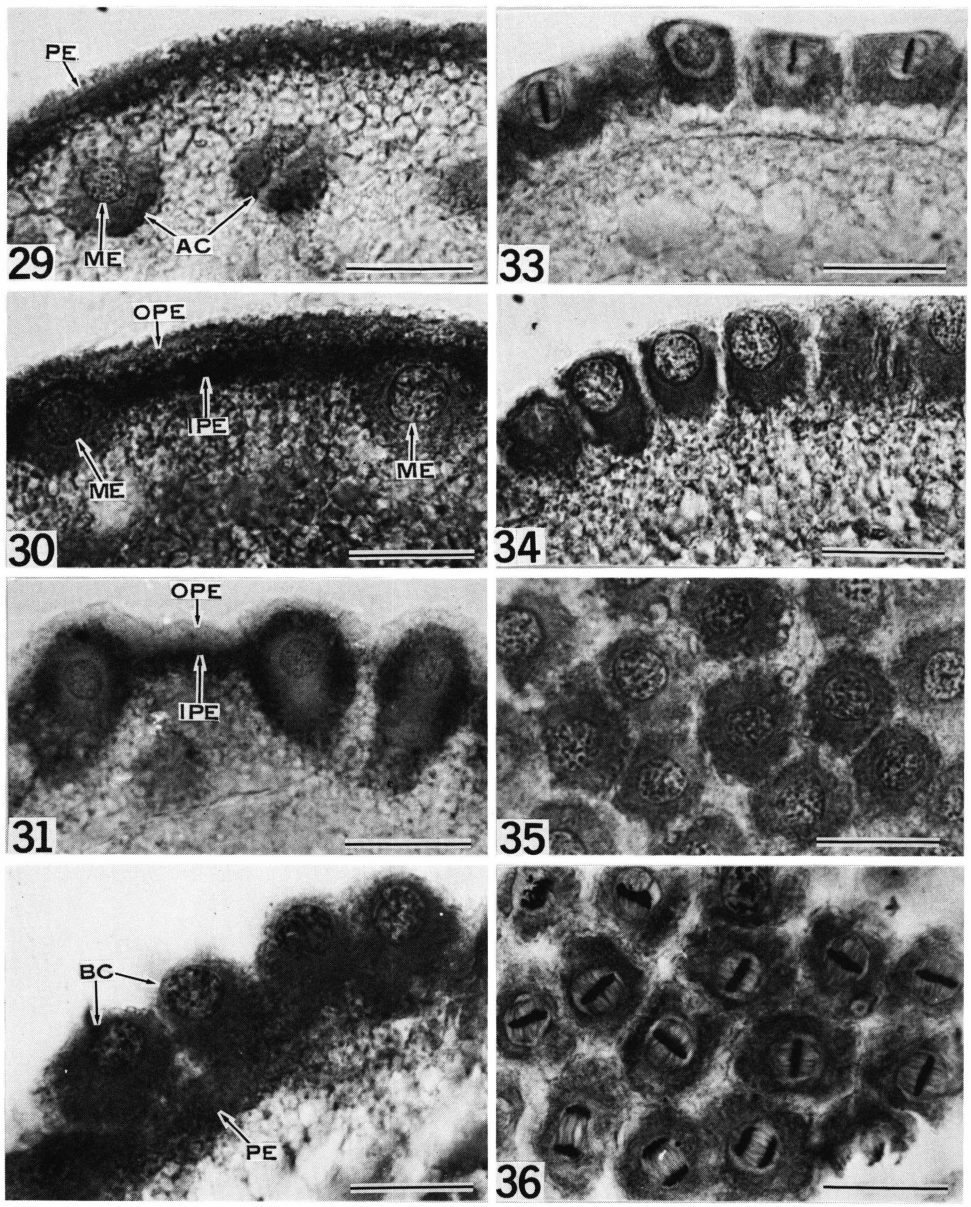
Figs. 17 – 19. Successive changes of polar bodies. Scale: 20 μm. 17. Polar bodies before union, about 2.5 hr after oviposition. 18. United nuclei of polar bodies, about 3 hr after oviposition. 19. Rod-like chromatin mass resulted from union of polar bodies, about 4.5 hr after oviposition.
CHR chromatin mass, PE periplasm.



Figs. 20 – 21. Surface structure of newly laid egg stained with Delafield's haematoxylin, schematic diagrams. 20. Lateral view. 21. Apical view.

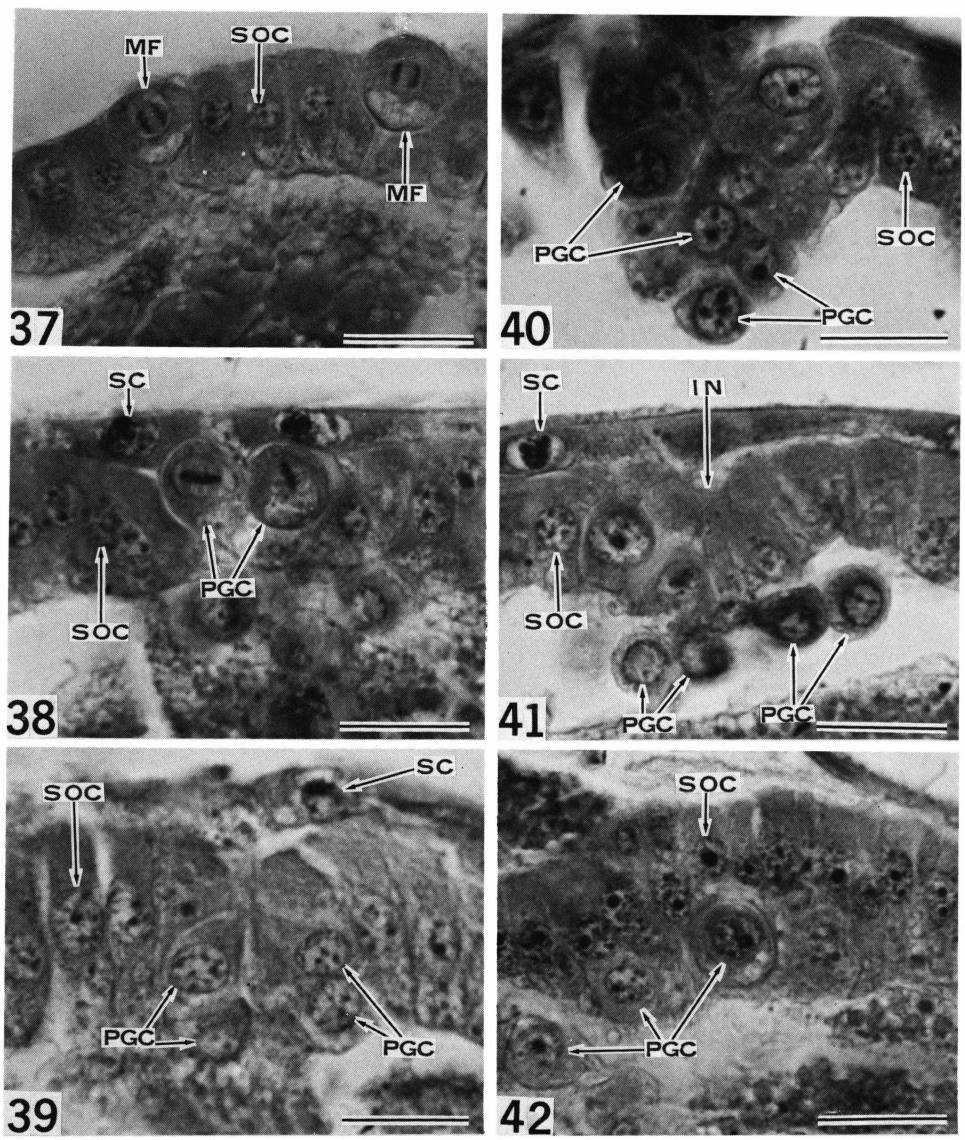
Figs. 22 – 28. Successive changes of anterior region of egg from fertilization to cleavage stage, schematic diagrams. 22. Anaphase of 1st maturation division and sperm, about 1 hr after oviposition. 23. Metaphase of 2nd maturation division and transformed sperm, about 1 hr after oviposition. 24. Four daughter nuclei in or near cytoplasmic island and transformed sperm, about 1.5 hr after oviposition. 25. Three polar bodies, female and male pronuclei, about 2 hr after oviposition. 26. Conjugation of male and female pronuclei, about 2.5 hr after oviposition. 27. Fusion polar bodies and mitotic figure of 1st cleavage, about 3 hr after oviposition. 28. Chromatin mass resulted from fusion of polar bodies and mitotic figures of cleavage nuclei, about 4.5 hr after oviposition.

AP anterior pole of egg, CHR chromosome, CM chromatin mass of polar bodies, CN cleavage nucleus, CON conjugating nuclei, DS dorsal side of egg, FP female pronucleus, MP male pronucleus, MV microvilli, PB_1, PB_2 1st and 2nd polar bodies, PE periplasm, PP posterior pole of egg, SP sperm, UP united polar bodies, VM vitelline membrane, VS ventral side of egg.



Figs. 29 – 34. Longitudinal sections through egg periphery, showing process of blastoderm formation, 9 – 12 hr after oviposition . Scale: 40 μm.

Figs. 35 – 36. Tangential sections through blastoderm. Scale: 40 μm. 35. Blastoderm cells at same stage shown in Fig. 32. 36. Mitotic figures of blastoderm cells at same stage shown in Fig. 33.
AC associated cytoplasm, BC blastoderm cell, IPE inner periplasm, ME migrating energid, OPE outer periplasm, PE periplasm.



Figs. 37 – 42. Longitudinal and cross sections through central region of germ band. Scale: 40 μm. 37. Mitotic figures of somatic cells, longitudinal section, about 18 hr after oviposition. 39 – 40. Group of primordial germ cells, cross sections, about 22 – 23 hr after oviposition. 41. Invagination of germ band and migrating primordial germ cells, cross section, about 25 hr after oviposition. 42. Primordial germ cells located in germ band, longitudinal section, about 32 hr after oviposition.

IN invagination of germ band, MF mitotic figure of somatic cells, PGC primordial germ cell, SC serosal cell, SOC somatic cell.

Yg1, Yg2 and Yg3 or Ygd by Takesue *et al.* (1980) respectively. However the fourth class of yolk granules composed of light-stained small granules and a few of dark-stained dot-like granules located in the central core are observed in the egg of *A. fortunei.*

Changes of the surface of egg

The microvilli or microprojections on the egg surface are seldom mentioned in lepidopteran insects, except for the silkworm, *B. mori* (Miya, 1978, 1980; Takei and Nagashima, 1975; Takesue *et al.*, 1980; Keino and Takesue, 1982). Takei and Nagashima described that the numerous microvilli were seen in the eggs from 1 to 3 hr after oviposition, but they disappeared 2 hr after oviposition. Takesue *et al.* and Keino and Takesue stated that the whole surface of the egg was covered with numerous microvilli up to 4 hr after oviposition, then they disappeared gradually and were replaced with ruffle-like microprojections at 6 hr after oviposition.

In *A. fortunei,* eggs immediately after oviposition the surface of egg was uniformly covered with numerous microvilli. And then the microvilli shrink and become irregularly winding microprojections by 1 hr after oviposition. Since the change of surface structure of the egg takes place very earlier in this species than in *B. mori*, it seems not to correspond with the stages of syngamy and cleavage.

Periplasm

In the newly laid egg the outer layer of the periplasm was divided into two areas; a large area encircling almost the entire equatorial periphery of the egg and a dumbell-shaped one covering a narrow dorsal surface and both poles of the egg. The former was somewhat thinner than the latter and contains a lot of tiny dot-like yolk granules dark-stained with Heidenhain's haematoxylin. These partitions are maintained until the migrating energids reach the periplasm, and the large area and the rest may correspond to the presumptive embryonic area and the presumptive extra-embryonic area respectively. This fact seems to indicate morphologically the predetermination of the embryonic and the extra-embryonic areas.

Polar bodies

Considerable works have been done on the formation and fate of the polar bodies in the various lepidopterous insects.

Eastham (1927, *Pieris*), Johannsen (1929, *Diacrisia*), Presser and Rutschky (1957, *Heliothis*) and Glavanakova and Dryanovska (1969, *Sitotroga*) described that two polar bodies resulted from the maturation divisions, while Hiue (1918, *Eudemis*), Sato *et al.* (1927, *Bombyx*), Bataillon and Su (1933, *Bombyx*), Gross and Howland (1940, *Prodenia*), Rempel (1951, *Mamestra*), Okada (1960, *Chilo*), Kobayashi and Ando (1982, *Neomicropteryx*) and etc. stated that three polar bodies occurred by subsequent divisions in the egg maturation.

Almost all students reported that the polar bodies fused to form a single vesicle and then disappeared in the early cleavage stage, but few ones described as to the fate of the fused vesicle (Gross and Howland, 1940; Sato *et al.* 1927; Bataillon and Su, 1933). In *P. eridania*

Gross and Howland described that the large, irregular chromatic strands formed by the fusion of polar bodies seemed to indicate an unsuccessful division within the vesicle and persisted until the cleavage nuclei migrated just beneath the periplasm. Bataillon and Su and Sato *et al.* observed the mitotic figures of the polar bodies in the eggs of *B. mori.*

In *A. fortunei* three distinct polar bodies were formed and fused to form a single chromatin group after the conjugation of pronuclei. At the stage of eight energids (after the third cleavage stage) the chromatin group was arranged in a row perpendicular to the surface of the egg and the spindle-like structure was rarely formed around the row. This structure resembled the metaphase of the mitotic division and remained unchanged until the migrating energids penetrating into the periplasm. Hence, the formation and fate of the polar bodies in *A. fortunei* are similar to those in *P. eridania.*

Blastoderm formation

In regard to the mode of the blastoderm formation several different results were reported. The first type is the formation of the blastoderm by the occurrence of cleavage furrows between the energids migrated into the periplasm, and is observed in the most species of the Lepidoptera (*Neomicropteryx,* Kobayashi and Ando, 1982; *Prodenia,* Gross and Howland, 1940; *Mamestra*, Rempel, 1951; *Bombyx*, Iwasaki, 1931, Miya, 1958, 1980; *Endoclita,* Tanaka, 1980; etc.). The second type is the formation of the blastoderm by the protrusion of energids beyond the initial level of the periplasm and is reported in *B. mori* (Takesue *et al.*, 1980; Takesue and Keino, 1981). In the third type the protrusion of energids occurrs beyond the initial level of the periplasm at first, but the boundaries between the energids are formed by cleavage furrows, and this type is observed in several species of lepidopterous insects (*Eudemis*, Huie, 1918: *Pieris,* Eastham, 1927; *Chilo,* Okada, 1960; *Papilio*, Vaidya, 1968; *Ancylolomia,* Tanaka, 1970).

In the *A. fortunei* egg, a mechanism of the blastoderm formation is similar to that described by Takesue *et al.* and Takesue and Keino in *B. mori.* Such diffe rence in the formation of the blastoderm may be closely connected with the strength of nuclear protrusion.

Primordial germ cells

Though many morphological observations concerning the time and the process of the primordial germ cell differentiation in the Lepidoptera have been done, the results obtained differ remarkably among the different species or authors. Those differences are probably caused by the difficulty in the distinction between the primordial germ cells and somatic cells just after differentiation. Generally, the primordial germ cells appear as a group of cells at some definite region of the germ band after the germ band formation, but before the inner layer formation (*Ephestia*, Sehl, 1931; *Solenobia*, Lautenschlager, 1932; *Bombyx*, Kawaguchi and Miya, 1943, Miya, 1952, 1958; *Heliothis*, Presser and Rutschky, 1957; *Epiphyas*, Anderson and Wood, 1968, Endoclita, Ando and Tanaka, 1979).

Miya (1950, 1952, 1958) described in *B. mori* that the germ cells were formed through the process of invagination at the ventral side of germ band when the blastoderm differentiated to the germ band and the extra-embryonic area, and the first multiplication of primordial germ cells occurred probably during the blastoderm formation and ceased at the

time of their appearance. Moreover, he (1950, 1957) presumed by the cauterization experiment that the cells destined to be differentiated to the germ cells were predetermined among blastoderm cells.

In *A. fortunei*, the primordial germ cells also differentiate at the definite region of the germ band at the similar period as in most species of the Lepidoptera. However unlike the result in *B. mori* the primordial germ cells of *A. fortunei* increase in number at the time of their first appearance as in *Solenobia triquetrella* (Lautenschlarber, 1932). Further it is suggested that the primordial germ cells in *A. fortunei* do not originate from one stem cell, because two or more mitotic figures are seen at their first appearance.

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