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Electron Microscopic Observation of Egg Apparatus of *Heloniopsis orientalis* (Liliaceae)

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Abstract. The subcellular structure of egg apparatus prior to fertilization in *Heloniopsis orientalis* was investigated using the transmission electron microscope. The egg apparatus which is composed of one egg cell and two synergids is overlaid with a nucellar epidermis. Large plastids filled with starch grains are contained in the cytoplasm of these three cells and also in the cytoplasm of the epidermal cells at the nucellar tip. The egg cell has a poor cytoplasm and there are few organelles in its cytoplasm. Furthermore, the organelles are immature in structure. Both the synergids have a richer cytoplasm than the egg cell and their organelles are more complicated in structure than the egg organelles. Characteristically, there are horseshoe-shaped Golgi apparatus in the synergid cytoplasm. The synergids seem to have a high activity in physiology and metabolism.

Our knowledge on the subcellular and electron microscopic structure of embryo-sac in Angiosperms has yet been very scanty. *Heloniopsis orientalis* has not yet examined embryologically, using not only an electron microscope, but also a light microscope. In the natural state, flowers of *H. orientalis* come out in very early spring. But we could create the individuals whose flowers come out in summer and another individuals whose flowers come out in autumn, by breeding in a growth chamber where temperature and hours of daytime are shifted from seasonal changes in their natural state by one or two seasons. That is, we have become to obtain ovules of *H. orientalis* time and again in a year. We have intended to investigate the subcellular structure of the developing and organized embryo-sac of *H. orientalis*. Our paper presents the subcellular structure of egg apparatus prior to fertilization in this species.

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Material and Method

Many individuals of *Heloniopsis orientalis* were collected at Kiyosato in Yamanashi Prefecture and at Hitachi City in Ibaraki Prefecture. Many of them have been planted and grown on the campus of the Yokohama National University, and some of them have been bred in the growth chamber above mentioned. The clusters of several ovules were excised from an ovary of flower which had come out. The clusters were fixed in 2.5% glutaraldehyde buffered with 0.1 M phosphate buffer and post-fixed with 1% osmium tetroxide in 0.1 M phosphate buffer. These fixed materials were dehydrated in a graded ethanol series and embedded in low viscosity epoxy resin (Spurr 1969). Many sections were stained with 2% uranyl acetate and lead citrate. For the detection of carbohydrate, some sections were stained by silver methanamine (Rambourg & Leblond 1967). The stained sections were examined under the JEM-100CX transmission electron microscope.

Observation

1. Nucellus

Heloniopsis orientalis has many anatropous ovules in an ovary. A nucellus of the ovules is crassinucellate and it is invested with two integuments. Several vegetative cells of the nucellus intervene between an epidermis at and near the nucellar tip and the micropylar end of developing embryo-sac. The intervening nucellar cells are gradually destroyed to change into the material with high electron density, as the embryo-sac develops. The micropylar tip of the organized embryo-sac is thinly overlaid with the material. Before fertilization some epidermal cells of nucellar tip usually divide periclinally to become 2-3 celled layers, and the partially multi-layered epidermis persists after fertilization. Several large plastids filled with several large grains (Fig. 1A) are contained in the epidermal cells at the tip of nucellus where an embryo-sac is organized. However, they are absent (Fig. 1B) or quite infrequent in ones at the flank of nucellus. If they are present in the epidermal cells at the flank, they are smaller than those in the epidermal cells at the nucellar tip. The grains contained in these plastids seems to be starch of carbohydrate, since they are stained with silver methanamine.

2. Egg apparatus

(a) Egg cell

An egg apparatus of the organized embryo-sac is constructed of one egg cell and two synergids. The egg cell (Fig. 2A) is enclosed with a thin but

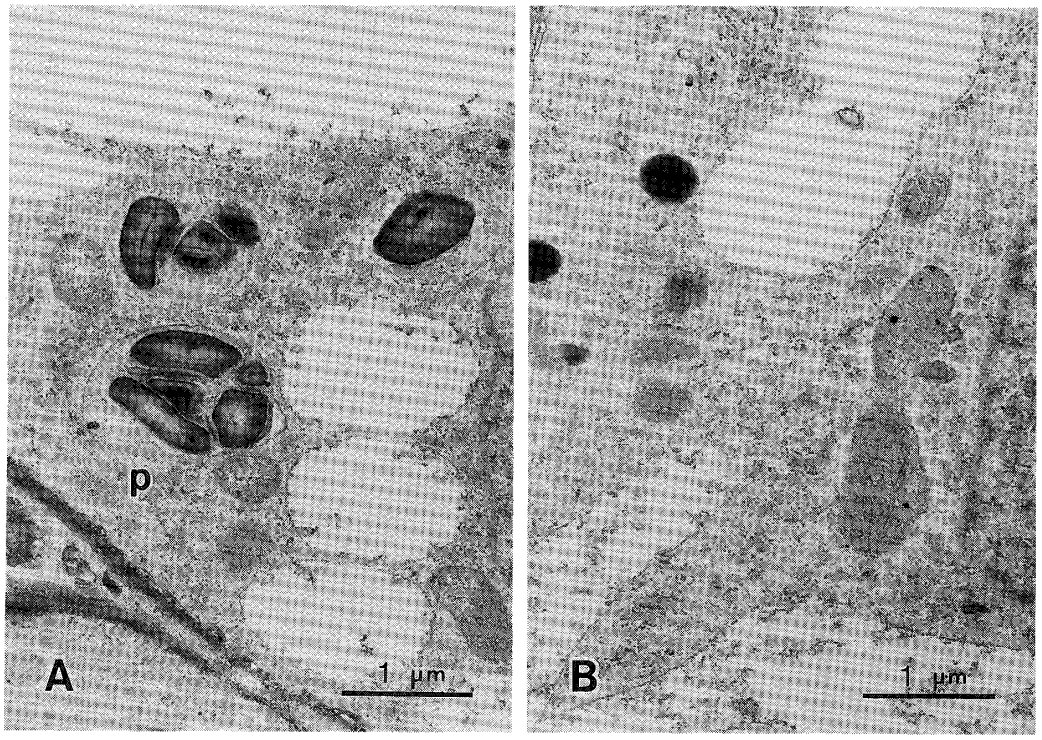

Fig. 1. Cytoplasm of epidermal cell of nucellus. A. Epidermal cell at tip of nucellus. Note the large plastid (p) filled with starch grains. B. Epidermal cell at flank of nucellus. Note an absence of plastid filled with starch grains.

prominent plasma membrane, but the occurrence of the cell wall has not been confirmed in the egg cell at the pre-fertilization stage. The cell is almost occupied with a large vacuole. The large vacuole pushes an egg nucleus to the chalazal end of the cell. Poor cytoplasm exists only around the nucleus. Kinds of organelles occur within the cytoplasm (Fig. 2B). That is, nearly all of the organelles within the egg cell are distributed near the nucleus. It is not difficult to distinguish a plastid from a mitochondrion, although these ellipsoidal organelles enclosed with a double membrane are usually small in volume and immature in structure. Large plastids (Fig. 2A) filled with a starch grain or grains, although they are not frequent, mingle among the immature ellipsoidal organelles. An endoplasmic reticulum (ER) with ribosomes permeates coarsely throughout the poor cytoplasm of the egg cell. A Golgi apparatus is composed of 4–6 stacked cisternae, but there are few Golgi vesicles at the periphery.

(b) Synergid

It seems that there is no difference between two synergids in volume, in

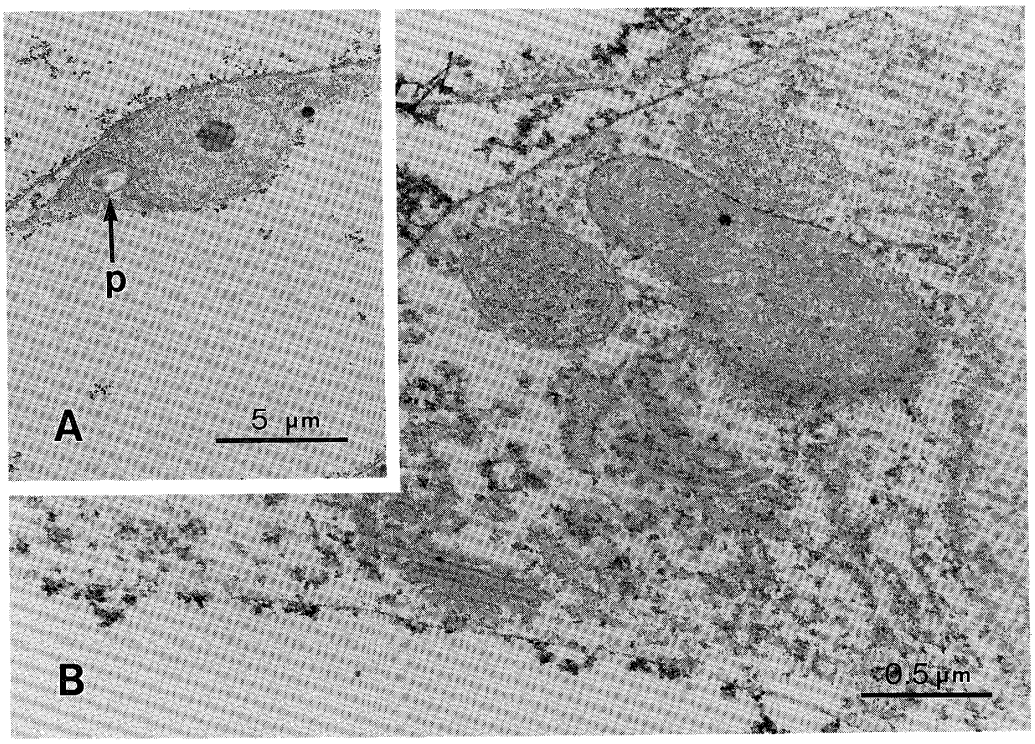

Fig. 2. Egg cell. A. Egg cell with poor cytoplasm where large plastid (p) occurs frequently. Large vacuole pushes the nucleus to the chalazal end. B. Cytoplasm near the egg nucleus.

shape, in structure and in appearance and distribution of organelles. In the synergids, a large vacuole is situated extending from their center to their chalazal end, and a nucleus is pushed toward the micropylar half by the vacuole. The cytoplasm gathers at the periphery of nucleus, but the vacuole is enclosed by the considerably rich cytoplasm as well (Fig. 4B): the cytoplasm is richer than the egg cytoplasm. A conspicuous filiform apparatus (Fig. 3A) well develops at the micropylar half of the synergids: the plasma membrane deeply and complicatedly curves into cell lumen, and the in-curved part is embedded with translucent material. In particular, the filiform apparatus well develops extending from the micropylar tip to the periphery of the nucleus.

There are more organelles within the synergid cytoplasm than within the egg cytoplasm. Organelles ontogenetically develop more further in the synergid cytoplasm than in the egg cytoplasm. Usually, therefore, it is easy to distinguish a mitochondrion from a plastid, although there are a few organelles that it is difficult to identify as a plastid or as a mitochondrion. Mitochondria (Fig. 3B) are ellipsoidal and small, while plastids (Fig. 3B) frequently are

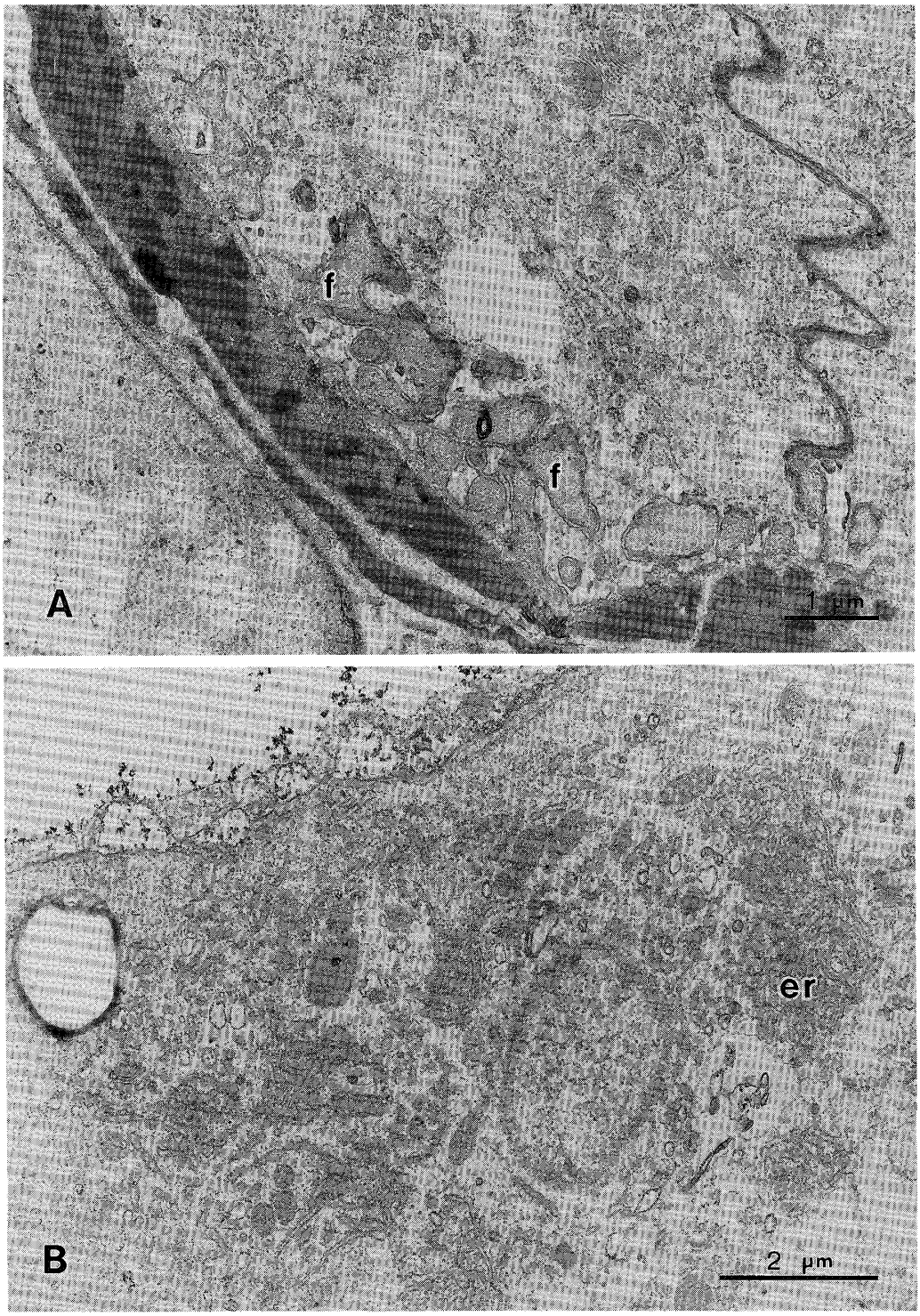

Fig. 3. Synergid. A. Well-developed filiform apparatus (f). B. Cytoplasm where there are numerous mitochondria and well-developed ER. Cisterna of ER (er) partially gathers in a lump which appears as tangled thread.

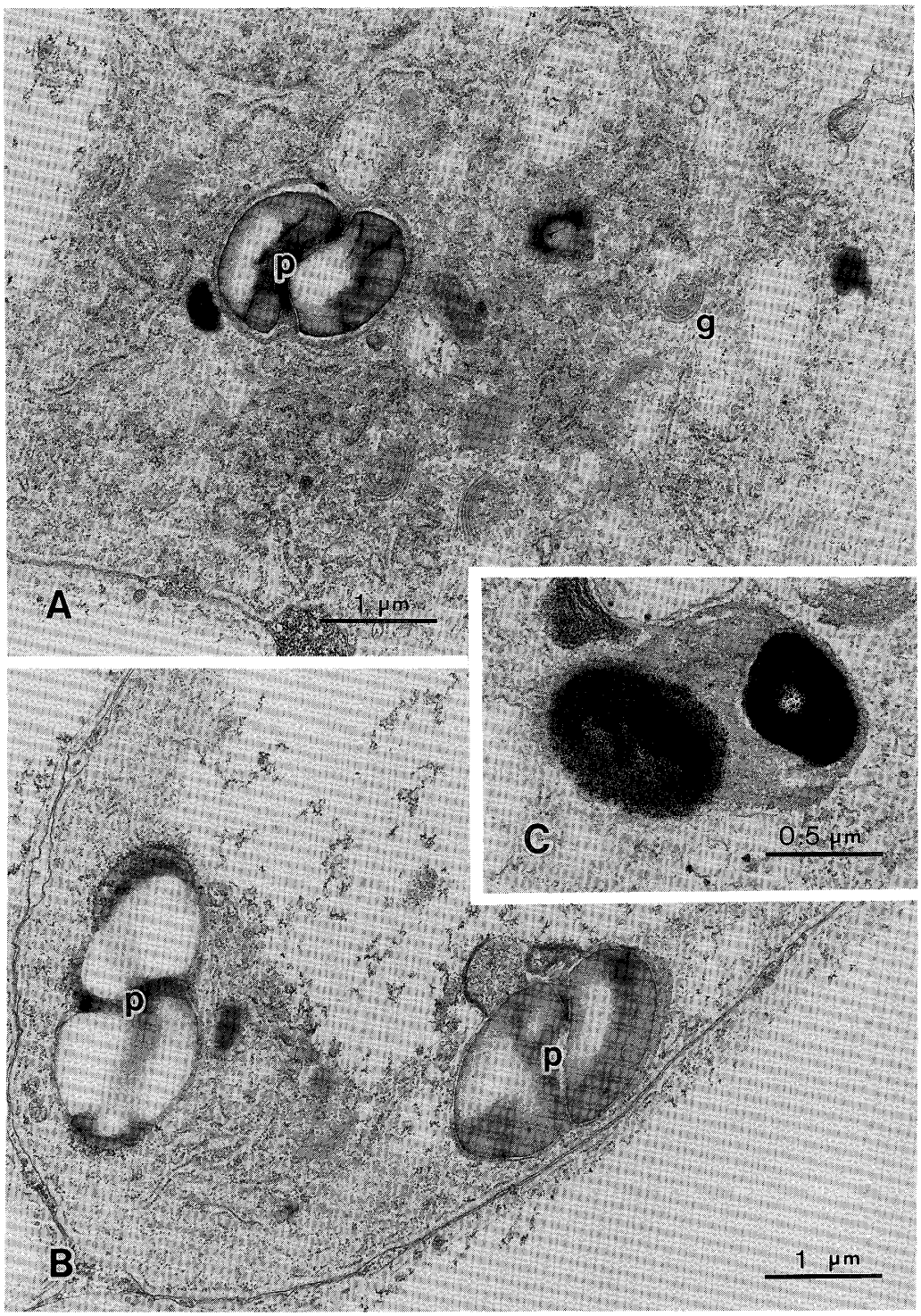

Fig. 4. Synergid. A. Cytoplasm near nucleus. Note the horseshoe-shaped Golgi apparatus (g) the large plastid (p) filled with starch grains. B. Cytoplasm at chalazal end. Note large plastid (p) filled with starch grains. C. Plastid with starch grains stained with silver methanamine.

irregular in shape and large. Mitochondria (Fig. 3B) meaningfully exceed plastids in number. Mitochondria are almost evenly distributed throughout the synergid cytoplasm. In nearly all of mitochondria, their cristae develop rather well and a few lipid globules are contained in their matrix. Many of the plastids are considerably larger than the mitochondria. Their inner membrane system, however, is very immature and several lipid globules are contained in the plastid matrix which is higher in electron density than the matrix of mitochondrion. Furthermore, some of the plastids (Figs. 4A, 4B) become greatly large and they are invariably filled with a starch grain or grains which are stained with silver methanamine (Fig. 4C). These plastids are evenly scattered throughout the synergid cytoplasm.

ER with numerous ribosomes permeates throughout the synergid cytoplasm and the cisterna of ER partially gathers thickly in a lump (Fig. 3B) which appears in sectional profile as tangled threads. Nearly all of the Golgi apparatus are distributed at the micropylar half of synergid. It appears in sections that several Golgi apparatus assemble to form Golgi fields in the synergid cytoplasm. Each Golgi apparatus is composed of 4–6 stacked cisternae like the Golgi apparatus within the egg cell. But different from the Golgi apparatus of egg cell, many of them within the synergids characteristically and strongly bend into a horseshoe in sectional profile (Fig. 4A). Very fine and osmiophilic vesicles are invested with the trans face of the Golgi stack shaped like a horseshoe. Ends of each cisterna of Golgi apparatus swell out, although there are few Golgi vesicles at the periphery of the apparatus.

Discussion

(1) Nucellus

The micropylar end of embryo-sac of *H. orientalis* is overlaid with the epidermis of nucellus before and after fertilization. An embryo-sac overlaid with a nucellar epidermis is well known for the angiospermous plants whose nucellus are crassinucellate. The micropylar tip of embryo-sac in *Aucuba japonica* (Satô 1976a) and *Stachyurus praecox* (Satô 1976b) is overlaid with an epidermal and some subepidermal layers of nucellus. Just before the pollen tube arrives at the tip of the nucellus through the micropyle, the epidermal cell or cells at the tip of nucellus change in shape and from this part the pollen tube enters into the nucellus. In *Spinacia*, Wilms (1981a, b) observed that the middle lamellae of the nucellar tissue between the embryo-sac and micropyle dissolve before the pollen tube arrives at the nucellar tip. In *H. orientalis* examined by us, the change of epidemal cell or cells in shape or/and

dissolution of the middle lamellae of the nucellar tissue have not been observed. But epidermal cells at the nucellar tip are different from ones at the flank of nucellus in appearance of plastids; plastids filled with starch grain are more and larger in the former than in the latter. It seems that the epidermal cell at the nucellar tip exceeds ones at the flank of nucellus in physiological and metabolic activity. This seems to bear some relation to the entry of pollen tube into the nucellus.

(2) Egg apparatus

The egg cell before fertilization has few organelles and also each of them is very immature in structure. Therefore, it seems that the egg cell is very low in physiological and metabolic activities. Within the synergid cytoplasm, on the other hand, the organelles such as mitochondria, ER with ribosomes and Golgi apparatus seem to function actively, since both the synergids, compared with the egg cell, are comparatively complicated in subcellular structure. That is, both the synergids before fertilization seem to have a high activity in physiology and metabolism. The subcellular structure of the egg apparatus in *H. orientalis* is nearly the same as the structure of apparatus which has been examined in other angiospermous plants (Willemse & Went 1984), although the egg apparatus of *Gossypium* where the egg cell has many mitochondria and well-developed ER with many ribosomes (Jensen 1965) is known as well. However, it is noteworthy that the egg apparatus of *H. orientalis* has the features as follows:

1. There are large plastids filled with large grain or grains of starch in the cytoplasm of egg cell and two synergids.
2. There are horseshoe-shaped Golgi apparatus in the cytoplasm of two synergids.

We will report in near future on the process of fertilization, the structure of embryo-sac after fertilization and so on in *H. orientalis*.

摘　　要

ショウジョウバカマ（*Heloniopsis orientalis*, ユリ科）の受精前の卵装置の構造を透過型電子顕微鏡を用いて観察した。胚株は倒生で，2枚の珠皮が厚層珠心を囲んでいる。珠心表皮は部分的に並層分裂を行い，2－3細胞層になり，受精後も消えずに生き残っている。卵装置はこの表皮で覆われていることになる。大きなデンプン粒で満たされた大形の色素体は，珠心側部の表皮細胞には含まれていても数は少ないが，珠心先端部の表皮細胞には必ず含まれており，数も多い。卵装置は1個の卵細胞と繊形装置の発達した2個の助細胞で構成されている。受精前の2個の助細胞には構造上の相違は特にはみられ

ない。卵装置を構成する3細胞にも，デンプン粒を含む大形の色素体が存在する。卵細胞の大部分は液胞で占められており，核は合点端に位置している。核の周辺にはいろいろな細胞内小器官がみられるが，数は少なく，構造も単純である。助細胞では，多数のリボソームを伴った粗面小胞体がよく発達し，もつれた糸が作る糸玉のように塊状に集まった小胞体が部分的にみられる。クリステの発達したミトコンドリアも量的にかなり多く存在している。繊形装置付近には多数のゴルジ体がみられるが，強く湾曲し馬蹄形状のゴルジ体が助細胞に特徴的に存在している。馬蹄形状のゴルジ体とデンプン粒を含む大形の色素体の存在が，ショウジョウバカマの卵装置の構造的な特徴と思われる。

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