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The sperm nucleus observed in the embryo sac of *Tricyrtis hirta* (Liliaceae)

Kazuaki KONDO and Yoshihiko SATO*

ホトトギス（ユリ科）の胚嚢内に観られた精核

近藤　和昭・佐藤　嘉彦

Abstract

The embryo sac before a pollen tube reaches, and the embryo sac after a pollen tube reaches were observed in *Tricyrtis hirta* of liliaceaous plant. Before the pollen tube reaches the embryo sac, two polar nuclei inside the central cell fuse, and become one large diploid nucleus. Moreover, an egg nucleus and the diploid nucleus have a globular form, and a nulear envelope of them spreads completely. But, looseness like a wave occurs in the nulear envelope of the egg nucleus and the diploid nucleus, when the pollen tube reached the embryo sac and a sperm nucleus was included in the egg cell and the central cell. The sperm nucleus immediately after it went into the egg cell and the central cell is thought to have an ellipsoid. As the sperm nucleus approaches the egg nucleus and the diploid nucleus, the side of the sperm nucleus which faces the egg nucleus and the diploid nucleus decreases in the degree of curving, and the shape of the sperm nucleus warps. The nulear envelope of the egg nucleus to face the sperm nucleus changes to go side by side with the nulear envelope which warped in the sperm nucleus in accordance with the deformation on the side of the sperm nucleus. The nulear envelope of the sperm nucleus, the egg nucleus and the diploid nucleus of the central cell maintains a double membrane structure while the sperm nucleus is not contact with to the egg nucleus and the diploid nucleus of the central cell.

An embryo sac just before the fertilization was observed by using the electron microscope in *Tricyrtis hirta* of Liliaceae. Researches of the fertilization of Angiosperm are reported up to today. But, sufficient informations haven't been collected yet.

The embryo sac in some speceis of *Tricyrtis* was researched by Ogura (1964 and 1966) by using the optical microscope. According to him, this embryo sac is formed in accordance with monosporeic 8-nucleate *Polygonum* type. Ando and Sato (1991) researched the megasporogenesis in *T. hirta* by using the electron

* Depertment of Science Education, Faculty of Education and Human Science, Yokohama National University, Tokiwadai, Hodogaya-ku, Yokohama, 240-8501, Japan

microscope. *T. hirta* has many ovules in one ovary. These ovules face the same direction, and they arrange regularly. The samples to observe the embryo sac embedded in the ovule with an electron microscope are easy to make for these reasons.

Material and Method

Many young flower buds were collected from individuals of *Tricyrtis hirta* grown on the campus of the Yokohama National University extending from September to October in 2001 and 2002. The clusters of several ovules were excised from an ovary of buds collected. They were fixed in 1.5% 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). Thin sections were stained with 2% uranyl acetate and lead citrate. the stained sections examined under the JEM-100CX transmission electron microscope.

Observation

1 . The embryo sac waiting for the pollen tube

(1) Synergid

The nucleus of the synergid (Fig. 1) is located at about the center of the cell. This nucleus almost has a globular form, and there is a large nucleolus at the center of the nucleus. Half on the chalaza side is occupied with a large vacuole, and a cytoplasm hardly exists. On the other hand, a cytoplasm exists abundantly in half on the micropyle side of the synergid. A mitochondrion, a plastid, a Golgi body, a rER, a vesicle, and so on can be observed in the cytoplasm. The plastid is larger than the mitochondrion. The plastid has various forms though the mitochondrion is the ellipsoid which is close to the globular form. The granule of starch is contained in many plastids. It seems that the Golgi body is the annual ring which consists of 5 - 6 layers in the sample of the microscope. Many Golgi vesicles exist at the central part of the body. A globular vesicle is the size of several hundred nm in the diameter, and the vesicle is surrounded by a single membrane. The inside of this vesicle is dotted with the part which has high electron density and which looks black. The vesicle exists in the synergid before the fertilization very especially much. These vesicles will be probably concerned with the formation of the filiform apparatus.

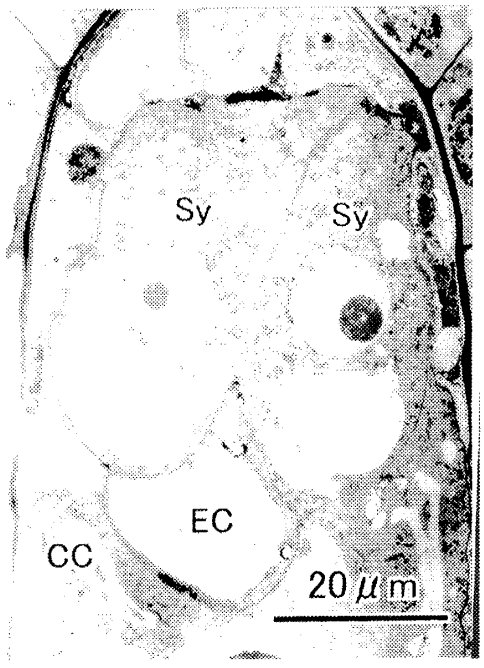

Fig. 1. The organized synergids (Sy) before the sperm nucleus enters it. EC: egg cell, CC: central cell.

The filiform apparatus is made on the cell wall which surrounds half on the micropyle side in the synergid. But, a filiform apparatus isn't made in half on the chalaza sides of the synergid. The plasmodesma to make contact with the egg cell, and the plasmodesma to make contact with the central cell exist in the cell wall of the synergid where the filiform apparatus doesn't develop. A difference concerning the cytology which it should make special mention of wasn't seen between two synergids just before the fertilization.

(2) Egg cell

The nucleus in an egg cell (Fig. 2) is the globular form which has the diameter of about 12 μm. The

nuclear outline is smooth, and it spreads, and not still uneven. A large nucleolus exists at the center of this nucleus. The nucleoplasm which has low electron density and which it seems to be light gray surrounds the nucleolus. A few cytoplasms are only distributed around the nucleus except for the cytoplasm which exists a little along the cell wall. The various kinds of organelle exist in the egg cell as well. But, there are a few organelles in number. The plastid which contains starch granule, that is, an amyloplast is outstanding existence in them. The wall of egg cell of this time is completed only around the end on the micropyle side, and the cell wall is very immature in other parts. The egg cell grows in the direction of the chalaza longer than two synergids.

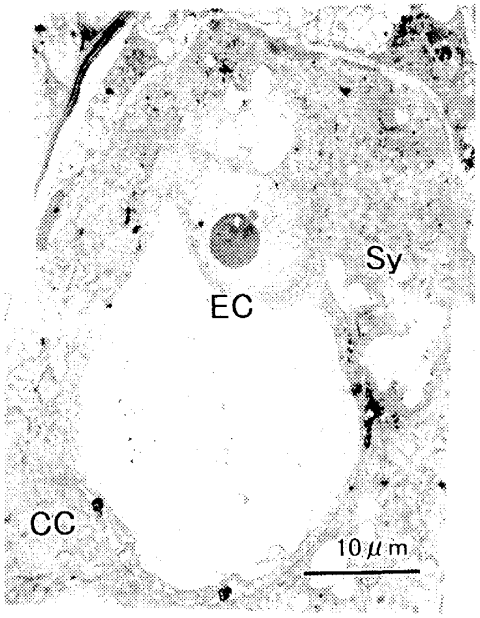

Fig. 2. The egg cell (EC) with its haploid nucleus before the sperm nucleus enters it. Sy: synergid, CC: central cell..

(3) Central cell

Two polar nuclei in the inside of the central cell fuse near the egg cell in the early time rather than a pollen tube reaches an embryo sac. Though the diploid nucleus that two polar nuclei fused almost has a globular form, the nuclear envelope isn't smooth, and it is undulating like a wave. This nucleus is about 30 μm in the diameter. The large nucleolus occupies the center of this nucleus. The part of the high electron density surrounds the part of the low electron density in this nucleolus. The various kinds of organelle exist in the central cell. A mitochondrion and a plastid exist especially abundantly even in them. Unlike the plastid in the egg cell, the plastid doesn't contain a starch granule.

2. The synergid that a pollen tube entered

The style of pistil begins to curve when 2-3 days pass after flowering. It continues to curve more for several days after beginning of the curvature. When the style began to curve, a pollen tube didn't reach the inside of the ovary yet. The pollen tube reached the ovule when 4-5 days passed after flowering and the style curved more strongly.

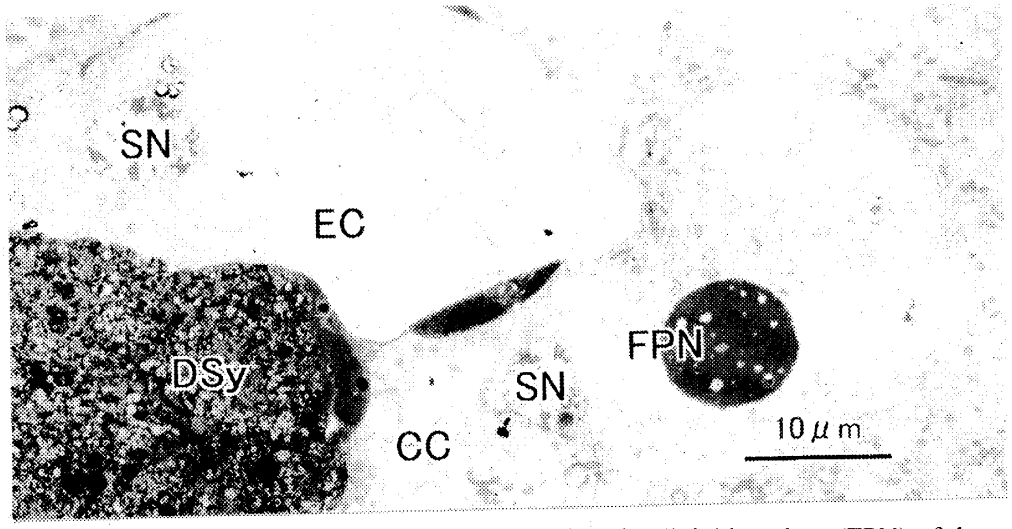

Fig. 3. The sperm nucleus (SN) which approaches the diploid nucleus (FPN) of the central cell (CC), and the sperm nucleus included into the egg cell (EC). DSy: degenerated synergid.

After the tip of the pollen tube passed through the micropyle, the tip reaches one of two synergids. Only this tip is conducted into that synergid. This tip of the pollen tube breaks, and the contents of the pollen tube are released in the synergid. The cytoplasm of the synergid which accepted the contents of the pollen tube rises in the electron density.

Two X-bodies which originated in the nucleus of the synergid and the vegetative nucleus in the pollen tube can be observed in the cytoplasm where electron density has rosen. A ribosome can be observed clearly. But, the cisternae of the ER decrease in the electron density, and it seems that the function of the ER is almost lost. Unfortunately, we couldn't observe a sperm cell or a sperm nucleus in the inside of the synergid in our investigation.

3. The egg cell that a sperm nucleus entered

A sperm nucleus contained in each of the egg cell and the central cell in the inside of the same embryo sac is shown in the figure 3. The egg cell has a circumference covered in the cell wall. The synergid which adjoined an egg cell and which is degenerating can be observed in this figure. This synergid has high electron density, and this is observed in black.

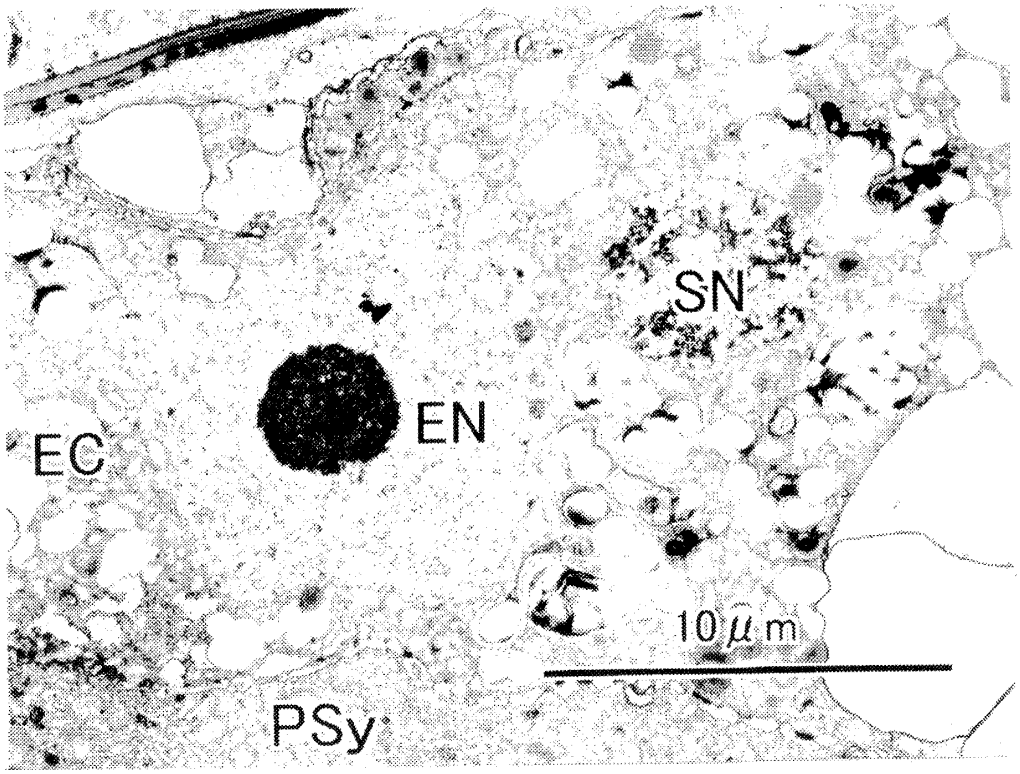

Fig. 4. The sperm nucleus (SN) in the egg cell (EC), which approaches the egg nucleus. (EN). PSy: persisting synergid

Another egg cell which is different from the egg cell shown in Fig.3 is shown in the figure 4. The figure 4 shows the egg cell which a large nucleus and a small nucleus exist in. The large nucleus almost has a globular form, and this nuclear diameter is 10-12 μ m. There is a large nucleolus whose electron density is high at the center of this nucleolus. Furthermore, the structure which has high electron density and which looks black can't be found in the nucleoplasm except for the nucleolus. This nucleus isn't thought to be structurally different from the nucleus of the egg cell which doesn't accept a nucleus and which was above mentioned. So, this nucleus can be judged the nucleus of the egg cell just before the fertilization. But, this nuclear envelope

undulates like a wave in the outline when it is compared with the nuclear envelope of the egg cell which doesn't contain a sperm nucleus. On the other hand, the small nucleus is an ellipsoid. The long diameter of this nucleus is 7 μ m from 8 μ m, and a short diameter is about 5 μ m. The chromatin which has high electron density and which looks black is observed clearly. So, it is judged that this small nucleus is the sperm nucleus sent in the egg cell. This sperm nucleus approaches an egg nucleus even in about 1.5 μ m. The sperm nucleus approaches an egg nucleus more. A distance between the sperm nucleus and the egg nucleus shown in the figure 5 is about 0.5μ m a little. The nuclear envelope which surrounds both nuclei almost goes side by side, and they are still maintaining a double membrane structure together. It seems that organelles inside the egg cell where a sperm nucleus has already been accepted become richer than the egg cell before accepting them. The inside of the cytoplasm which surrounds the egg nucleus and the sperm nucleus is especially rich in a mitochondrion and a plastid which contains starch granule.

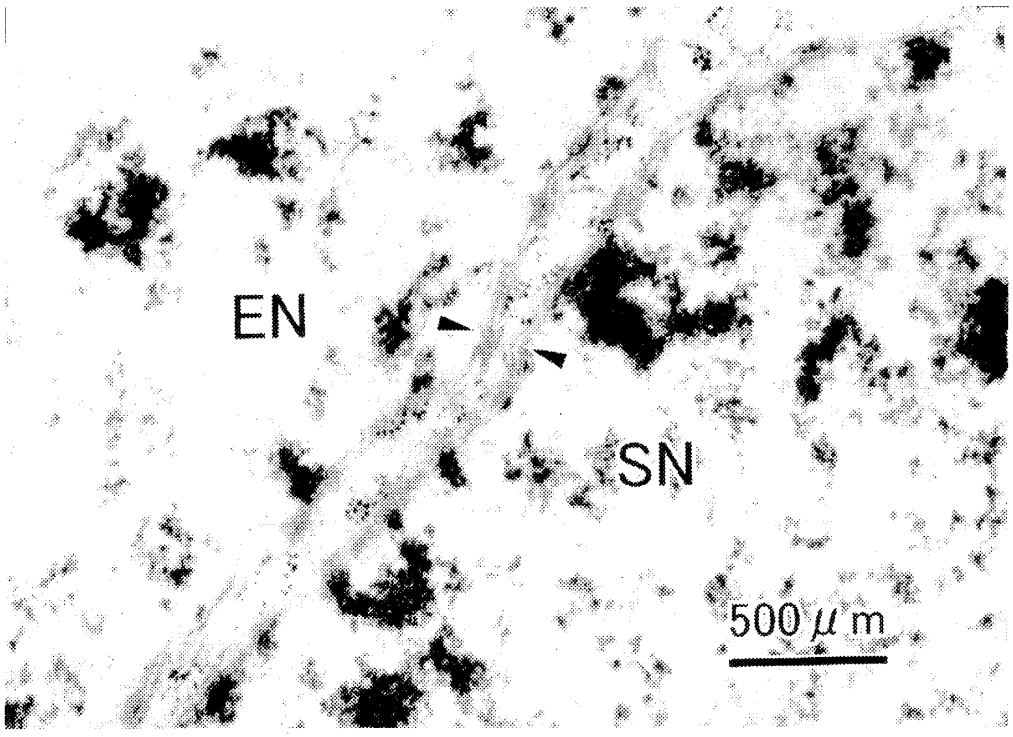

Fig. 5. Each nuclear envelope of the sperm nucleus (SN) and the egg nucleus (EN) just before the connection.

4. The central cell which accepted a sperm nucleus

A figure 6 is a photograph from another sample that it was made from the same embryo sac shown in the figure 3. A small nucleus approaches just the even neighborhood of the diploid nucleus of the central cell in the figure 6. Though the diploid nucleus of the central cell is a globular form mostly, the nuclear outline isn't smooth, and it undulates like a wave greatly. The traces which a sperm nucleus or a sperm cell passed through weren't observed in the cell wall of the central cell near the egg apparatus. A small nucleus can be observed in the position where about 0.1 μ m left it from this diploid nucleus. This small nucleus has the long diameter of about 6 μ m. The chromatin which has high electron density and which looks black is observed in this nucleus. This nucleus is judged to be the same as the small nucleus observed in the egg cell from these points. In other words, this small nucleus is considered a sperm nucleus. There is a difference in the degree of curving between the side which faces the diploid nucleus of the cetral cell, and the side of the opposite side in this

sperm nucleus. The degree of curving of the side of the former is lower than the latter. On the other hand, the nuclear envelope of the egg nucleus which faces a sperm nucleus changes to go side by side on its side of the diploid nucleus of the cetral cell.

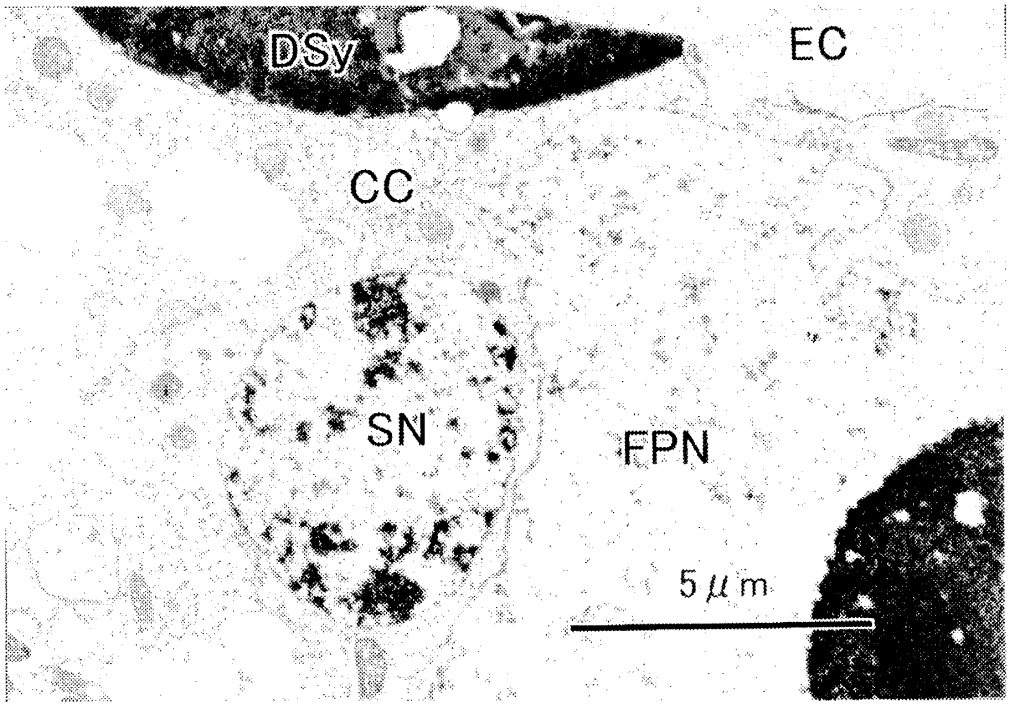

Fig. 6. The sperm nucleus (SN) which approaches the diploid nucleus (FPN) of the central cell. CC: central cell, DSy: degenerated synergid, EC: egg cell.

Discussion

Before a pollen tube reaches an embryo sac, it is said that one of two synergids begins to degenerate (Jensen 1965). But, such a phenomenon wasn't confirmed in *Tricyrtis hirta*. More precise research in this species is necessary in the future.

The traces which a sperm cell or a sperm nucleus passed through weren't recognized in each cell wall of the egg cell and the central cell which had accepted a sperm nucleus alreadg. A proposal concerned with the way that a sperm cell or a sperm nucleus enters in the egg cell and the central cell was made by Went et al. (1984). From our obsevation it may be able to guess that either of the following processes happens in the egg cell and the central cell.

1. The sperm cell or a sperm nucleus enters in the egg cell and the central cell before the cell wall of these cells is formed.
2. The wall is repaired at once right after a sperm cell or a sperm nucleus passed through the cell wall of the egg cell and the central cell.

A sperm nucleus in the inside of the egg cell and the central cell was observed. Unfortunately, the fusion of the sperm nucleus and the egg nucleus and the fusion of the sperm nucleus and the diploid nucleus of the central cell couldn't be observed.

The haploid nucleus of the egg cell, the diploid nucleus of the central cell and the sperm nuclei which entered in the egg cell and the central cell cells are in the following condition.

1. Observed sperm nuclei aren't surrounded in the cytoplasm and the cell wall. They exist under the naked

condition in the egg cell and the central cell.

2. Chromatin can be observed clearly though the nucleolus of the sperm nucleus isn't clear.
3. One large nucleolus always exists in the egg nucleus and the diploid nucleus of the central cell.
4. Each nulear envelope of the egg nucleus and the nucleus of the central cell which a sperm nucleus is approaching undulates like a wave, and not smooth.
5. A sperm nucleus may have an ellipsoid or a globular form right after the penetration into the egg cell or the central cell. But, as approaching an egg nucleus or the diploid nucleus of the central cell, the sperm nucleus changes a form. The degrees of curving are made to decrease on the side of the sperm cell which faced an egg nucleus and the diploid nucleus of the central cell.
6. Each nulear envelope in the egg nucleus and the diploid nucleus of the central cell changes to go side by side with the nulear envelope of the sperm nucleus that the degrees of curving decreased, too.
7. When a sperm nucleus doesn't touch perfectly to the egg nucleus and the diploid nucleus of the central cell yet, each nulear envelope which surrounds the sperm nucleus, the egg nucleus and the diploid nucleus of the central cell respectively maintains a double membrane structure.

A conspicuous large nucleolus exists in the egg nucleus and the diploid nucleus of the central cell. A large nucleolus is made before the active composition of RNA (Scott 2001). Besides there are a few cytoplasms in the egg cell, this cell has only the organelle of a small quantity. The large nucleolus of the egg nucleus is supposed to shoulder some important roles. It seems that the proposal made by Scott can be accepted.

Just before the nulear envelope of these nuclei sticks, the double membrane structure of the nulear envelopes in the nuclei which will fuse is being maintained. A change will happen in each nulear envelope after the time when we observed it. The photograph of the fusion of two polar nuclei is adopted in the 'Atlas' edited by Cresi et al.(1992). This photograph was taken by Wilms. Furthermore, Went and Willems (1984) is successful in taking a photograph that a sperm nucleus and a diploid nucleus fuse in the central cell. The phenomenon shown in these photographs will progress at the time of the fertilization of *Tricyrtis hirta* as well.

摘　要

ユリ科植物のホトトギスを材料として、花粉管が到達する前の胚嚢と到達後の胚嚢を観察した。花粉管が到達する前に、中央細胞内の２個の極核は融合し、大きな１個の複相の核になる。また、卵核と複相核は球形をしており、核膜は張りきっている。しかし、この両核は時間の経過と共に次第に変化し、花粉管が助細胞に到達し、精核が卵細胞と中央細胞に入ったときには、卵核と複相核の核膜は波を打ったようにたるみが出来る。卵細胞と中央細胞に入った直後の精核は楕円体をしていると思われる。精核が卵核や複相核に近づくにつれて、卵核や複相核に面している精核の側面が湾曲の程度を減少させ、精核の形がいびつになる。精核の側面の変形に合わせて、精核に面している卵核と複相核の核膜は精核の変形した核膜に並行するように変わる。精核が卵核と中央細胞の複相核に接着する直前まで、精核と卵核、複相核のそれぞれの核膜は２重膜構造を維持している。

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