Yoshihiko SATÔ* & Takehiro ARIMA**: Development of spore and gametophyte in *Liriope platyphylla* Wang et Tang

佐藤嘉彦*・有馬武裕**: ヤブランの胞子および配偶体の形成

Liriope is usually treated as a genus of the family Liliaceae, but Huber (1969) assigns it to the Convallariaceae. Unfortunately *Liriope* has not been studied embryologically. We have, therefore, intended to investigate the developmental mode of the spore and the gametophyte in *Liriope platyphylla*.

Occurrence of callose during sporogenesis has been investigated in some angiospermous plants, since it was demonstrated by Waterkeyn (1962) during microsporogenesis, and by Rodkiewicz & Górska-Brylass (1967) during megasporogenesis. It is suggested that secretion of callose on a cell wall may be related to a decrease of permeability of the wall to macromolecules (Heslop-Harrison 1966). We have investigated also the behavior of callose during development of the spore and the gametophyte in *L. platyphylla*.

Material and Methods Many individuals of *Liriope platyphylla* were gathered in the environs of Shinsei-ko in Hatano City, Kanagawa Pref. and planted on the campus of Yokohama National University. From them, many flowers were collected at appropriate intervals extending from June to Augast in 1980–1983, and fixed with formalin-acetic-alcohol (FAA). The fixed materials were dehydrated in ethyl alcohol-*tert*. butyl alcohol series and embedded in paraffin (m.p. 57–60 C). They were sectioned serially at 6–8 μm thick. The sections were stained with Heidenhain's iron alum hematoxylin and fast green combination or saffranin and fast green combination.

Materials to detect callose were also fixed with FAA and were sectioned according to the same procedure as the above-mentioned one. The sections were stained with aqueous solution of aniline blue (Smith & McCully 1978) and observed under the fluorescence microscope. Callose emits bright-yellow fluorescence when treated with the fluorochrome.

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To observe the development of pollen tubes, pollen grains were put onto the surface of an agar medium containing about 12% sucrose. The pollen tubes were stained with 1% aceto-orcein to observe the vegetative nucleus, generative cell and sperm cells, and callose in the wall of the tube was detected by treatment with aniline blue.

Observation Development of microspore and microgametophyte. Sporogenous cells divided mitotically and gave rise to microsporocytes (Fig. 1A). They began to disperse one another in the anther locule and gradually changed into a prolate shape during the first meiotic division (Fig. 1B). A spindle of this division was usually formed along the long axis of the microsporocyte. After the first division two dyad cells (Fig. 1C) were separated by a cell wall. After the second meiotic division a separating wall was formed in each of the two cells; the spindle formed in one dyad cell was either parallel with or perpendicular to that in another cell (Fig. 1D). Consequently, the arrangement of the microspores in tetrad was either isobilateral (Fig. 1E) or decussate. The isobilateral arrangement was seen more usually than the decussate. The microsporogenesis in *L. platyphylla* followed the successive type of cytokinesis.

Soon after the microsporocytes entered the first meiotic division, a massive deposit of hyaline substance came to appear along the inside of their walls and before the end of the first division the substance completely enclosed the microsporocyte. It also deposited on the wall separating two dyad cells and on the two walls formed after the second meiotic division. Consequently, each of the four tetrad cells was surrounded and isolated by the hyaline walls (Fig. 1E). The microspores were released from the tetrad by the dissolution of these hyaline walls. The released microspore increased in size and a large vacuole or vacuoles were formed in its cytoplasm (Fig. 1F-a). The microspore nucleus was displaced from its original central position toward the wall on the side of the microspore away from a furrow, where it would divide mitotically (Fig. 1F-b) to form a small generative cell lying next to the spore wall and a much larger vegetative cell (Fig. 1G), the former being separated from the latter by a curved thin wall. A little later, the generative cell became detached from the pollen wall by means of the gradual decrease of the contact area with the intine, so that the free generative cell became surrounded by the cytoplasm of the vegetative cell. In *L. platyphylla*, the pollen grain had one slender furrow and was shed from the anther in a two-celled state.

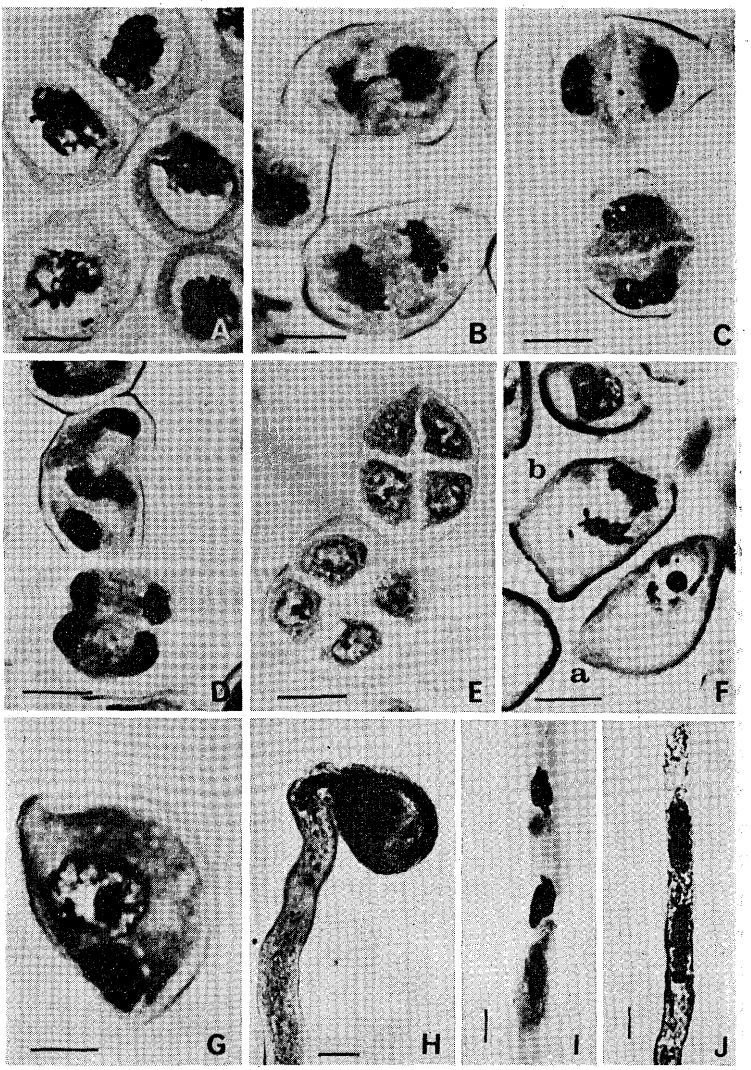

Fig. 1. Development of microspore and microgametophyte in *Liriope platyphylla*. A. Microsporocytes. B. Microsporocytes in division. C. Dyads. D. Two dyad cells, each in division. E. Tetrads. F. Microspores with large vacuole (a) and its nucleus in division (b). G. Pollen grain with large vegetative nucleus and generative cell. H. Entry of generative cell into pollen tube. I. Portion of pollen tube. The vegetative nucleus proceeds two sperm cells. J. Portion of pollen tube. The generative cell proceeds the vegetative nucleus. (All scales equal 10 μm)

Pollen grains began to germinate at about 15 min after they were put onto the surface of the agar medium and after 60 min in culture, pollen tubes were formed in more than 95% of the grains. When the tube attained a length of about 100 μm, the generative cell began to enter the developing pollen tube (Fig. 1H) and it divided to form two sperm cells in the pollen tube. Since it was difficult to differentiate the vegetative nucleus from the cytoplasm in most of the pollen tubes stained with aceto-orcein, it was very nearly impossible to make the behavior of vegetative nucleus clear. But in the pollen tubes 250–300 μm long, the vegetative nucleus was rarely observed. In some of these pollen tubes, it proceeded the generative cell or sperm cells (Fig. 1I); in others it was found behind the generative cell (Fig. 1J).

Deposition and disappearance of callose during development of microspore and microgametophyte. Hyaline wall formed during the microsporogenesis always exhibited the callosic reaction when treated with aniline blue. The deposition and disapperance of this wall were understood in more detail by applying fluorescence test for callose.

Callose began to fluoresce on the wall of microsporocyte at the leptotene stage of the first division (Fig. 2A). Before this stage, callosic fluorescence was never observed on any walls of microsporocyte. Usually, callose began to deposit on the wall of microsporocyte near the poles of spindle which would be formed in process of the first division. The callosic wall thickened rapidly in the poles (Fig. 2B) and it extended to line the whole of the microsporocyte wall before the end of this division, though deposition of callose considerably lagged behind along the equator of the microsporocyte wall which the cell plate formed during the first division was expected to join. The callosic reaction was also observed on the wall formed after the first division (Fig. 2C) and on the further walls formed after the second division (Fig. 2D). Consequently, each of the tetrad cells was completely enclosed by the callosic wall.

Shortly after the meiosis was over, the microspores were released from the tetrad by complete dissolution of callosic wall around the tetrad (Fig. 2E). Thus, the callosic fluorescence completely disappeared from the microspores for a short period. But it was temporarily observed on the curved thin wall formed after the division of microspore nucleus (Fig. 2F) and disappeared again soon after; the fluorescent wall did not surrounnd the generative nucleus and its associated cytoplasm. Before the mature pollen grains, in which the generative

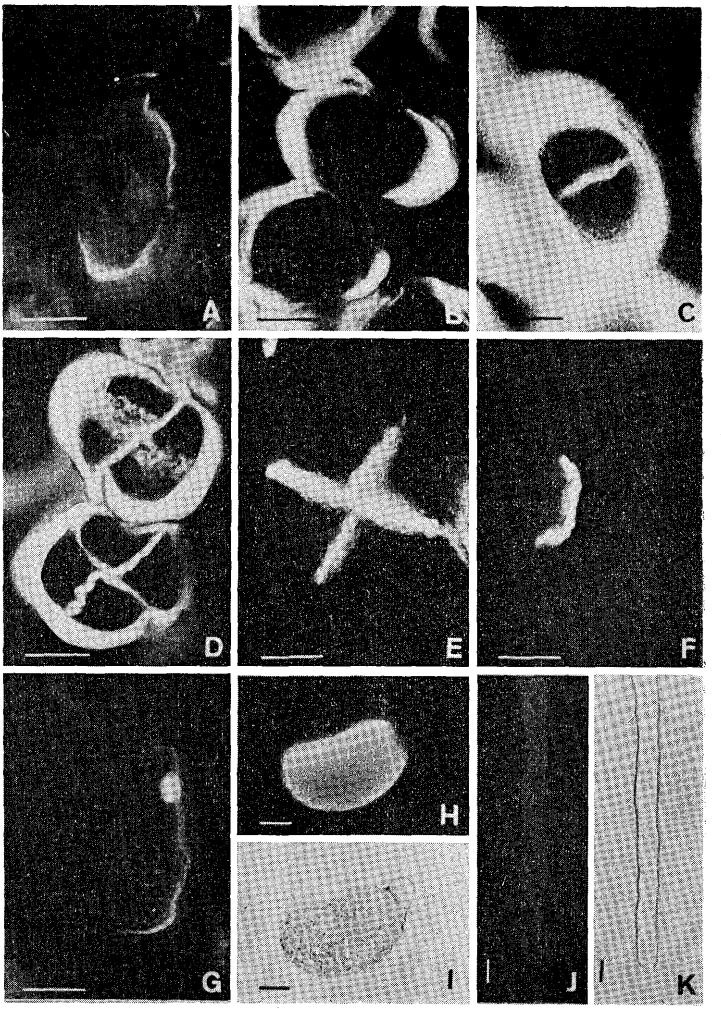

Fig. 2. Deposition of callose during microsporogenesis and microgametogenesis in *Liriope platyphylla*. A. Microsporocyte in leptotene stage. B. Microsporocytes in diplotene stage. C. Dyad. D. Tetrads. E. Tetrad shortly before the microspores are released. F. Callosic wall formed after the division of microspore nucleus. G. Mature pollen grain. Callose accumulates at a subpolar region of furrow. H & I. Pollen grain after about 15 min in culture. H is the fluorescence photomicropraph of the grain shown in I. J & K. Pollen tube in culture. J is the fluorescence photomicrograph of the tube shown in K. The apical part of elongating pollen tube does not show the fluorescent reaction. (Scales equal 10 μm except H and I where scales equal 30 μm)

cell had already been detached from the pollen wall, were shed, the accumulation of callose occurred at a subpolar region of the furrow from which the pollen tube would emerge (Fig. 2G).

Immediately after the pollen tube emerged from the grain, callosic fluorescence was not observed in the wall of pollen tubes (compare Fig. 2H with Fig. 2I). But, in the pollen tubes which reached a length of more than 20 μm, the fluorescence began to be found at the proximal portion of them. After this stage, it was observed in the wall of developing pollen tube except for a distance of about 20 μm from the tube apex (compare Fig. 2J with Fig. 2K). No callosic fluorescence was observed in this apical portion when the pollen tube continued to elongate actively, but when the tube had stopped elongating, callose rapidly deposited in this portion as well.

Ovule. The ovary was incompletely divided into three loculi with three parietal placentae. Two ovular primordia initiated on each placenta and the ovule attained nearly an anatropous condition before the end of megasporogenesis. A nucellus was enclosed by two integuments (Fig. 3K), of which the inner initiated earlier than the outer. The former was always composed of two layers of cells except its apical portion and grew rapidly to form a micropylar cannal, while the latter grew up to the level of the micropyle before fertilization and elongated greatly to form a testa after fertilization.

In some ovules (Fig. 3A, C) the megasporocyte was situated directly below the nucellar epidermis, while in others (Fig. 3B, D) it was separated from the epidermis by a parietal cell or cells. A tenuinucellate and a crassinucellate type of nucellus occurred in *L. platyphylla*, the latter occuring about twice as many as the former. Some of the epidermal cells at the nucellar tip divided periclinally to become two or three layers of cells. Cells at the lower portion of nucellus and near the chalaza differentiated into a hypostase. From about the time when the meiosis was over, they began to become poor in cytoplasmic contents and to have helical thickenings on their walls. But, when treated with aniline blue, wall of the nucellar cell or cells below the megasporocyte in some ovules had already begun to emit weak bluish-white fluorescence different from the callosic one (Fig. 4A). As the megasporogensis and the megagametogenesis proceeded, this bluish-white fluorescence (Fig. 4C) became strong gradually and the cells with fluorescent wall increased in number.

Embryo sac formation. A young megasporocyte (Fig. 3A, B) differentiated

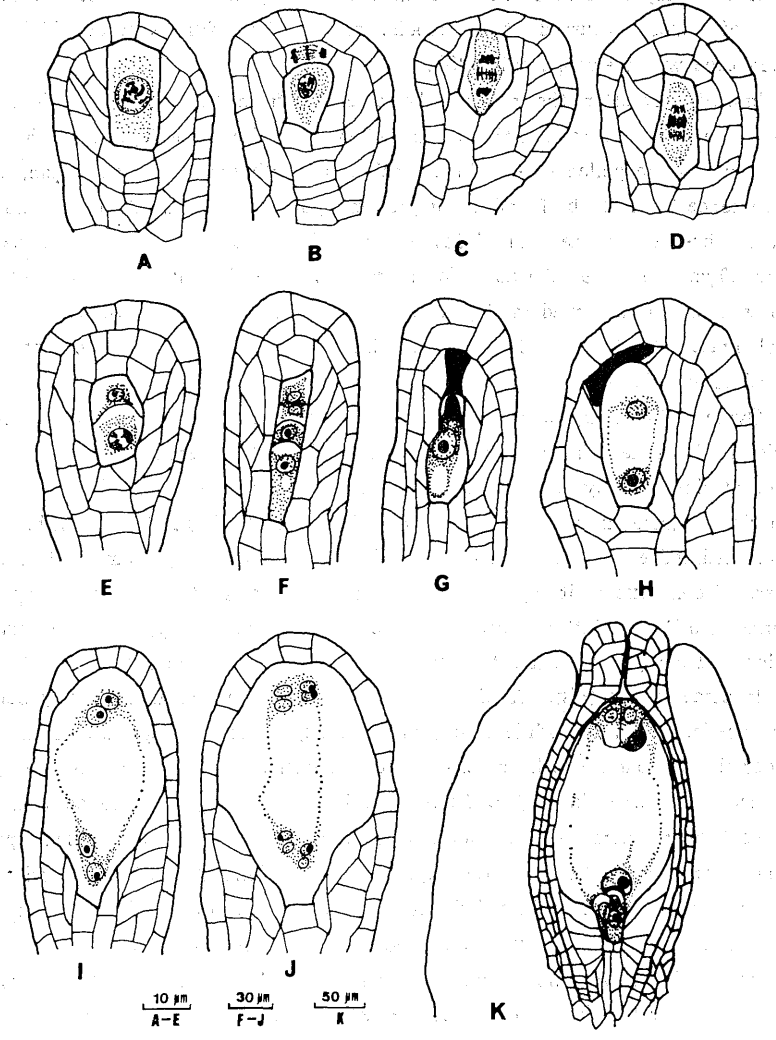

Fig. 3. Development of megaspore and megagametophyte in *Liriope platyphylla*. A. Tenuinucellate nucellus with megasporocyte. B. Crassinucellate nucellus with megasporocyte. C. Tenuinucellate nucellus with megasporocyte in division. D. Crassinucellate nucellus with megasporocyte in division. E. Nucellus with dyad. F. Nucellus with tetrad. G. Nucellus with functional megaspore. H. Nucellus with 2-nucleate embryo sac. I. Nucellus with 4-nucleate embryo sac. J. Nucellus with 8-nucleate embryo sac. K. Portion of ovule with mature embryo sac.

in the nucellus of the tenuinucellate or the crassinucellate type began to elongate and the outline of its wall came to have some of a tinge of round. The elongated megasporocyte underwent the first meiotic division (Fig. 3C, D) to form two dyad cells (Fig. 3E), of which the chalazal was somewhat larger than the micropylar. They then underwent the second meiotic division, resulting in the formation of four-celled tetrad (Fig. 3F), arranged in line. A chalazal megaspore, farthest from the micropyle, of the four tetrad cells became functional and the micropylar three of them degenerated (Fig. 3G). The chalazal half of the functional megaspore, whose nucleus lay in the micropylar half of it, was usually occupied by a large vacuole.

The functional megaspore underwent the first division of its nucleus to form a two-nucleate embryo sac (Fig. 3H). The two nuclei moved apart toward the opposite poles of the embryo sac and a vacuole intervened between them. Each of them divided at each pole of the embryo sac to form four nuclei (Fig. 3I). A division of them produced a total of eight nuclei (Fig. 3J), arranged in quartets, at the micropylar and chalazal ends of the embryo sac. Three of the quartet at the micropylar pole became differentiated as an egg apparatus (Fig. 3K) : this consisted of an egg cell, which was flanked by two synergids with filiform apparatus. At the chalazal end of the sac, three of the quartet differentiated as antipodal cells (Fig. 3K). The two remaining nuclei migrated from the opposite ends of the sac into a central cell and they became polar nuclei. They fused before fertilization to become one diploid nucleus (Fig. 3K). It always lay very near the antipodal apparatus. The antipodal cells persisted after fertilization and degenerated after the formation of two- or three-celled proembryo. The embryo sac of *L. platyphylla* was organized according to the monosporic eight-nucleate *Polygonum* type of development.

Deposition of callose during megasporogenesis. In process of megaspore formation, the wall where callose deposited was observed only under the fluorescence microscope. Callose was never detected in any walls of cells composing a very young ovule. Callose first appeared in the wall at micropylar end of megasporocyte in the leptotene stage of the first meiotic division. Then, it appeared in the wall at chalazal end of megasporocyte. When this division proceeded up to the zygotene or pachytene stage, callose began to deposit in the lateral wall of megasporocyte (Fig. 4A). Before the end of this division, a whole wall of megasporocyte emited callosic fluorescence. Callose deposited in the wall of

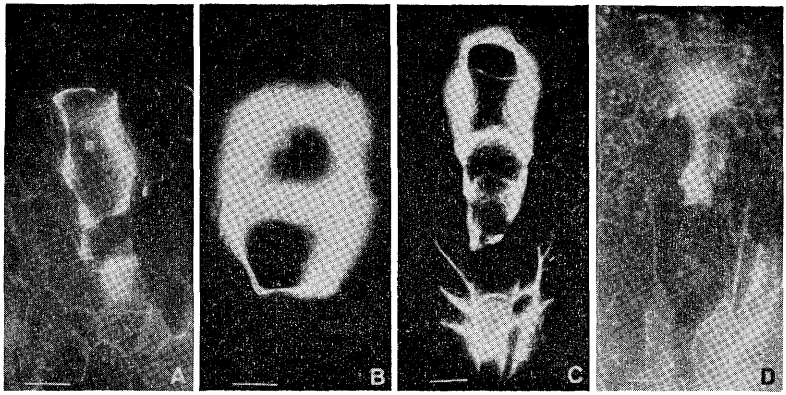

Fig. 4. Deposition and disappearance of callose during megasporogenesis in *Liriope platyphlla*. A. Megasporocyte in zygotene stage. B. Dyad. C. Tetrad and hypostase. D. Functional megaspore and degenerating megaspores. (All scales equal 10 μm)

megasporocyte did not disappear and was preserved throughout megasporogenesis. The callosic fluorescence rapidly increased in intensity in the lateral wall of the elongated megasporocyte. It remained weakly in the wall at the chalazal pole of megasporocyte, while the wall at its micropylar pole fluoresced strongly at the time when the first division had been over. Callose rapidly deposited in the transverse wall separating two dyad cells as well (Fig. 4B). The callosic fluorescence of this wall was very strong as well as that of the lateral walls and micropylar end-wall. Callosic reaction was observed in two transverse walls formed after the division of two dyad cells as well, but the fluorescence was very weak (Fig. 4C). As the micropylar three megaspores of tetrad began to degenerate, the fluorescence in their walls increased in intensity and then it extended in their cytoplasms. On the other hand, as the functional megaspore began to increase in size, the callosic fluorescence began to disappear from walls at the opposite ends of the megaspore. Callose remained relatively long in the lateral wall, facing the vacuole, of the functional megaspore. But this callose also disappeared before the division of its nucleus (Fig. 4D). After that, callosic fluorescence induced by aniline blue was not observed in the process of megagametogenesis.

Discussion Sporogenesis, gametogenesis and ovule. Davis (1966) has represented that the Liliaceae of Hutchinson's sense is variable in respect of the

developmental mode of spore and gametophyte. In *Liriope platyphylla*, the microsporogenesis follows the successive type of cytokinesis and the embryo sac is formed according to the monosporic eight-nucleate *Polygonum* type of development.

Davis (1966) has stated that the Hutchinson's Liliaceae is one of the families in which the nature of the nucellus constitutes a generic or specific character, and Dahlgren & Clifford (1982) have also stated that variable types of nucellus occur in the Convallariaceae of Huber's sense where *Liriope* is assigned. In *L. platyphylla* the tenuinucellate ovule, where an archesporial cell develops directly into a macrosporocyte, coexists with the crassinucellate one, where an archesporial cell cuts off a primary parietal cell. In this species, the epidermal cells at and near the nucellar tip divide periclinally to become two or three layers of cells. The nucellus of this species is of the same nature as that of *Ophiopogon wallichianus* (Maheshwari 1934). This nature of nucellus common between *Liriope* and *Ophiopogon* is considered to be one of characters which represent a near relation of these two genera.

Behavior of callose during sporogenesis and gametogenesis. The mode of callose deposition during microsporogenesis in *L. platyphylla* almost coincides with that in *Lilium henryi* (Heslop-Harrison 1966). But in *L. platyphylla* the deposition usually starts from the wall of microsporocyte near the poles of spindle which will be formed in process of the first division. It is suggested from Waterkeyn's illustrations (1962) that the onset of the deposition of callose in *Helleborus fortidus*, where the microspore is formed according to the simultaneous type of cytokinesis, is similar to that in *L. platyphylla*.

Górska-Brylass (1967) has represented it for several species of *Chlorophytum* and *Tradescantia* that the generative cell is still enclosed by a callosic wall after it moved away from the pollen wall. In *L. platyphylla*, however, callose which has deposited in the thin wall formed after the division of microspore nucleus usually disappears before the generative cell moves away from the pollen wall; the generative cell of *L. platyphylla* is not enclosed by a callosic wall. It is suggested that, in angiospermous pollen grains, there is a variation in the length of time during which callose exists in the wall of generative cell.

Currier (1957) reported that callose accumulated at the germ pores of ungerminated mature pollen grains in *Garrya elliptica* and seven other angiospermous species. This accumulation of callose, however, has not been confirmed in other

angiospermous pollen grains (Kapil & Tiwari 1978). In *L. platyphylla*, callose accumulates at the subpolar region of the furrow from which the pollen tube will emerge, while the pollen grain still remains in the anther. In this species, at least, it may be quite all right to consider that the site where the pollen tube begins to emerge is already determined precisely before the pollen grain is shed. There is need of further investigations as to the accumulation of callose at the germ pore.

Investigations of the behavior of callose during megasporogenesis are very meager. Kapil & Tiwari (1978) have summarized the knowledge of the behavior during megasporogenesis. According to them, in the species whose embryo sacs are formed according to the monosporic type of development, callose is invariably secreted in the wall of megasporocyte, two dyad cells and four tetrad cells, but the portion where callose is absent occurs invariably in the wall of these cells. The mode of deposition of callose during megasporogenesis in *L. platyphylla* is different from the mode found in plants examined to date. In this species callose deposits in the whole wall of megasporocyte after it has started to deposit in the wall at the micropylar end of the megasporocyte. And each of the dyad cells and each of the tetrad cells are wholly enclosed by the callosic wall. In this respect, the mode of deposition of callose during megasporogenesis in this species, as compared with the mode in other plants examined to date, is similar to the mode found in process of microspore formation. The rormation of callose may be closely related to the severing of cellular connections between the cell with callosic wall and the cells adjacent to it (Heslop-Harrison 1966). *L. platyphylla* seems to have a higher degree of isolation of the megasporocyte, each of the dyad cells and each of the tetrad cells from the nucellar cells adjacent to them than plants examined to date have.

It is suggested that there occurs a variation with regard to the behavior of callose during sporogenesis and gametogenesis in angiospermous plants. Embryology has contributed not a little forward bringing taxonomical problems to solution (Maheshawari 1950, etc.). If the knowledge of the callosic behavior is added to that obtained by the traditional embryological method, embryological features will have a greater significance in the systematics and the taxonomy of Angiosperms than they used to.

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ヤブラン (*Liriope platyphylla*) の胞子と配偶体の形成過程を観察し，さらにこの過程でみられるカロース (callose) の消長を調べた。カロースの沈着した細胞壁は高分子物質に対する透過性が著しく減少すると考えられている (Heslop-Harrison 1966)。小胞子は漸次膜形成型 (successive type) に従って作られ，1本の細長い発芽溝をもつ2細胞性の花粉に発達する。雌性配偶体は単胞子性8核タデ型に従って作られる。珠心には厚層型 (crassinucellate type) と薄層型 (tenuinucellate type) がみられ，珠心先端部の表皮細胞はしばしば並層分裂を行ない数層に発達する。珠心のこの特徴はジャノヒゲ属 (*Ophiopogon*) でも知られている (Maheshwari 1934)。

小胞子母細胞も大胞子母細胞も減数分裂細糸期になると，後に紡錘体の両極が位置すると思われる付近の細胞壁からカロースが検出される。細糸期前の両母細胞の壁から

はカロースは検出されない。ヤブランでは，両母細胞とも第1分裂がすすむにつれ，カロースの沈着は壁全体に拡大し，カロースの検出されない壁の部分はみられない。小胞子の形成過程でも大胞子のそれでも，2分子や4分子の各細胞の壁全体からカロースが検出される。大胞子形成過程の母細胞と2分子や4分子の各細胞がカロース壁で完全に囲まれる例は，ヤブラン以外ではまだ知られていない。

大胞子の形成を終了した雌性配偶体形成過程からは，カロースの検出は全くできない。一方，ばらばらになった小胞子からは一時的にカロースを検出できなくなるが，その後の雄性配偶体形成過程ではカロースの検出される時期がある。花粉中の生殖核と栄養核を分ける細胞壁にカロースが沈着する。ヤブランでは生殖細胞が花粉壁を離れる前に，このカロースは消え，生殖細胞をカロース壁が囲むことはない。今までに調べられた植物では，生殖細胞は花粉壁を離れてからもカロース壁で囲まれている。生殖細胞の壁にカロースが沈着している時間的長さには変異があると思われる。裂開前の葯の中にある花粉の発芽溝の一方の極付近にカロースの顕著な集積がみられる。Currier (1957) もこの集積を報告しているが，ほかには報告がなく，今後の調査を必要とする。寒天培地の上で成長中の花粉管の壁にもカロースが沈着するが，先端部約 20 μm からはカロースは検出されない。

被子植物の胞子及び配偶体の形成過程でのカロースの消長には多様性が認められる。このカロースの消長様式の情報を加えることによって，発生学は今まで以上に系統学や分類学に役立つ情報を提供できるようになると思われる。

○ケハギの白花型ユキハギ（秋山　忍・大場秀章）　Shinobu Akiyama & Hideaki Ohba: A white-flowered form of *Lespedeza patens* Nakai

ケハギの白花型を山崎富佐子氏が新潟県小千谷で採集された。このハギに対し，山崎敬博士はユキハギの和名を用意された。小千谷周辺で稀に栽培されている。ケハギの白花型には茎に伏した毛のあるシロバナタテヤマハギ *L. Thunbergii* (DC.) Nakai var. *acutifolia* (Nakai) Hiyama ex Murata f. *leucantha* Murata があるが，これはケハギとは異なる。詳しいことは別の機会に発表したい。ユキハギの材料の提供と生育地の状況等について御助言をいただいた山崎敬博士にお礼申し上げます。

Lespedeza patens Nakai f. **nivea** S. Akiyama et H. Ohba, f. nov.

Haec forma ut typo caules pilis patentibus habet sed a typo floribus albis differt.

Nom. Jap. Yukihagi (Yamazaki, nov.).

Habit. Japan. Honshu: Niigata Pref., Ojiya (Fusako Yamazaki s. n. 20 Sept. 1966, Holo- et iso-typi in TI).　　(東京大学 総合研究資料館植物部門)