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# Occurrence of Callose during Embryo Sac Development in *Stachyurus praecox* Sieb. et Zucc.

by

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**Abstract.** The deposition pattern of callose during the embryo sac development was investigated in *Stachyurus praecox*. Although two chalazal megaspores of a tetrad began to develop in some of the tetrads examined, it was only one megaspore nearest the chalaza that developed into an eight-nucleate embryo sac. Callose was detected only in wall separating two dyad cells and in walls separating four tetrad cells. This deposition pattern of callose has not been known in process of the embryo sac development of the monosporic type studied hitherto. According to Kapil and Tiwari (1978), in process of the development of bisporic type, the deposition of callose is restricted in the separating wall formed after the first meiotic division. In this respect, the deposition pattern of callose in *S. praecox* seems to bear a resemblance to that found in process of the development of bisporic type. The Stachyuraceae have been considered to have a near relation to the Flacourtiaceae by mordan taxonomists. It is necessary to consider a relation of the Stachyuraceae to the Theaceae as well, because only the Theaceae among the families which have been considered to have a relation to the Stachyuraceae by taxonomists have the bisporic development of embryo sac.

## Introduction

The genus *Stachyurus* has been treated as only one genus of the family Stachyuraceae by almost all of the mordan taxonomists. But there has been disagreement about the taxonomic position of the Stachyuraceae. For example, Hutchinson (1973) included the family in the order Hamamelidales, Cronquist (1968) in the order Theales, and Melchior (1964), Takhtajan (1969, 1980) and Cronquist (1981) in the order Violales.

The embryological studies on *Stachyurus* have been performed by Maurizon

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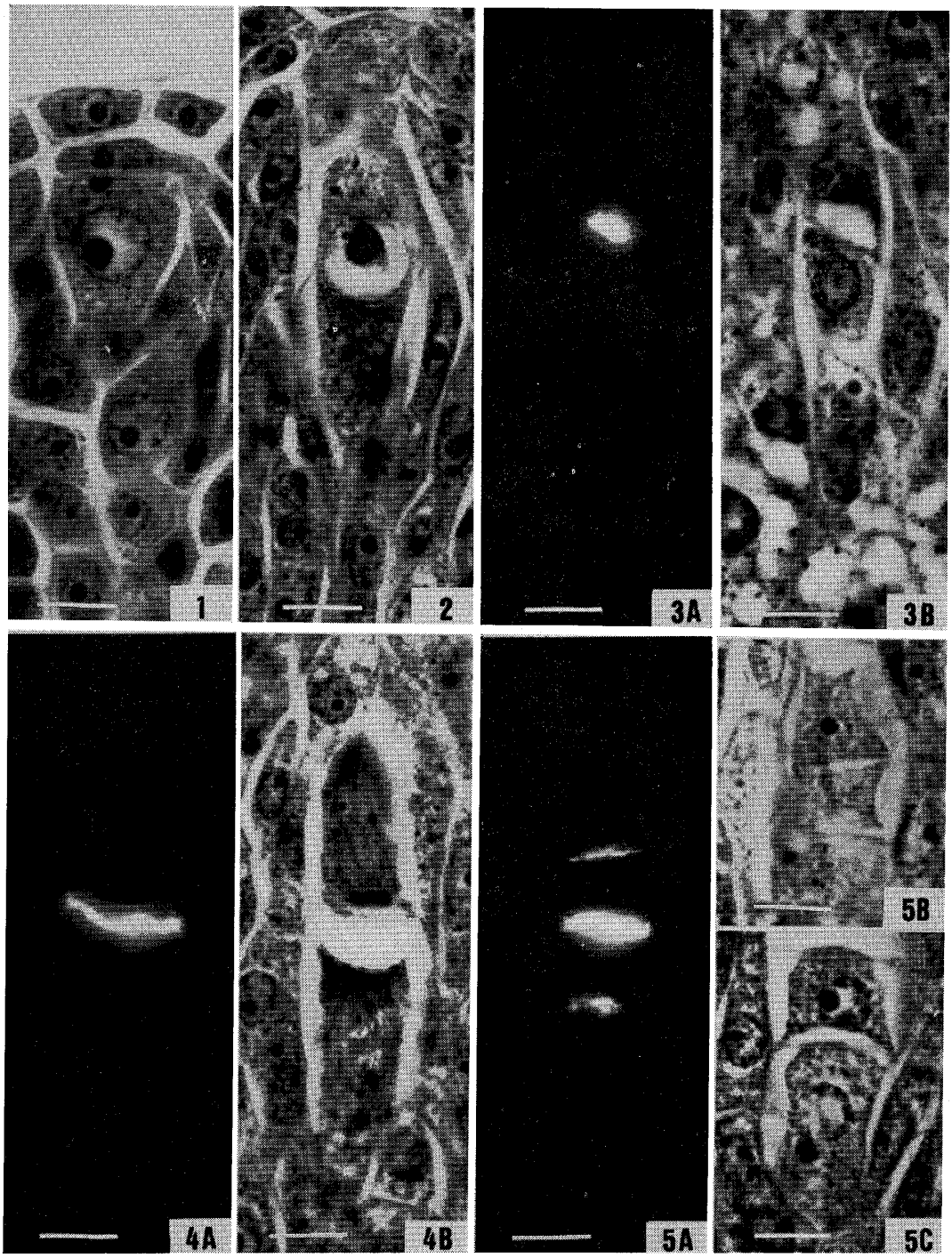


Fig. 1. Nucellus with parietal cell and sporogenous cell. No callose exists in these cells. Scale equals 10 μm.

Fig. 2. Nucellus with megasporocyte, whose nucleus is interphase. No callose exists in the megasporocyte. Scale equals 10 μm.

Fig. 3A, B. Dyad. A is the fluorescence photomicrograph of the dyad shown in B. Scale equals 10 μm.

Fig. 4A, B. Dyad in second meiotic division. A is the fluorescence photomicrograph

(1936), Satô (1976) and Mathew and Chaphekar (1977). Judging from their reports, *Stachyurus* has embryologically prominent features as follows. Only a megaspore, nearest the chalaza, of the tetrad cells becomes functional and develops into an eight-nucleate embryo sac. That is, the embryo sac is formed according to the monosporic eight nucleate Polygonum type of development. But, although a tetrad cell which lies directly above the functional megaspore does not develop into an eight-nucleate embryo sac, it frequently undergoes a division of its nucleus once or twice. Besides the monosporic type of the embryo sac development, Mathew and Chaphekar (1977) suggested the rare occurrence of the bisporic Endymion type of development.

Satô and Arima (1984) conjectured that, if the knowledge of the deposition pattern of callose during sporogenesis and gametogenesis in the angiospermous plants was added to that obtained by the traditional embryological method, embryological features would have a greater significance in the systematics and the taxonomy of Angiosperms than they used to. So, the pattern of embryo sac development in *Stachyurus praecox*, which had been studied once by Satô (1976), was confirmed again and, furthermore, to acquire a considering knowledge of taxonomic position of the Stachyuraceae, we investigated the pattern of the depoistion and disapperance of callose during the embryo sac development. The results obtained will be reported in this paper.

## Material and Method

Many buds and flowers of *Stachyurus praecox* were collected at appropriate intervals extending from April to May in 1984, from a female plants growing wild on the campus of the Yokohama National University, Hodogaya-ku, Yokohama City. They were 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 aqueous solution of aniline blue (Smith and McCully, 1978) and observed under the fluorescence microscope. Callose emits bright-yellow fluorescence when treated with the fluorochrome. Callosic fluorescence was photographed using Kodak Tri-X film (ISO, 400). After the photographs had been taken, the sections

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of the dyad shown in B. Scale equals 10 μm.

Fig. 5A, B, C. Linear tetrad. In B, two micropylar megaspores are in focus, but two chalazal ones are out of focus, though the extrimity of them is observed in this section. C, which is the next section to B, shaws two chalazal megaspores. A is the fluorescence photomicrograph of the tetrad shown in B. Scale equals 10 μm.

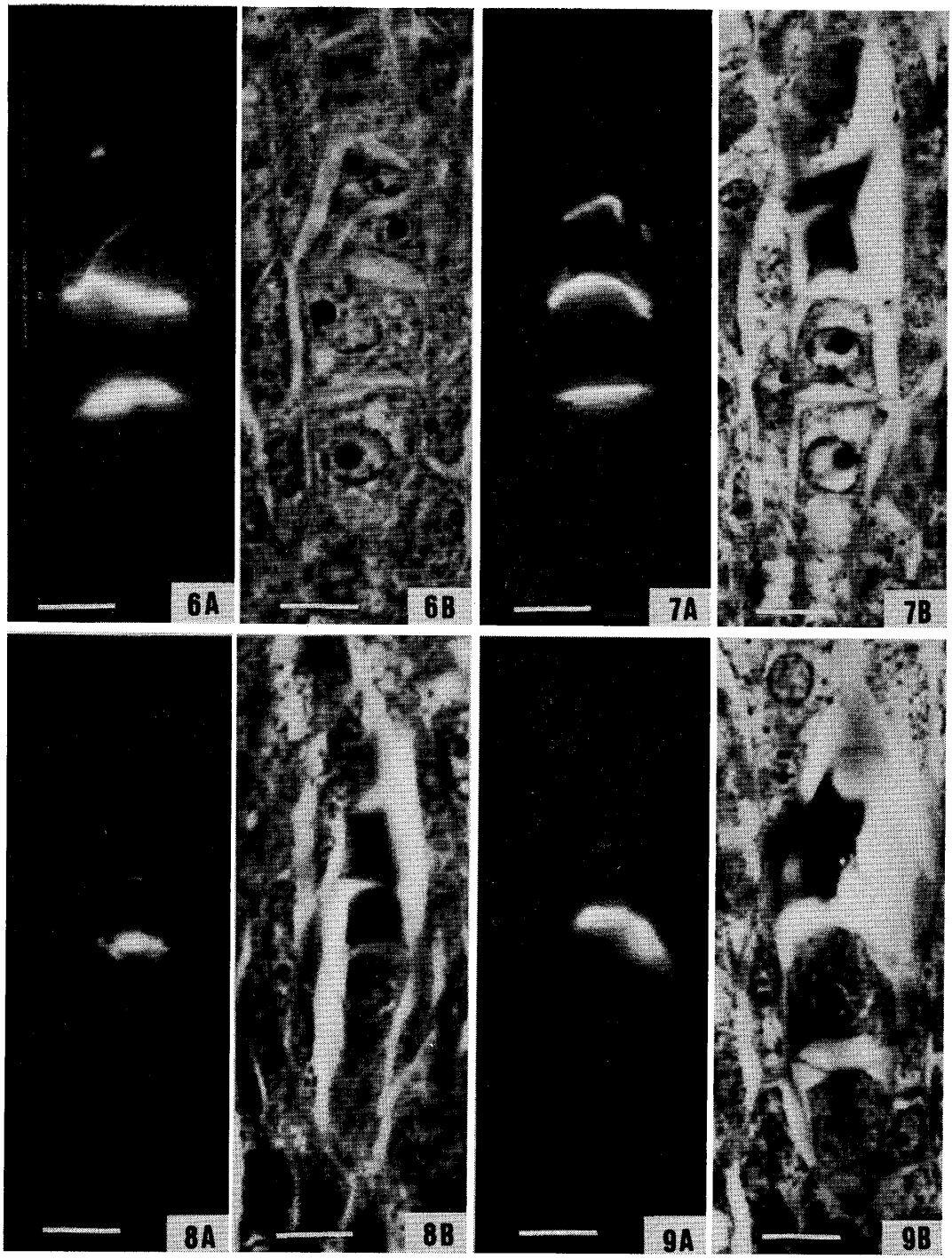


Fig. 6A, B. Oblique tetrad. A is the fluorescence photomicrograph of the oblique tetrad shown in B. Scale equals 10 μm.

Fig. 7A, B. Tetrad, of which two micropylar megaspores are degenerating. A is the fluorescence photomicrograph of the tetrad shown in B. Scale equals 10 μm.

Fig. 8A, B. Two chalazal cells of tetrad; two micropylar cells of tetrad have degenerated and thier residuum is still persisting. A is the fluorescence photomicrograph of the chalazal cells shown in B. The callosic fluorescence of the

were well washed in running water. Then they were dehydrated in ethyl alcohol series and stained again with Heidenhain's iron alum hematoxylin and fast green. After then the same cell or cells that fluorescence had been observed were photographed again under the optical microscope with a tungsten bulb as the illuminator.

## Observation

An archesporial cell divided to form a sporogenous cell and a primary parietal cell (Fig. 1). The former became larger and differentiated into a megasporocyte (Fig. 2), while the latter repeated a mitotic division and formed a well-developed parietal tissue. It was composed of one to two files of cells, each of which consisted of seven to eight cells. There was no occurrence of callose in any walls of these parietal cells and the megasporocyte which did not enter the first division of meiosis yet (Fig. 2). Although callose was never detected in the wall of the megasporocyte in which the first division of meiosis was proceeding, it was invariably detected in the transverse wall separating two dyad cells which had been produced after the first division (Figs. 3A, B). The two dyad cells underwent the second division of meiosis (Figs. 4A, B), resulting in the formation of a four-celled tetrad. The tetrad cells usually arranged in line (Figs. 5B, C), but two micropylar cells of them occasionally arranged obliquely (Fig. 6B). Of three walls separating the four tetrad cells, callose persisted in the separating wall formed after the first division of meiosis and it was, in addition, detected in the two separating walls formed newly after the second division of meiosis (Figs. 5A, 6A, 7A). The separating wall, which had been formed after the first division, emited much stronger callosic fluorescence than the two walls, which had been produced after the second division. Thus, only the separating wall, by which the respective cells constructing the dyad and the tetrad were separated, became callosic, but callose was not detected in any other portions of wall enclosing the dyad and tetrad cells.

The megaspore nearest the chalaza alone became functional and developed into an eight-nucleate embryo sac. In all tetrads examined, the two micropylar cell degenerated soon after formation of the tetrad (Figs. 7B, 8B, 9B). In some tetrads (Fig. 10), however, the cell which lay directly above the functional megaspore soon degenerated without any nuclear divisions, following the degeneration of the

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separating wall nearest the chalaza is much stronger than that of the upper wall. Scale equals 10 μm.

Fig. 9A, B. Two chalazal cells of tetrad, which are at the same stage as those shown in Fig. 8. A is the fluorescence photomicrograph of the chalazal cells shown in B. The callosic fluorescence of the separating wall nearest the chalaza is much stronger than that of the upper wall. Scale equals 10 μm.

two micropylar cells of the tetrad. In other tetrads (Fig. 12), which were more frequently than the tetrad just mentioned above, it persisted long after the two micropylar cells of the tetrad had degenerated, and furthermore, became a two-nucleate (Fig. 13) or a four-nucleate state. Although it never developed into a mature embryo sac, the nuclear division of it usually proceeded earlier and faster than that of the functional megaspore. As a result, frequently, a two-nucleate cell or a four-nucleate cell lay directly above the functional megaspore, or a four-nucleate cell lay above a two-nucleate embryo sac.

Before the functional megaspore or the tetrad cell directly above it became a two nucleate state, the callosic fluorescence disappeared perfectly from three separating walls of the tetrad. The disappearance of callose in these walls proceeded as follows. The callosic fluorescence of the separating wall nearest the micropyle first disappeared together with degeneration of the two micropylar megaspores of tetrad. After then, however, in some of the degenerating tetrads, the fluorescence of the separating wall nearest the chalaza temporarily became stronger (Fig. 8A) than that of the separating wall which had been formed after the first division of meiosis,

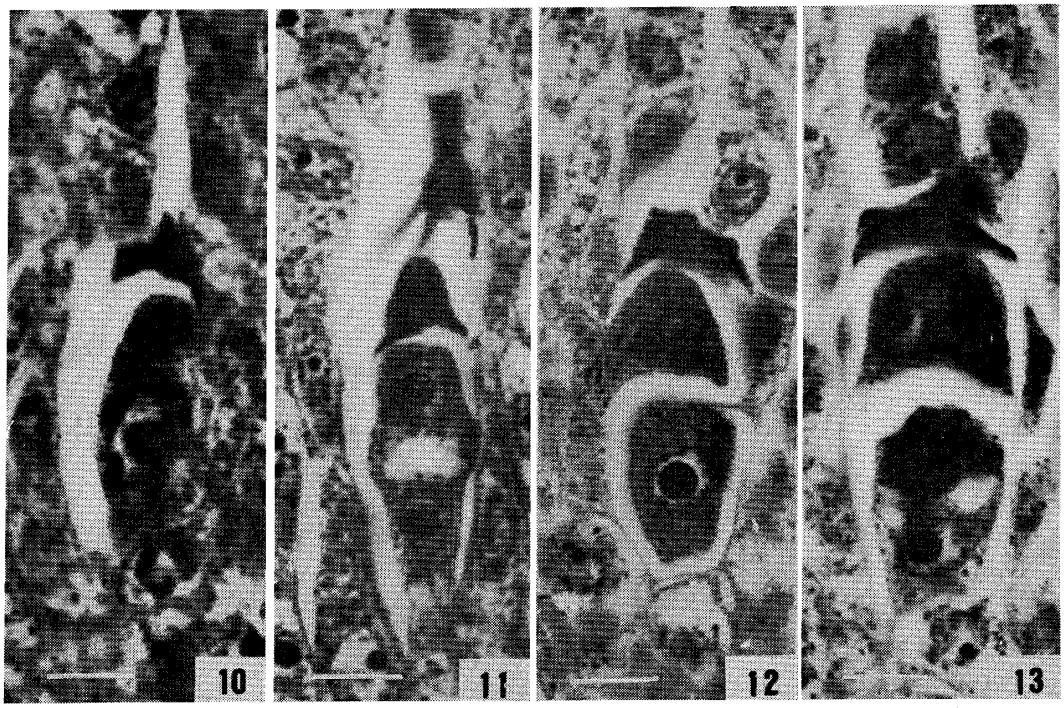


Fig. 10. Elongating functional megasphore; three micropylar megaspores degenerate already. Scale equals 10 μm.

Fig. 11. Two-nucleate embryo sac. No callose exists in it. Scale equals 10 μm.

Fig. 12. Two chalazal megaspores, each of which is elongating. No callose already exists in them. Scale equals 10 μm.

Fig. 13. Two nucleate embryo sac, above which a two-nucleate cell lies. No callose exists in them. Scale equals 10 μm.

while in others it became gradually weak (Fig. 9A). In the former, the tetrad cell directly above the functional megaspore had not increased in size conspicuously. It was suggested that it would degenerate soon after the degeneration of the two micropylar cells of tetrad. In the latter, it had increased in size. It was suggested that it would develop into multinucleate state. These suggestions were made as in the following. Formation of callose is closely related to the severing of cellular connections between the cell with callosic wall and the cell adjacent to it (Heslop -Harrison, 1966). For this reason, in the latter an information to make the tetrad cell nearest the chalaza develop into a female gametophyte was transmitted to the cell lying directly above it, while in the former, transmission of such an information was prevented by the wall with strong fluorescence of callose.

After then no callosic fluorescence could be observed in process of the embryo sac development (Figs. 10—13).

## Discussion

The developmental pattern of embryo sac in *S. praecox* quite agreed with that which had been reported for this species by Satô (1976). In *S. praecox*, the deposition of callose during the embryo sac development is invariably restricted in a wall separating two dyad cells and in three walls separating four tetrad cells. The deposition pattern of callose during the embryo sac development of the monosporic type (Polygonum type and Oenothera type) is summarized as follows: callose is secreted not only in a separating wall of dyad and tetrad, but also in a wall encloseing a megasporocyte, two dyad cells and four tetrad cells, though the portion where callose is absent occurs in the wall of these cells in almost all of the species examined hitherto (Kapil and Tiwari, 1978). The deposition pattern of callose found in *S. praecox* is obviously different from that found in process of the development of the monosporic type examined hitherto. The pattern in which only a separating wall becomes callosic is found in process of the embryo sac development of bisporic type (Rodokiewicz, 1968; Kapil and Tiwari, 1978). That is, the deposition pattern of callose in *S. praecox* bears a resemblance to that found in process of the development of bisporic type. In prosess of the development of embryo sac in *S. preaecox*, two megaspores begin to undergo the nuclear division. It may be premature to ralate unquestioningly this phenomenon with the developmental pattern of the bisporic type. But, considering the deposition pattern of callose, it seems that the developmental pattern of embryo sac in *S. praecox* has a characteristic common to that of bisporic type.

Occasionally in *S. chinensis*, also, two megaspores of tetrads begin to undergo a nuclear division (Mathew and Chaphekar, 1977). Mathew and Chapekar (1977)

suggested, on the basis of the occurrence of dyad which is composed of a three-nucleate micropylar cell and a two-nucleate chalazal cell, that in *S. chinensis* there is the bisporic Endymion type of embryo sac development as well as the monosporic type of development. They further stated that this developmental pattern of the bisporic type is more frequent in *S. praecox* than in *S. chinensis*. But they did not observe all stages of embyro sac development of this type. We could not have observed such a phenomenon as suggesting the occurrence of the bisporic type of development. There is no ground for denying the occurrence of the bisporic type of development. However, it is premature to conclude the occurrence of the Endymion type without observation of a series of the developmental stages of embryo sac, because frequently in *Stachyurus*, two chalazal megaspores of the tetrad undergo the nuclear division, which proceeds earlier and faster in the micropylar one of the two than in the chalazal one.

Hutchinson (1973), who placed the Stachyuraceae in the order Hamamelidales, did not grasp characters of the family correctly as mentioned by Mathew and Chaphekar (1977). Melchior (1964) and Takhtajan (1969, 1980) treated the Stachyuraceae as one of the families in the order Violales. According to Lawrence (1951), the Stachyuraceae were once united with the family Theaceae. Cronquist (1968, 1981) stated that the boundary between the Theales and the Violales is arbitary and the Stachyuraceae have a near relation to the Flacourtiaceae of the Violales. In *Idesia* of the Flacourtiaceae (Tohda, 1971), two megaspores of the tetrad have a tendency to develop, though the embryo sac is formed according to the monosporic type of development. Corner (1976) considered that the seed structure of the Stachyuraceae is compatible with that of the Theaceae, not the Flacourtiaceae. The developmental pattern of the embryo sac in the Flacourtiaceae is monosporic, while that the Theaceae, especially in *Camellia*, is bisporic (Davis, 1966). The deposition pattern of callose in *S. praecox* is common to that found in the species examined hitherto in which the embryo sac is produced according to the bisporic type of development. Thus, the Stachyuraceae have a characteristic common to the Theaceae and the Flacourtiaceae. At present, it is difficult from the embryological point of view to solve the problem whether the Stachyuraceae should be placed in the Violales or in the Theales, or whether it should be placed in other order established newly, because the knowledge of the embryology of the Theaceae, the Flacourtiaceae and their allied families is too meager to solve the problem. However, it is probably certain that the group composed of the Theaceae and their allied families and the group composed of the Flacourtiaceae and their allied families are phylogenetically connected by the Stachyuraceae.

# キブシの胚嚢形成過程におけるカロースの沈着様式

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## 摘　要

キブシの胚嚢形成過程におけるカロースの沈着様式について調査した。キブシの胚嚢は単胞子性8核タデ型に従って形成される。4個の大胞子のうち最も合点側の大胞子が8核からなる胚嚢に発達するが，この大胞子のすぐ上に位置する大胞子も，しばしば2核や4核の細胞に発達する。カロースは二分子細胞を分ける隔壁と四分子細胞を分ける隔壁にだけ検出される。このようなカロースの沈着様式は，単胞子性の胚嚢形成過程では，これまでにはまったく知られていなかったものである。しかし，二胞子性の胚嚢形成過程におけるカロースの沈着は，異形分裂の後に作られる隔壁に限られている。キブシでみられるカロースの沈着様式は，大胞子形成過程の隔壁にしかカロースが沈着しないという点で，二胞子性の胚嚢形成過程でのそれと共通する性質をもつものと思われる。キブシ科との類縁関係が議論されている科の中で，特にイイギリ科はキブシ科と近い関係をもつと考える研究者が多い。しかし，キブシ科との類縁関係が議論されている科の中で，二胞子性の胚嚢形成が知られている科はツバキ科である。今回の調査から得られた発生学的な特徴はツバキ科との関係を示すものと思われる。

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