THE OCCURRENCE OF A POLLEN TUBE WITH FOUR SPERMS AND TWO TUBE NUCLEI IN POLYGONATUM[1]

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Variations of the sporophytic phases of higher plants are familiar to many biologists, but studies of variations of gametophytic phases are indeed limited (Wulff and Maheshwari 1938). During the past five years approximately 10,000 pollen tubes of *Polygonatum canaliculatum*, prepared by a technique previously outlined by the writer (Eigsti 1940a), have been examined in the laboratory. In all of these cases, with two exceptions, 2 sperms (fig. 1, s_1, s_2) and 1 tube nucleus (fig. 1 t) appeared in the pollen tube (fig. 1-2), and of these exceptions one pollen tube had 3 sperms and 1 tube nucleus (Eigsti 1940b), while the other here reported had 4 sperms and 2 tube nuclei (fig. 3-4).

Sax (1935) reported gametophytic variations in microspores of plants of *Tradescantia* subjected to high and low temperatures prior to development of the microspores. He found three kinds of nuclear and cytoplasmic abnormalities following treatment of plants, viz., a division of the tube nucleus, disturbance of polarity of the cell, and unusual extension of the microspore on the ventral side. The plants from which pollen for the present investigation was obtained were not previously treated, but irregularities in nuclear activities may have been caused by some disturbance of the nuclear and cytoplasmic processes within the pollen grain (fig. 3-4). A bi-nucleate microspore, or an additional division of the generative cell and tube nucleus, might account for the pollen tube with 4 sperms and 2 tube nuclei. It is evident from the photomicrographs that the formation of sperms occurred in the pollen tube (fig. 4).

The number of chromosomes of the nuclei was not determined but a comparison of nuclear volumes (fig. 1-4) indicates that no quantitative change in nuclear material had occurred. It is conceivable that such gametophytic variations occurring under natural conditions prior to fertilization may induce irregularities in the subsequent development of endosperm, embryo, and finally new sporophyte.

Artificial media can be used successfully for the production of slides showing a pollen tube with nuclei (Eigsti 1940a). Aceto-carmine for staining and fixing, tertiary butyl alcohol for dehydrating, xylol for clearing, and balsam for permanent mounting provide preparations with pollen tube walls, nuclei, and plasm, well differentiated (fig. 1-5). This method of making aceto-carmine slides permanent, as yet unpublished, will be given in greater detail in a forthcoming paper.

By regulating temperatures during germination of pollen the division of the generative cell can be induced without the usual formation of a pollen tube (fig. 5). This species, *P. canaliculatum*, has binucleate pollen grains and the generative cell divides in the pollen tube. Certain species of plants have a 3-nucleate pollen grain. The nuclear and cytoplasmic activities are not affected in the same manner under a given set of environmental con-

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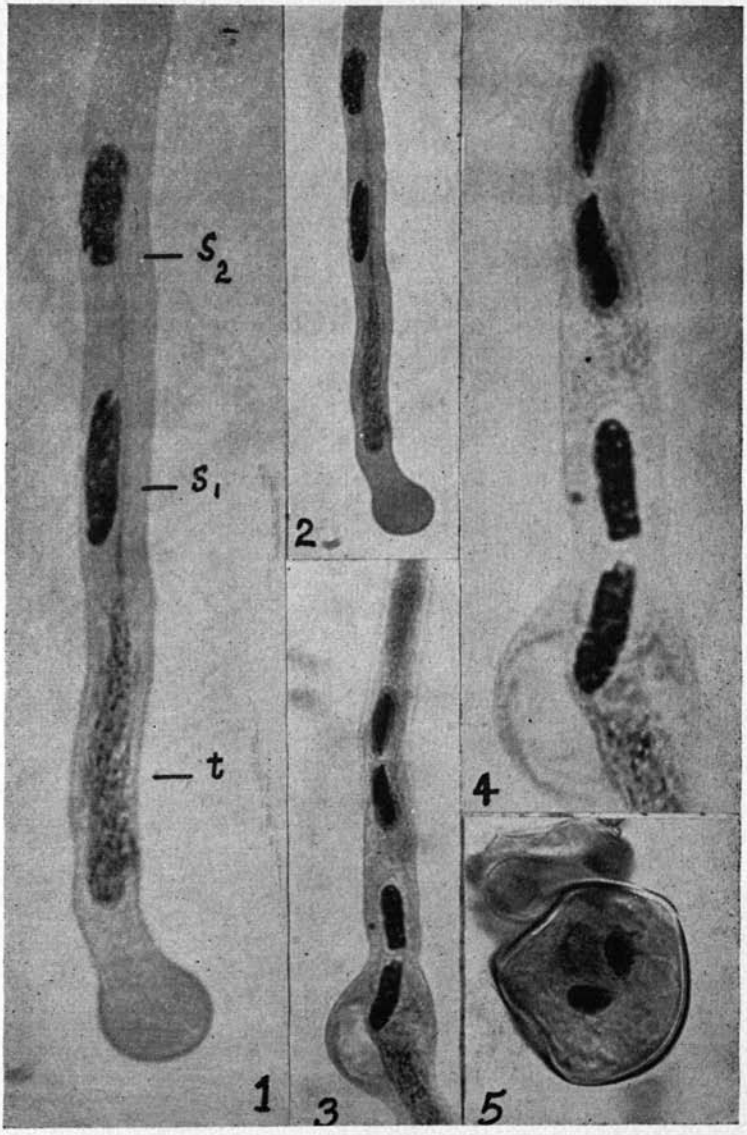

Fig. 1-5

ditions. Sax (1935) emphasizes this feature of synchronization of the activities of the nucleus and cytoplasm in his studies of the effect of temperature upon nuclear differentiation in the development of microspores.

SUMMARY

1. A pollen tube with 4 sperms and 2 tube nuclei is reported.
2. Photomicrographs demonstrate the adequacy of the techniques for making pollen tube preparations with differentiated nuclei.
3. The division of the generative cell may occur in the pollen grain of *Polygonatum* prior to pollen tube formation.

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Legend for explanation of figures:

FIG. 1-5. *Polygonatum,* pollen tube and nuclei stained with aceto-carmine, mounted in balsam.—FIG. 1. Pollen tube; *t,* tube nucleus; s_1, sperm, s_2 sperms, ×1350.—FIG. 2. Same as Fig. 1. ×700.—FIG. 3. Pollen tube, 4 sperms, 2 tube nuclei. ×700.—FIG. 4. Same as Fig. 3. ×1360.—FIG. 5. Pollen grain with 2 sperms, tube nucleus. ×700.