THE MORPHOLOGY OF POLLEN TUBES IN ANGIOSPERMS

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The development of the pollen tubes of twenty species of angiosperms was studied. Two methods were employed for the study, viz., 1) the microscope slide culture technique (Trankowsky, 1930; Maheshwari and Wulff, 1937; Beatty, 1937); and 2) the dissected style whole mounts technique (Buchholz, 1931; Eigsti, 1937). With these methods, the entire pollen tube with its contents could be studied. Vital dyes were used as an aid in studying the migration of nuclei and the activity of the cytoplasm in the pollen tube. The use of vital dyes prior to fixation made it easier to determine the stage of development of the nuclei within the pollen tube. It is well known that the nucleus of the microspore divides to form the generative nucleus and tube nucleus before the pollen is shed. The division of the generative cell which forms the two microgametes completes the development of the microgametophyte. In some plants this division of the generative cell occurs before the development of the pollen tube, whereas in other plants, the division takes place in the pollen tube. The tube nucleus ordinarily precedes the generative cell or the microgametes into the pollen tube. However, there are exceptional cases where the generative cell migrates into the pollen tube ahead of the tube nucleus.

The study of pollen tubes in angiosperms includes observations of twenty different species: *Lilium canadense*, *L. auratum*, *L. speciosum*, *Hyacinthus orientalis*, *Reseda odorata*, *R. lutea*, *R. alba*, *Gleditsia triacanthos*, *Trillium erectum*, *Datura stramonium*, *Podophyllum peltatum*, *Tradescantia reflexa*, *Hemerocallis fulva*, *Smilacina racemosa*, *Clintonia borealis*, *Papaver somniferum*, *Helianthus annuus*, *Iris versicolor*, and *zea mays*.

The nature of the division of the generative cell offers a problem worthy of consideration in this study of pollen tube morphology. The nuclear phase of the division of the generative cell which involves the division of the chromosomes, does not differ from an ordinary mitosis. However, the division of the generative cell (cytokinesis) offers a problem of greater complexity and one which shows numerous variations for the different species studied. Cooper (1936) reviewed this phase of development in *Lilium regale*, *L. auratum*, and *L. Philippinense*. He concluded that a metaphase plate, spindle fibers, and cell plate were formed in the division of the generative cell. His conclusion supported the earlier work of Guignard (1891), Strasburger (1908), and others. Upcott (1936), working with *Tulipa*, also concluded that a metaphase plate and cell plate were formed in the division of the generative cell. Trankowsky (1931) found a metaphase plate formation and spindle fibers in the division of the generative cell of *Hemerocallis fulva*, but could not find these structures in *Convallaria majalis*. Wulff (1933) presented another view, namely, that the division of the generative cell is accomplished by means of a constriction (furrow) and the metaphase and cell plates are without any special function. This view had been held by a number of previous workers in essentially the same aspects as presented by Wulff. Recently, Wulff and Maheshwari (1938) gave a critical review of the development of the male gametophyte in angiosperms which summarized the earlier as well as the more recent conclusions regarding the nature of the division of the generative cell. After making a critical study of the problem, which included their own research with the division of the generative cell, they stated (p. 123):

"Work of more comprehensive and comparative nature, than has been done hitherto, is necessary to elucidate this problem."

In this survey of the development of the microgametophytes of angiosperms, the salient features of development found for a few representative species are as follows: (1) In *Lilium canadense, L. speciosum, L. auratum*, the chromosomes become arranged on a metaphase plate, but spindle fibers are difficult to demonstrate; (2) a definite cell plate has been demonstrated for *L. canadense, Polygonatum commutatum* and *Convallaria majalis;* (3) *Tradescantia reflexa* shows numerous irregularities of the division of the generative cell; (4) definite metaphase plates, spindle fibers, and cell plates have been found in *Tradescantia reflexa;* (5) the cytoplasmic sheath of the generative cell or microgametes was not found in *Tradescantia* but were demonstrated definitely in *Polygonatum* and three species of *Lilium;* and (6) in all cases studied the division of the chromosomes does not appear different from an ordinary mitosis.

The study of pollen tubes growing in the style did not reveal enough differences to warrant a detailed comparison of the division of the generative cell in tubes growing in the style as compared to this division in tubes grown in culture. Wulff (1936), working with *Narthecium ossifragum*, maintains that the difference in pollen tube diameter is an important factor in the development of the spindle fibers and the cell plate. No spindle fiber and cell plate formations have been found in the pollen tubes grown in the style because the pollen tubes are very narrow, whereas, the pollen tubes of the same species grown in culture showed these formations because the pollen tubes were broad. Cooper (1935) did not find significant differences between the pollen tubes of three species of *Lilium* grown in culture and pollen tubes grown in the stylar tissue.

It had been shown by Dustin (1934), Lits (1934), and other more recent workers, that colchicine specifically inhibits cell division. The nuclear phase (the division of the chromosomes) apparently is not influenced or inhibited by this treatment. The spindle fiber mechanism, the formation of a metaphase plate, and the formation of a cell plate are definitely inhibited by colchicine. Thus, the application of colchicine to the pollen tubes containing dividing cells should give information regarding the nature of the division of the generative cell as compared with the division of somatic cell.

A 1 percent and a .1 percent aqueous solution of colchicine were added to slides containing germinating pollen of *Lilium auratum*. The slides were placed in a germinating pan and kept there for 48, 72, and 96 hours respectively. At each of these respective intervals, samples of treated and untreated material were fixed and stained to determine the effects of colchicine upon the division of the generative cell. The results of a comparison of the treated and untreated material are as follows: (1) The division of the chromosomes occurs in both treated and untreated material at the 48 and 72 hour intervals; (2) colchicine inhibits the division of the generative cell; (3) a restitution nucleus, apparently polyploid, is formed in the treated material; (4) microgametes were not found on the slides of treated material; (5) an increase in diameter and decrease in the length of the pollen tube was observed on slides treated with colchicine; and (6) a chromosomal break appeared in the treated preparations which is of interest in the light of other experimental work which shows that changes other than polyploidy are induced by colchicine.

SUMMARY

1. The microscope culture method and the dissected style whole mount method were employed in this study.
2. The nuclear phases of the division of the generative cell are similar to the nuclear phases in ordinary mitosis.
3. Cytokinesis of the generative cell is variable in the different species studied and does not occur the same as in ordinary mitosis.
4. Significant differences between the pollen tubes grown in culture and pollen tubes grown in the style were not found in this study.
5. Colchicine does not inhibit the nuclear phases of the division of the generative cell.
6. Cytokinesis of the generative cell is inhibited by colchicine treatment.
7. A large restitution nucleus is formed in the tubes of treated material whereas microgametes are found in corresponding slides of untreated material.
8. An increase in diameter and decrease in length of the pollen tube was observed on slides treated with colchicine.
9. A chromosomal break was observed in one division figure of the generative cell which had been treated with colchicine.

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