OBSERVATIONS ON THE DEVELOPMENT OF THE MALE GAMETOPHYTE IN CERTAIN MONOCOTS

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In view of the extensive literature dealing with the various cytological and taxonomic aspects of Angiosperms it is rather surprising that a comprehensive study of the male gametophyte has apparently never been made. Certain isolated aspects of this subject, as the structure of the chromosomes during meiosis, chronological descriptions of the meiotic process, the asymmetry of the microspore mitosis, etc., have been worked out and commented upon in detail. Consequently these are omitted in this report. But since the development of the male gametophyte has received such scattered attention it is the purpose of this paper to give a coordinated presentation in regard to such easily observed features as vacuoles, refractive granules, cell walls, nuclear shape and volume, and cell size in its development in certain monocots and particularly in Tradescantia.

The plants used in the present study were: Tradescantia hirsutiflora, T. paludosa, unnamed triploid and tetraploid Tradescantia hybrids, and Aloe sp. Correlated observations
were made on species of *Rhoeo*, *Gasteria*, and *Sansevieria*. The plants were obtained from the Missouri Botanical Garden and grown under greenhouse conditions. Acetocarmine smear preparations of pollen mother cells, microspores, and pollen grains were used throughout the investigation. Hanging drop slides, smears being made on the cover slip and later inverted so that the material could float free in acetocarmine, were made to study tetrad shape. The work was carried on in the laboratories of the Henry Shaw School of Botany of Washington University, most of it as part of a course in cytology. We are indebted to other members of the class for various observations, and to Dr. Edgar Anderson for his direction in this study and his helpful suggestions.

Meiosis in *Tradescantia* shows the phenomena recorded for the various species, the nucleus of the pollen mother cell passing through two divisions and producing four daughter cells. These daughter cells are arranged in tetrads, which in *Tradescantia* are of two types. The type more often found is shown in fig. 1,A. In this case the spindle axes of the second meiotic division were parallel. Fig. 1,B shows the result of the corresponding division in which the spindle axes were perpendicular to each other. The ratio of type A to type B was found to be approximately 4:1 in *Tradescantia*. Type B is often found in groups on the slide in which none of type A are ap-

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**Fig. 1.** (A) Tetrad in which the spindle axes of the second meiotic division were parallel; (B) in which the spindle axes of the second meiotic division were perpendicular; (C) in which the walls dividing the cells were formed after both meiotic divisions had taken place.
parent. This perhaps indicates that all in the group were
developed in a particular part of the anther which gave rise
only to that type. The tetrads of *Rhoeo* are similar to those of
*Tradescantia* but here the two types occur in about equal num-
bers. In *Gasteria* and *Aloe* the four cells of the tetrad are
tetrahedrally arranged (fig. 1,C). The walls dividing the cells
are evidently formed after both meiotic divisions have taken
place.

In *Tradescantia* and *Rhoeo* the wall of the pollen mother cell
about the tetrad is an ordinary spherical coat and shows no

Fig. 2. Diagram of a typical tetrad of *Gasteria, Aloe, or Sanse-
vieria* surrounded by the heavily flanged pollen mother cell wall,
represented as if upper half of wall were removed. Figured by
H. A. McQuade.

resemblance to the same structure in *Gasteria, Aloe,* and
*Sansevieria,* in which two heavy flanges are at opposite ends of
the cell wall (fig. 2). In *Sansevieria* and *Aloe* the flanges of
adjacent pollen mother cell walls fit together and form a long
chain of dyads or tetrads in the anther according to the stages
examined (fig. 3). When the tetrads are freed these flanges
are no longer visible on empty pollen mother cell walls or frag-
ments of these walls; neither are they visible when the individ-
uals of the tetrad are ready to separate. There is a gradual
reduction in the size of the flanges from the first meiotic di-
vision until the tetrads are freed (fig. 4). Exactly what func-
tion these thickenings have is not known, but it is quite possible that they are gradually digested by the cells forming within the pollen mother cell. It is suggested that their function is twofold: (1) For the retention of cells within the anther, and (2) for food storage. The chemical nature of these flanges is likewise not known but they do not seem to be of cellulose. Crude microchemical tests and observations under polarized light with crossed Nicols fail to give positive cellulose reactions even though the epidermal cell walls of the anther do.

The microspores of *Tradescantia*, in the tetrad, regularly have the shape of the quadrisection of a sphere. While the cells are united in fours, and immediately after they separate, the nucleus is in the middle of the cell (pl. 4, fig. 1). There are no vacuoles and the cytoplasm appears clear and watery. In
radial view the cell at this stage is nearly triangular with a continuous, smooth, distinct wall. As the microspore develops there is a differentiation of the wall. The wall which was innermost in the tetrad remains flattened and smooth, but the outer wall rounds out and becomes definitely sculptured. The inner wall rounds out much more gradually until the pollen grain is nearly ellipsoidal by the time it is mature. When seen in tangential view the sculptured part of the wall of the microspore shows at each end, while the long side walls are smooth.

A study was made of microspores of *Tradescantia paludosa* to determine the change in position of the nucleus between the time of the breaking up of the tetrad and the first mitotic division. Soon after the microspores become separate the nucleus moves from the middle to the end of the cell (pl. 4, fig. 2). A large vacuole usually occupies the other end and the middle region of the cell. It is during the migration of the nucleus from the middle to the end of the cell that the wall sculpturing first becomes apparent. From this time on it becomes more and more distinct.

Just before prophase of mitosis the nucleus is again in the middle of the cell, closer to the flat wall than to the curved sculptured one, when seen in radial view (pl. 4, fig. 3). In some of the cells there appeared one large vacuole, enlarged at each end of the cell with a narrower connecting portion squeezed between the nucleus and the sculptured wall. These cells probably represent a slightly earlier stage than those usually observed which contained two vacuoles, one in each end of the cell. The vacuoles usually appeared broad and blunt at the end toward the smooth flat wall of the cell and tapered off toward the curved wall.

The asymmetry of the mitotic figures in the microspore of *Tradescantia* is noticeable from the very early stages of prophase (pl. 4, fig. 4). Throughout their formation the vegetative nucleus and the generative nucleus can be distinguished by their different appearance as well as by their position in the microspore. When the chromatids begin to differentiate, the nucleus is spherical in form. As soon as the chromosomes
become more definite and begin to loosen up and pull apart, the nucleus loses its regular shape and becomes more nearly ovoid. The small end is pressed close against the middle of the flat wall and the broad end reaches nearly to the other side of the cell. The asymmetric shape of the prophase nucleus is due to the fact that at the small end the chromosomes are closely massed together, with very little space between them, while at the broad end they are much more scattered and pulled apart. Apparently, even before the chromosomes line up on the division plate there is some force which holds them in this asymmetric arrangement. The chromosomes become very much shortened and thickened before metaphase.

The equatorial plate in mitosis is close to the smooth flat wall of the microspore cell and parallel to it. The six chromosomes are approximately equal in length, two of them with median, and four with sub-median attachment constrictions. At metaphase they are arranged with their attachment constrictions in a rough circle on the plate. The ends of the chromosomes may or may not lie within the plate.

In anaphase the chromosomes split to form two dissimilar groups. The group which is to form the generative nucleus is close to the smooth inner wall of the cell and is very small and compact. The other chromosomes migrate almost to the outside wall of the cell to form a much larger and much more loosely constructed group, which is to become the vegetative nucleus. The contraction of the chromosomes, begun in prophase, continues through anaphase.

In the two-nucleate stage of the microspore the compact, darkly staining generative nucleus is close to the smooth inner wall of the cell and is surrounded by a non-staining area. A smooth membrane cuts it off from the rest of the cell. The vegetative nucleus is much larger, light staining, somewhat irregular, and often rather vague in outline.

Later stages observed show that the membrane about the generative nucleus is no longer present. The generative nucleus swells somewhat and is next seen farther from the wall, larger and spherical. It seems as though the nucleus at this
stage is already in the prophase of the next mitotic division. The generative nucleus in mature pollen has no definite position but may lie in any region of the cell. It elongates rapidly and curls and twists about the vegetative nucleus, which is becoming smaller. There is definite indication that separate chromosomes are arranged more or less parallel at this stage. This nucleus is often seen split from one end nearly its full length, the two halves lying parallel and close together. (Fig. 5.) A definite organization was also observed in the vegetative nucleus of pollen grains taken from flowers of *Tradescantia paludosa*.

Fig. 5. Camera-lucida sketches of generative nuclei from several pollen grains of *Tradescantia paludosa*: (A) nuclei from young pollen grains; (B) nuclei at time of pollination. (x approx. 345).

*paludosa* which were mature and shedding pollen and also in a tetraploid *Tradescantia* hybrid (pl. 4, fig. 9). The ends of the chromosomes could be seen sticking out from the main body of the nucleus and in some cases could be distinguished entirely across the nucleus. In no case could more than twelve ends be counted (except in the tetraploid where twenty-four would be expected) and in two cases this number could be definitely seen. It seems reasonable to believe that these are separate chromosomes.

The presence of refractive granules in the cytoplasm of the pollen mother cell has been noted by several authors, but Sax and Edmonds are the only workers who make any statement as to their varying presence or as to their chemical composition. Crude microchemical tests show that in *Tradescantia* these
Fig. 6. Showing variation in volume in the development of the male gametophyte of *Tradescantia paludosa*. Note the great increase just before mitosis begins and after mitosis when the pollen is ripening. The decrease from its greatest volume until the time of pollination is probably due to a gradual desiccation as the anther opens. Length of time for the development of the various stages from Sax and Edmonds ('33).
granules are neither starch (Sax and Edmonds, '33) nor lipoids. In Aloe the granules are not as definite and distinct as in Tradescantia and at times they are hardly visible. Furthermore, there seem to be two types of granules in Aloe; one kind very clearly distinguishable and seemingly of the nature of those in Tradescantia; another kind rare in occurrence and not so distinct in appearance. These latter granules seem to be lipo-protein in nature, being soluble in alcohols, esters, and di-ethyl ether, slightly soluble in chloroform and benzene, soluble in concentrated hydrochloric acid and sodium hydroxide. They give a positive orange-red reaction with Sudan III, a brownish coloration with osmic acid, and a positive Biuret test. Reactions characteristic of other types of compounds give negative results. Granules of this type were present in few cells, but there can be no doubt that some granules, lipo-protein in nature, occasionally occur in Aloe and are absent in Tradescantia.

The relative number of refractive granules appearing in the various stages has never been estimated. Accordingly, comparative counts of every stage in the development of the male gametophyte of Tradescantia hirsutiflora were made, taking into account the following considerations: cells of equal age were selected for each type; counts were made from the same view in every case; and slides were kept at the same relatively low temperature during the study (below 20° C.). Since moderate heating produces better differentiation in staining between nucleus and cytoplasm, heating is often employed to produce better slides. But as granules are more or less, sometimes entirely, destroyed in this way, slides subjected and those not subjected to this treatment must not be compared.

Refractive granules are absent in the pollen mother cell and in prophase appearing between prophase and metaphase I of meiosis. From metaphase I there is a gradual decrease in the number of granules per cell throughout the entire meiotic process, the smallest number being present in the individual cells united in the tetrad (fig. 7). The cells making up the tet-
Fig. 7. Showing the average number of refractive granules found in unvacuolated cells in the development of the male gametophyte of *Trades-cantia hirsutiflora*. Length of time from Sax and Edmonds ('33).
rad then split, and comparisons made with unvacuolated cells in prophase of mitosis show that an enormous increase in volume of the cell and a corresponding increase in the number of granules has taken place. In the cells united in the tetrad and in the young microspore the nucleus is seen in the center of the cell; the granules are very large and no vacuoles are present (pl. 4, fig. 1). Later the nucleus is found at the end of the cell, and at this stage the microspore characteristically has a very large vacuole (pl. 4, fig. 2) and almost no granules. Before prophase of mitosis the nucleus is again at the center of the cell and the number of granules depends upon the presence or absence of vacuoles. If a very large vacuole is present granules are usually lacking, whereas if vacuoles are absent the cytoplasm is filled with granules. Intermediate stages with small vacuoles and few granules compared to the same stage in its unvacuolated condition can be found. Thus the data show a distinct correlation between the number of granules and the presence or absence of vacuoles.

Although granules are often found in all stages of mitosis it is apparently customary for cells in this division to be greatly vacuolated and to be practically lacking in granules. Counts made during mitosis in *Tradescantia hirsutiflora* in cells in which vacuoles were absent show a steady decrease (paralleling meiosis) from prophase to telophase, a rather large number being present in telophase (fig. 7). There is an increase from telophase to mature pollen. If the telophase stage indicated in the graph is very late there is evident in the two processes (meiosis and mitosis) a parallel decrease in the number of granules throughout both divisions, and a corresponding increase after division. At no time are more granules present than in unvacuolated mature pollen. The granules of the mature pollen grain are usually smaller and less definite than in the microspore, it is true, and they may even be different in chemical character, but there can be no doubt that they are present in abundance in the mature pollen of the specimens of *Tradescantia* and *Aloe* studied.
SUMMARY

1. A consecutive account of the development of the male gametophyte in species of *Tradescantia* is presented.

2. Appearance and disappearance of vacuoles in *Tradescantia* is correlated with the variation in number of refractive granules.

3. Wall sculpturing in *Tradescantia* begins with the sudden growth of the young microspore, just before prophase of mitosis.

4. There are two definite periods in which increase in size is apparent in the development of the male gametophyte in *Tradescantia*: (1) Just before mitosis begins, and (2) after mitosis, when the pollen is ripening.

5. It has been possible to observe separate chromosomes in vegetative nuclei of mature pollen in *Tradescantia paludosa* and in a tetraploid *Tradescantia* hybrid.

6. It is believed that the elongated generative nucleus in *Tradescantia* is composed of separate chromosomes.

7. A decrease in the number of refractive granules during the processes of meiosis and mitosis in *Tradescantia hirsutiflora* is shown by comparisons of the average number present in unvacuolated cells.

8. The flanges noted on the pollen mother cell wall of *Gasteria*, *Aloe*, and *Sansevieria* seem to be digested by the cells developing from the pollen mother cell.

BIBLIOGRAPHY


Explanation of Plate

PLATE 4

Diagrammatic review of the development of the male gametophyte of *Tradescantia*, showing simultaneously the changes in nucleus, vacuoles, refractive granules, cell wall, and cell size. The figures are all drawn to the same scale from acetocarmine smears. The wall outlines are from enlargements of camera-lucida drawings. The nuclei and vacuoles are drawn to scale either from the same cell from which the size was obtained or from other typical cells of the same stage. (x approx. 985.)

Figs. 1–8. *Tradescantia hirsutiflora*.

Fig. 1. Microspore soon after separation of tetrad, nucleus in the middle, granules very large, no vacuole, cell wall not differentiated.

Fig. 2. Older microspore after nucleus has migrated to the end, granules very scanty, one large vacuole, wall sculpturing first apparent.

Fig. 3. Very early prophase of mitosis, cell and nucleus both much larger.

Fig. 4. Prophase of mitosis, chromosomes optically double. Note the asymmetry of the nucleus.

Fig. 5. Metaphase of mitosis, tangential view of the cell (polar view of the plate).

Fig. 6. Anaphase of mitosis, the two groups of chromosomes very different in compactness.

Fig. 7. Two-nucleate microspore.

Fig. 8. Mature pollen grain, some structure visible in the generative nucleus, many small granules, no vacuoles.

Fig. 9. Tangential view of the mature pollen grain of a tetraploid *Tradescantia* hybrid showing visible organization in the vegetative nucleus.

The figures are frankly diagrammatic. They are an attempt to integrate and summarize all observations.