

## THE EFFECT OF TEMPERATURE ON NUCLEAR DIFFERENTIATION IN MICROSPORE DEVELOPMENT

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*With one text figure and plate 150*

"HEREDITY is effected by the transmission of a nuclear preformation which, in the course of development, finds expression in a process of cytoplasmic epigenesis" (Wilson, 1925). The evidence from genetic and cytological investigations has proven conclusively that nuclear preformation is dependent upon the genic constitution of the chromosomes. The mechanism of expression in cytoplasmic epigenesis is more obscure. The problem is difficult because it is not subject to direct attack. A comparison of induced and hereditary effects has provided a method for studying certain developmental processes in *Drosophila*. An analysis of the effect of temperature on developing microspores has provided some information regarding nuclear cytoplasmic relations in differentiation and development, and has some bearing on the problem of genic expression.

Normal microspore development in *Tradescantia* has been described in detail by Sax and Edmonds (1933). The young microspore contains a centrally located nucleus surrounded by cytoplasmic granules. The granules disappear, and the nucleus migrates to the end of the oval-shaped microspore. The cytoplasm is massed around the nucleus, and at the other end of the cell there is a large vacuole. There is then a migration of cytoplasm and vacuole so that two vacuoles are formed, one at each end of the cell. Most of the cytoplasm lies between the vacuoles so that the longer axis of the cytoplasmic mass lies in the short axis of the cell. The nucleus at this time lies toward the heavy or dorsal wall of the microspore, — originally the inner wall at the time of tetrad formation. When the nucleus divides, the daughter nucleus near the heavy wall of the microspore is enclosed by a thin temporary wall which includes little cytoplasm. This nucleus does not pass into the typical resting stage, but retains its chromaticity and finally elongates to form the generative nucleus. The other nucleus formed near the center of the cytoplasmic mass enlarges to form the inactive tube nucleus. Shortly after the division of the microspore nucleus, the vacuoles disappear, and the cytoplasm appears to be rather homogeneous.

The normal development of the microspore of *Pseudolarix amabilis*

resembles that of *Pinus*, described in detail by Coulter and Chamberlain (1901). Soon after the release of the microspore from the wall of the microsporocyte, the wings develop rapidly on opposite ends of the spore towards the ventral side of the cell, while the dorsal side of the spore, which was formed during meiosis, becomes thickened. At the time of the first nuclear division, the nucleus lies near the dorsal wall surrounded by most of the cytoplasm, and the region towards the wings and the ventral side of the spore are more vacuolate. The first division occurs across the short axis of the microspore, and the nucleus near the dorsal wall is cut off and degenerates. The other nucleus divides again in the same axis, and another prothallial cell is cut off. The third division produces the generative nucleus and the tube nucleus of the mature pollen grain. The generative nucleus lies near the inner or dorsal wall of the pollen grain and is cut off by a thin wall which encloses little cytoplasm, while the large tube nucleus lies free near the center of the cell.

#### THE EFFECT OF TEMPERATURE ON NUCLEAR DIFFERENTIATION

Both high and low temperatures are effective in producing abnormal development in the microspores of *Tradescantia*. Plants were placed in a constant temperature chamber where the temperature was maintained at about 6°C. for the cold treatment, or at about 35°C. for the heat treatment. Three days' treatment was sufficient to produce abnormal development at either temperature range, and doubtless a shorter time would be effective at the higher temperature. The microspores were examined soon after exposure to abnormal temperatures, and for several subsequent days after they had been placed in the normal greenhouse environment.

Three types of abnormalities were produced. Under normal conditions, only two nuclei are formed in the pollen grain,—the generative nucleus and the tube nucleus. The tube nucleus normally does not divide and is inactive in further development. The heat treatment occasionally causes the tube nucleus to divide in *Tradescantia* (Figs. 1 and 2). The subsequent fate of the daughter nuclei is not known. Apparently no great deviation from normal environment is required to produce a second division, since this behavior was observed by Mr. R. H. Goodwin in *Tradescantia* plants grown in the greenhouse at the Biological Institute of Harvard University.

The second type of abnormality is produced by either cold or heat treatment. The polarity of the microspore is disturbed so that the nuclear division is no longer oriented across the short axis of the cell.



In extreme cases the division is at right angles to the normal axis, the daughter nuclei are not differentiated, and the temporary cell wall is formed across the center of the microspore (Fig. 3). In most cases there is partial differentiation of the daughter nuclei, but the more compact nucleus does not elongate to form the typical generative nucleus (Fig. 4). The degree of differentiation of the two nuclei is closely associated with the angle of division. With a smaller degree of variation from the normal axis of division there is increased differentiation of the generative nucleus (Fig. 5). In a single anther all degrees of differentiation are found, including the normal condition (Fig. 6).

In order to determine more accurately the relation between the angle of division and the differentiation of the nuclei, a statistical study was made. All microspores measured were from a single flower taken from a plant which had been kept at a temperature of about 36°C. for three days. Camera lucida drawings were made of 163 microspores selected at random among those which showed the two nuclei in the same focal plane. The angle between the normal axis of division across the short diameter of the microspore and the line drawn through the centers of the two nuclei was taken as the angle of division. The length of the outline of the "generative" nucleus was measured in millimeters. This work, as well as the tabulation and analysis of the data, was done by my wife, Dr. Hally Jolivette Sax. The relation between the angle of division and the length of the generative nucleus is shown in Table 1. The high correlation of  $-.81 \pm .02$  shows that the degree of nuclear differentiation is closely associated with the angle of division.

A third type of aberrant development was found in microspores which began to "germinate" before the division of the nucleus. In some cases one of the daughter nuclei was found in the original microspore and the other in the newly-formed outgrowth (Figs. 8 and 10). In these cases a thin cell wall divided the cytoplasm into approximately equal parts, and there was no indication of nuclear differentiation. The division may occur so that neither daughter nucleus remains in the original microspore (Figs. 7 and 9). If the division is oriented lengthwise of the cytoplasmic mass, there is no nuclear differentiation, but if it is oriented across the short diameter of the outgrowth, so that one daughter nucleus is near the cell wall, there is a differentiation which resembles that following normal division in a normal microspore (Fig. 7).

The precocious growth of the microspores of *Tradescantia* is unlike normal pollen-tube growth. The pollen-tube usually grows from the end of the pollen grain adjacent to the heavy dorsal wall, while the aberrant outgrowth occurs at the ventral side of the microspore. Per-

haps this abnormal growth is the first stage in the development of an embryo-sac-like structure such as Stow (1930, 1933) has found in the anthers of *Hyacinthus*. Unfortunately, a study of the further development of the abnormal growth in *Tradescantia* microspores could not be continued because the anthers dehisced and disintegrated so soon. Possibly these peculiar microspores could be developed further in a nutrient solution.

TABLE I.

THE RELATION BETWEEN ANGLE OF DIVISION AND THE DIFFERENTIATION OF THE NUCLEI IN MICROSPORES OF *TRADESCANTIA*

Angle of division

| Length of generative nucleus | Angle of division |    |    |    |    |    |    |    |    |    |  |
|------------------------------|-------------------|----|----|----|----|----|----|----|----|----|--|
|                              | 0                 | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 |  |
| 8-11                         | 2                 |    | 1  | 2  |    | 2  | 2  | 3  | 3  | 3  |  |
| 11                           |                   |    |    | 2  | 1  | 5  | 3  | 8  | 3  | 9  |  |
| 14                           | 1                 | 1  | 3  | 1  | 1  | 4  | 2  | 2  | 2  |    |  |
| 17                           |                   | 5  | 4  |    | 3  |    | 3  | 2  |    |    |  |
| 20                           | 4                 | 7  | 7  | 2  | 2  | 2  | 1  | 1  |    |    |  |
| 23                           | 10                | 4  | 5  | 1  | 1  | 1  | 1  |    |    |    |  |
| 26                           | 9                 | 3  | 3  |    |    | 1  |    |    | 1  |    |  |
| 29                           | 3                 |    |    | 1  |    |    | 1  |    |    |    |  |
| 32                           | 3                 |    |    | 1  |    |    |    |    |    |    |  |
| 35                           |                   |    |    |    |    |    |    |    |    |    |  |
| 38-40                        | 4                 | 1  |    |    |    |    |    |    |    |    |  |

$$n = 163$$

$$r = -.81 \pm .02$$

The development and differentiation of the microspores of *Pseudolarix amabilis* is also affected by environmental conditions. Branches containing male flowers were placed in a warm corner of the greenhouse for about two weeks. During this time meiosis occurred, and the microspores developed to maturity. Most of the microspores were normal in their development (Figs. 11 and 12), but various types of abnormalities were observed. If the first division occurs lengthwise of the cell in the axis of the wings, there is no differentiation of the nuclei if each is an equal distance from the cell wall (Fig. 13). If, however, one nucleus lies near the cell wall, regardless of the orientation of the division spindle, this nucleus tends to remain small and form a prothallial cell, while the nucleus near the center of the cytoplasmic mass remains large and divides again (Figs. 14 and 16). The first division may occur in the normal position, but the second division may be aberrant in orientation (Figs. 15 and 16). Several years ago Mrs. Sax found a mature pollen grain of *Picea* which contained four undifferentiated nuclei of



approximately equal size. Apparently relatively slight changes in environmental conditions can cause abnormal development of conifer microspores. Nuclear differentiation in these microspores appears to be entirely dependent upon the orientation of the division spindles and the position of the nuclei in relation to the cytoplasmic mass.

Nuclear differentiation in the microspores of *Tradescantia* and *Pseudolarix* appears to be determined by the nuclear cytoplasmic relationships. In *Tradescantia* it is possible to observe the relations of nuclei, vacuoles, and cytoplasm in the living microspores. Observations at various stages of development show that normal development is dependent upon the synchronization of cytoplasmic and nuclear activities.

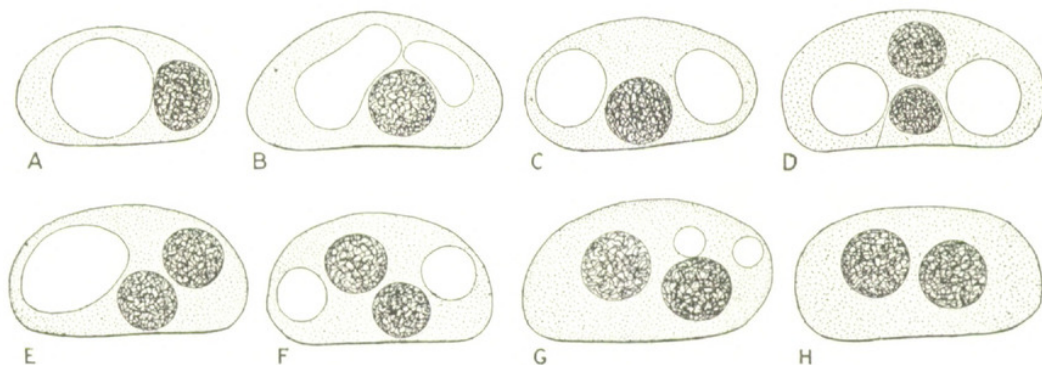
In the microspores which develop under normal conditions, the cell contents show a gradual shifting in position before the nucleus divides. A large vacuole is formed at one end of the microspore, and the nucleus and most of the cytoplasm move to the opposite end. The vacuole then extends towards the opposite end, near the ventral side of the spore, and finally forms two vacuoles, one at either end of the cell. Meanwhile the cytoplasmic mass and the nucleus migrate towards the center of the cell. The cytoplasm extends between the ventral and dorsal walls so that the length of the cytoplasmic mass is across the short diameter of the cell. Some cytoplasm extends around the entire periphery of the cell. The nucleus lies near the dorsal wall at the time of division. After the division the nucleus near the dorsal wall is cut off by a thin temporary wall, and then develops into the elongated generative nucleus. The other nucleus enlarges, loses its chromaticity, and becomes the inactive tube nucleus. The sequence of early development of the normal microspore is shown in text figures a, b, c, and d, which are camera lucida sketches drawn from living material.

When the microspore develops at low temperatures, the same cycle of development begins, but the nucleus divides before the vacuole, cytoplasm, and nucleus reach their normal positions. (Text figure e.) Since the cytoplasm migrates towards the center of the cell along the dorsal wall of the microspore, the length of the cytoplasmic mass is at an angle to the normal axis of division. The nuclear spindle is oriented in the long axis of the cytoplasmic mass, and the daughter nuclei lie towards one end of the microspore and do not undergo complete differentiation.

The exposure to high temperatures for several days appears to accelerate the cytoplasmic movement without causing a corresponding activity of the nucleus. At the time the cell constituents are in the position usually associated with nuclear division (Text figure c), the nucleus may remain inactive. The vacuoles then become smaller or may dis-

appear entirely before nuclear division. As a result, the long axis of the cytoplasmic mass is not oriented in the short axis of the cell, and the division may occur at various angles, depending in part upon the cytoplasmic distribution (Text figures f, g, and h).

In a single flower, following heat treatment, the angle of division may vary from 0 to 90 degrees. Camera lucida drawings were made from a random sample of these cells, and the angle of division was determined in relation to the distribution of cytoplasmic mass. The length of the



TEXT FIGURE. Development of the microspore under normal and abnormal conditions.

All figures are from camera lucida drawings of living microspores.

Figures a, b, c, and d show the movement of the cell contents during early development of the microspore under normal conditions. The vacuole becomes extended along the ventral wall and finally forms two vacuoles. The nucleus at the time of division becomes oriented near the dorsal or heavy wall of the microspore.

Figure e shows the nuclei formed by nuclear division before normal orientation of the cell constituents is attained. This microspore developed at a low temperature, which seems to retard cytoplasmic movement without retarding nuclear division.

Figures f, g, and h are drawings of microspores which had been subjected to a high temperature. The vacuoles are small or absent at the time of nuclear division, and the axis of division tends to occur in the long axis of the cytoplasmic mass.

cytoplasmic mass was determined for the long axis of the cell, and the width was measured across the short axis of the cell. For example, in a normal microspore (fig. d) the length of the cytoplasmic mass is the distance between the vacuoles, and the width is the distance across the microspore in the axis of division, and the angle of division is very small. In this cell the length-width ratio is about 0.5. In figure e the length-width ratio is about 1.0, and the angle of division is about 45 degrees, while in figure h the length-width ratio of the cytoplasmic mass is about 2.0, and the angle of division is about 80 degrees. The length-width



ratio of the cytoplasmic mass correlated with the angle of division gave a value of  $r = .66 \pm .03$ . It is evident that there is a strong tendency for the nucleus to divide in the long axis of the cytoplasmic mass, although as the volume of cytoplasm increases, there is not a corresponding tendency for the nucleus to divide in the longer axis. In general, however, the direction of division in the microspore is controlled by the distribution of the cytoplasm in accord with Hertwig's rule, and the nuclear differentiation is controlled by the position of the daughter nuclei in relation to the cytoplasmic mass.

It is not possible to follow the cytoplasmic movements in the living cells of conifers, but judging from the description of normal development (Ferguson, 1904) and the behavior of the nuclei in abnormal microspores, the failure of normal differentiation is also based on the disturbed relations of nucleus, cytoplasm, and vacuoles.

A comparison of nuclear differentiation in *Tradescantia* and *Pseudolarix* microspores and in the embryo sacs derived from microspores in *Hyacinthus* shows a good deal of similarity in polarity. In both *Tradescantia* and *Pseudolarix* the center of activity in early microspore development is near the dorsal wall which was formed during microsporogenesis. If a second division occurs in the *Tradescantia* microspore, the nucleus nearer the center of the cell divides, as is the case in normal microspore development in the conifers. The vegetative nucleus is always the one nearer the ventral wall and is surrounded by a large amount of cytoplasm, while the generative or sexual nucleus lies near the dorsal wall and is enclosed by a thin temporary cell wall which includes little cytoplasm. The "embryo sacs" which develop from microspores of *Hyacinthus* (Stow, 1933) show the exine of the microspore at the egg or sexual end of the embryo sac, while the polar or vegetative nuclei lie in the center of the embryo sac, apparently surrounded by a relatively large amount of cytoplasm.

Stow was able to induce embryo sac-like structures in anthers of *Hyacinthus* by subjecting the bulbs to a temperature of 28°C. for 18 to 24 hours at the time of planting in the fall. The abnormal development observed in the following spring may have been induced either by the temperature treatment or by the effects produced by the large number of degenerating sterile microspores. At any rate the differentiation of the microspore to produce a normal pollen grain or an embryo sac appears to depend upon environmental conditions. We are inclined to believe that the precocious growth of *Tradescantia* microspores is the first stage in embryo sac formation, and that the complete structure could be developed, under temperature control, if the cells could be kept alive over a long period of time, as is the case in *Hyacinthus*.



## FACTORS IN DEVELOPMENT AND DIFFERENTIATION

According to Osterhout (1921), life is dependent upon a series of reactions which normally proceed at rates which bear a definite relation to each other. If for life we substitute development and differentiation, we have an hypothesis which seems to explain development and differentiation. Certainly the differentiation of the microspore nuclei appears to be dependent upon the relative rates of cytoplasmic migration and nuclear activity. Less direct evidence indicates that the difference between a pollen grain and an embryo sac may be dependent upon the same type of timing relationships. If sex can be determined by the timing relationships of different reactions, effected either by environmental conditions or hereditary factors, we have indirect evidence that genic expression may be effected by differential reactions.

More direct evidence regarding the mechanism of genic expression is found in the behavior of the chromosomes at meiosis. A failure of chromosome pairing, or asynapsis, may be caused by genetic factors or by environmental conditions. Genetic asynapsis has been found in *Drosophila*, *Zea*, *Triticum*, *Rumex* and *Datura*. Induced asynapsis can be effected in *Rhoeo* and *Datura* by subjecting the plants to low temperatures for several days, and it has been obtained in *Tradescantia* following treatment at low and high temperatures. Both the hereditary and induced effects are similar in their expression. The chromosomes are unpaired at meiosis, the divisions are irregular, diploid gametes may be produced, and there is a high degree of pollen sterility in the asynaptic plants. Both types of asynapsis may be attributed to the same cause. If chromosome development is not synchronized with other cell activities, the chromosomes may not be effectively paired before nuclear division is initiated.

An exceptionally clear case of the timing factor in genic activity was found in *Aquilegia* by Anderson and Abbe (1933). The "compacta" mutant of *Aquilegia* is dependent on a single genetic factor. In the mutant type the branches are more erect and numerous, and the flowers are upright from the beginning. The dwarf type is caused by the precocious thickening of the cell walls, and the somatic expression is simply the result of disturbed timing relationships, — "the precocious initiation of a normal feature of normal development." Further aspects of the relation between genes and development in *Drosophila* have been discussed by Schultz (1935) and by Goldschmidt (1935), and similar work is being done on the cucurbits by Sinnott (unpublished).

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## SUMMARY

The subjection of *Tradescantia* plants to low and high temperatures may produce three kinds of abnormalities in microspore development. (1) The tube nucleus, which in normal microspores is inactive and ultimately degenerates, may divide. (2) The polarity of the cell may be disturbed so that the division of the microspore nucleus is not oriented in the normal axis. The angle of deviation is closely correlated with the differentiation of the daughter nuclei. (3) The microspore may become greatly extended on the ventral side, and the nuclear division may occur in this new outgrowth. This abnormality may be the first step in the transformation of a microspore to an "embryo sac," as found in *Hyacinthus* by Stow.

When *Pseudolarix* microspores are developed at a relatively high temperature, there is a failure of normal differentiation of the nuclei. The differentiation of prothallial cells and generative and tube nuclei is dependent upon the nuclear cytoplasmic relationships in the developing microspore.

The normal differentiation in *Tradescantia* microspores is dependent upon the synchronization of cytoplasmic movements and nuclear activity. There is some evidence that many differences in development and differentiation, induced either by genetic factors or by environmental conditions, are dependent upon differences in reaction rates of different processes.

## LITERATURE CITED

- ANDERSON, E. and L. B. ABBE (1933). A comparative anatomical study of a mutant *Aquilegia*. (Amer. Nat. **67**: 380-384.)
- COULTER, J. M. and C. J. CHAMBERLAIN (1901). Morphology of spermatophytes. (D. Appleton and Co. New York.)
- FERGUSON, M. C. (1904). Contributions to the knowledge of the life history of *Pinus*, with special reference to sporogenesis, the development of the gametophytes, and fertilization. (Proc. Wash. Acad. Sci. **6**: 1-202.)
- GOLDSCHMIDT, R. (1935). Gen und Ausseneigenschaft. (Zeit. f. Ind. Abstam. u. Vererb. **69**: 38-131.)
- OSTERHOUT, W. J. V. (1921). The mechanism of injury and recovery of the cell. (Science N. S. **53**: 352-356.)
- SAX, KARL and H. W. EDMONDS (1933). Development of the male gametophyte in *Tradescantia*. (Bot. Gaz. **94**: 156-163.)
- SCHULTZ, J. (1935). Aspects of the relation between genes and development in *Drosophila*. (Amer. Nat. **69**: 30-54.)
- STOW, I. (1930). Experimental studies on the formation of the embryo-sac-like giant pollen grain in the anther of *Hyacinthus orientalis*. (Cytologia, **1**: 417-439.)
- (1933). On the female tendencies of the embryosac-like giant pollen grain of *Hyacinthus orientalis*. (Cytologia, **5**: 88-108.)
- WILSON, E. B. (1925). The cell in development and heredity. (The Macmillan Co. New York.)

## DESCRIPTION OF PLATE 150

Photographs of aceto-carminc preparations of abnormal microspores of *Tradescantia* and normal and abnormal microspores of *Pseudolarix amabilis*. Figures 1 to 6 inclusive, magnified  $\times 800$ . The other figures are magnified  $\times 600$ .

## TRADESCANTIA

Figures 1 and 2. Division of the tube nucleus of the microspore.

Figure 3. The division of the microspore nucleus has occurred at right angles to the normal axis of division, and the daughter nuclei do not become differentiated.

Figures 4, 5, and 6. The nuclear divisions have occurred at various angles followed by a corresponding amount of nuclear differentiation. These microspores were developed at a high temperature.

Figures 7, 8, 9, and 10. Abnormal microspores produced by heat and cold treatment.

## PSEUDOLARIX

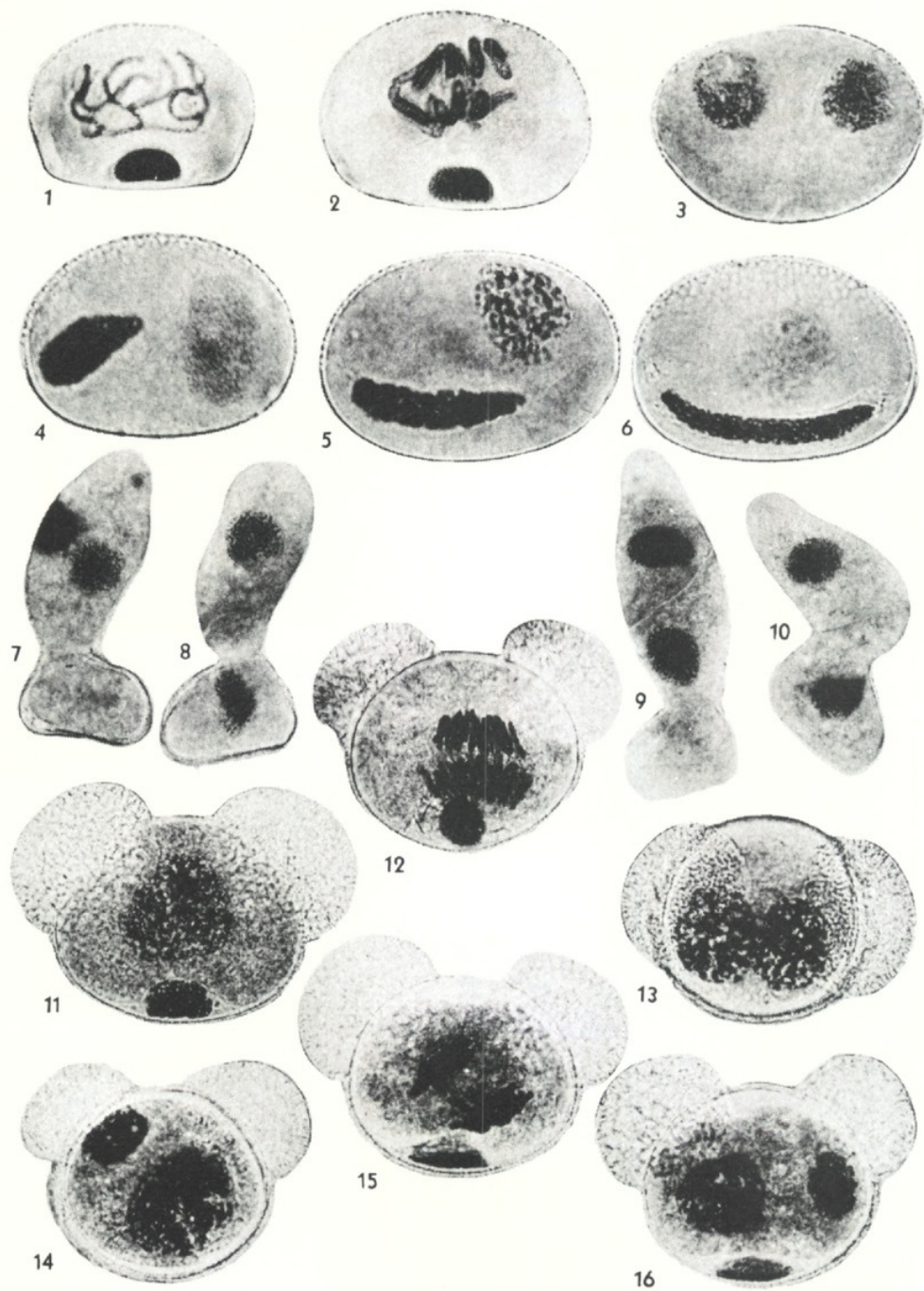
Figures 11 and 12. Stages in the normal development of the microspore.

All divisions are across the short axis of the cell, and the prothallial cells are always cut off near the heavy dorsal wall.

Figures 13, 14, 15, and 16. Abnormal development induced by heat treatment. The nuclei may divide at various angles followed by various degrees of differentiation of the daughter nuclei. The prothallial cells may be cut off at any point along the cell wall.

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TEMPERATURE AND NUCLEAR DIFFERENTIATION



Sax, Karl. 1935. "The Effect of Temperature on Nuclear Differentiation in Microspore Development." *Journal of the Arnold Arboretum* 16(3), 301–310.  
<https://doi.org/10.5962/p.324578>.

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