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STUDIES ON THE COMPARATIVE CYTOLOGY OF THE ANNUAL AND BIENNIAL VARIETIES OF MELILOTUS ALBA.

by

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A thesis submitted to the Graduate Faculty

for the Degree of

DOCTOR OF PHILOSOPHY.

Major subject (Plant Morphology)

No. 19.

Approved

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1924.

STUDIES ON THE COMPARATIVE CYTOLOGY OF THE ANNUAL AND BIENNIAL VARIETIES OF MELILOTUS ALBA.

Introduction.

Biennial white sweet clover has been known in the United States for many years and was, according to Gray (19), originally naturalized from Europe. In 1856, Prof. Tutweiler of Green Springs Academy, Alabama, received a small quantity of white sweet clover seed from the Secretary to the United States Consul in Chile (7). Whether this represents the first introduction of the seed into the United States is a matter of question.

The first evidence of the existence of an annual variety of white sweet clover seems to be somewhat obscure. It was probably observed by S. M. Tracy as early as 1898 for, in "Forage Plants and Forage Resources of the Gulf States", he says of it: "a few plants will produce seed the first year and a few will live three years" (46). Sometime before 1916, Dr. W. B. Gernert, then of the Illinois Experiment Station, discovered the annual variety and propagated it (46). Pieters (46) states that the variety was also observed in Arkansas in 1916. The attention of agronomists was first called to the new variety, however, by Professor H. D. Hughes (46) of the Department of Farm Crops of the Iowa State College, who, sometime previous to the spring of 1916, in the experimental plots of the common white sweet clover, found a form which produced an annual root

and died at the close of the season. Nothing is known as to where the seed, from which this annual white sweet clover originated, came from. Coe (8) states that in the winter of 1916 a quantity of Melilotus alba seed which had been grown in Hale Co., Alabama, the previous summer, was purchased for experimental purposes. Approximately five per cent of the plants grown from this seed flowered abundantly and matured seed in September, 1916. All the other plants from this seed produced first year's growth typical for the ordinary biennial variety. None of the plants which flowered the first year lived through the winter of 1916-1917, whereas only a small per cent of the typical biennial plants winter-killed. Seed was collected from the plants which flowered the first year and, when planted, produced typical annual plants and flowered the first season. The annual variety has been so uniform in structure and behavior that Coe (8) has described it as a new variety, naming it Melilotus alba Desr. var. annua n. var. (Annual White Sweet Clover)

The annual may be distinguished from the biennial variety in that in the annual the food and energy are used to form a central vertical stem which flowers the first season, whereas in the biennial they give rise to numerous lateral branches which die down at the end of the first season without flowering. From buds on the crown of the biennial plant, lateral branches arise during the second season and produce numerous flowers.

The present investigation was undertaken for the purpose

of ascertaining whether any cytological differences exist between the two varieties.

Materials and Methods.

Material was collected from plants grown both in the greenhouse and in the field. The entire study was made from young flowering racemes of both the annual and biennial varieties, taken when the racemes were from .5 to 1.5 cm. long. Stages varying from the very earliest, when the anther was a mere mass of meristematic tissue, to mature pollen grains, could easily be secured on the same section by cutting the racemes longitudinally. The material was killed and fixed in chromacetic acid of medium strength, or in medium chromacetic acid to which were added ten drops of two per-cent osmic acid per 50 cc. of solution. Sections were cut 3-10 u thick, stained either with Haidenhain's iron-alum haematoxylin or Fleming's triple stain. Much of the material, especially in the study of the pollen-mother-cell wall, the special wall formed around the microspores, and the walls of the pollen grain were studied in living as well as fixed condition. Chromosome counts were made both in sections of fixed material, and in living condition by the use of Belling's (4) iron-aceto-carmine method.

The cytological situation found in the annual variety will be described first, and statements regarding the biennial

will be made at the end of the discussion of the annual.

THE ANNUAL VARIETY.

Development of the Anther.

The development of the anther is, in general, similar to that described by Warming (66), who furnished the first detailed account of microsporangium development. In Melilotus the anther first appears as an oval mass of meristematic cells which soon becomes four lobed in cross section. In each lobe there is a layer of radially elongated hypodermal cells (fig. 1), the archesporium, the cells of which are easily distinguished by their large size, dense cytoplasm, large nuclei and conspicuous nucleoli. These cells soon divide by periclinal walls forming two layers, the outer of which is the primary parietal, and the inner the primary sporogenous layer (fig. 2). The cells of the primary parietal layer divide further, eventually forming three concentric parietal layers (fig. 6), the outermost of which develops into the endothecium, whereas the innermost layer becomes the tapetum, and the middle layer remains undifferentiated. Contrary to the usual situation, the spore-mother-cells of Melilotus are formed by the continued division of but a single longitudinal row of these sporogenous cells, rather than by the division of the whole transverse row. In cross section, this cell appears considerably larger than the adjoining cells of what is ordinarily regarded as the primary

sporogenous layer (fig. 3). In longitudinal section, it appears as a row of from five to eight cells (fig. 4). By successive divisions (fig. 5) this large sporogenous cell forms a mass of from six to eight mother-cells in cross section (fig. 6), and from six to ten cells in longitudinal section.

Resting Stage and Synizesis

In the sporogenous cells just previous to the formation of the mother-cells the chromatin appears in small clumps, most of which are in contact with the nuclear membrane. Two nucleoli are frequently seen in each nucleus at this stage.

The pollen-mother-cells are polyhedral in shape and their cytoplasm is more dense than that of the surrounding cells. The nuclei in the resting stage are 5-7 u in diameter. The chromatin is at first rather clumped around the nuclear membrane but it soon assumes the form of a loose reticulum (fig. 7), in the midst of which is a single nucleolus, which exhibits a differentiation of structure into a central region which is somewhat transparent, and a peripheral region which stains deeply (fig. 7). As nuclear development proceeds the central region of the nucleolus becomes more transparent. (fig. 13). During the resting stage, as well as succeeding stages, the nucleolus shows little buds (fig. 7-10) being

constricted off at its surface, suggesting that nucleolar material is given off into the nuclear sap and that it may be used in the further development of the nucleus. Montgomery (43) in his paper on the morphology of the nucleolus has given a comprehensive review of the literature covering animal and plant nucleoli down to 1897, and Wager (65) has in a similar manner reviewed the botanical literature on the subject up to 1904. Montgomery, as a result of his study of the literature, and of his own work, concludes that the ground-work of the nucleolus is variable in consistency and that vacuoles are often present. In animal egg-cells two kinds of nucleoli are often found, the nucleolus proper, and the paranucleolus. In some cases, a double nucleolus is found, each part of which represents a true nucleolus, and this double nucleolus may be composed of a true nucleolus, and a chromatin-nucleolus, the latter bearing a close relation to the chromatin. He considers that the nucleolus has its origin in the first place from the cytoplasm and that it consists of substances taken into the nucleus from the cell body. These substances are probably related to the nutrition of the nucleus, and are either nutritive in function or are waste products. In general, he finds no evidence that the nucleoli bear a genetic relation to the chromatin. Wager (65) concludes from his studies on Phaseolus that nucleoli

may be composed of plastin, a non-chromatin homogeneous refractive slightly stainable substance, or "of plastin combined with chromatin in varying quantities". He finds that when the chromatin network is prominent the nucleolus may be absent or, if present, it gives little or no reaction for chromatin, but when the chromatin thread is not prominent, the nucleolus is large and gives a strong reaction for chromatin. He thinks the nucleolus is not an independent organ of the nucleus but is a part of the nuclear network which stores or elaborates chromatin, which is concerned in the formation of the chromosomes and possibly of the spindle, and that in some cases a part of it may be extruded into the cytoplasm, where it disappears. In the reconstruction of the daughter nuclei he finds that most of the chromatin ultimately passes into the nucleolus. He is of the opinion that the vacuolar structure of nucleoli is general, and that there is at least a partial separation of the nucleolar substance into plastin and chromatin, or a greater accumulation of chromatin in the peripheral layer. Nichols (44), working on the formation of the pollen from the pollen-mother-cell in Sarracenia, concludes that the nucleolus elaborates chromatin. She finds that after synizesis the nucleolus often shows a central dark region and a peripheral light region, and that from the central mass minute spheres are budded off which escape into the nuc-

lear sap and are absorbed and distributed along the reticulum. She finds that there is not only a physical, but also a chemical change in the nucleolus, as is shown by its reaction to stains. Gregory (21), studying pollen development in sweet peas, observed that the nucleolus serves as a store-house for most of the chromatin during the resting stages between mitosis.

The behavior of the nucleolus in Melilotus indicates that the nucleolus is composed of a peripheral region of chromatin and a central more transparent region which may be composed of plastin, as suggested by Wager (65), and that chromatin is elaborated by the nucleolus and is cast off into the nuclear sap, where it is used in the thickening of the reticulum, and that the nucleolus serves as a store-house for chromatin during interkinesis.

Early in the heterotypic prophase, the leptonema stage is initiated, and during the development of this stage the nuclear reticulum assumes a more distinctly thread-like appearance (fig. 8.) Because of the small size of the nucleus, the writer has been unable to follow further the behavior of these threads and can make no statement as to whether or not they pair at this stage. The first evidence of synizesis is the appearance of a clear space at one side of the nucleus and the thickening of the threads of the

chromatin network. The threads now contract forming a very tight knot at one side of the nucleus, in which the nucleolus is at first entirely imbedded but from which it soon emerges (fig. 9). The synizesic ball evidently remains intact for a relatively long time, as shown by the large number of nuclei found in this stage. Santos (52) has disproved the contention of Lawson (35) that synizesis is due to a sudden growth of the nucleus rather than to a contraction of the reticulum, for he finds by accurate measurement, that there is not only an enlargement of the nucleus but also a contraction of the reticulum during synizesis. In Melilotus there is a definite contraction of the reticulum as well as an increase of 2-4 u in the diameter of the nucleus during synizesis. The synizesic ball now loosens and appears as a system of anastomosing threads (fig. 10) which are double. The threads become rather uniformly distributed throughout the nucleus and are certainly now much thicker in the pachynema (fig. 11). No second contraction stage was observed.

Diakinesis.

The thread now breaks up into a number of irregular pieces, the chromosomes (fig. 12), which soon become more regular in outline, and, either bend in such a way as to have one end of the chromosome fold over the other end, or assume the shape of a figure U. In either case a break occurs at the

bend of the chromosome, thus forming the two components of the bivalent chromosome. These components of the bivalent chromosome at first become so arranged as to form a figure X or V or O, but eventually come to be in contact throughout their entire length (fig. 13). In diakinesis the writer has been able to establish beyond doubt, both by counts in sections of fixed material, and by the use of Belling's (4) iron-aceto-carmine method for living material, that the number of bivalent chromosomes is eight (fig. 13). This diakinesis figure clearly shows that the chromosomes are double. One of these chromosomes is U shaped and is in the stage just previous to breaking at the point of greatest curvature, to form the two components of a bivalent chromosome. The nucleolus at this stage shows a sharp demarcation into the inner transparent, and the outer deeply staining zone.

The Heterotypic Division.

As the heterotypic division is initiated the nuclear membrane gradually becomes thinner and finally disappears. The nucleolus also disappears entirely at this stage. The chromosomes at first become arranged parallel with the long axis of the heterotypic spindle on which they soon assume various positions, however, (fig. 14). Figure 15 represents a section cut at right angles to the long axis of the heter-

otypic spindle through the equatorial region, and shows a cross section of the eight bivalent chromosomes. This is the most favorable stage for determining the number of chromosomes and many of the preparations, in this stage, show the number of bivalent chromosomes to be eight. The members of the bivalent chromosomes now separate along their line of contact and the components of each bivalent chromosome go to the opposite poles. In the anaphase of the heterotypic division the chromosomes are closely grouped together (fig. 16), presenting a rather flat chromatic mass, in which it is often impossible to distinguish the individual chromosomes. They soon become separated, however, and the group assumes a more spherical form. Before the nuclear membrane can be distinguished around each chromosome group, one can without difficulty see that there are eight chromosomes at each pole. Each nuclear mass now becomes enclosed by a membrane and it is still possible to see that there are eight chromosomes in the nucleus (fig. 17). As the daughter nuclei enlarge slightly, the chromosomes become more or less completely fused together (fig. 18), but soon break up into numerous small clumps. A nucleolus is formed in each of the daughter nuclei which now show a more or less definite chromatin network (fig. 19). Thus, contrary to the usual situation at the end of the heterotypic division, the nuclei of Melilotus

are completely reconstructed. By this time, the spindle fibers of the heterotypic spindle have entirely disappeared and there is no evidence of even a trace of a cell plate or wall or cleavage furrow across the spindle at any time.

The Homotypic Division.

The daughter nuclei remain in the resting stage (fig. 19) for only a short time. The next succeeding stage the writer has found in his preparations is represented by figure 20, in which the univalent chromosomes of the daughter nucleus are seen to be separated at their ends. A deeply staining nucleolus is also seen. Just what the procedure between the resting stage (fig. 19) and the reappearance of the univalent chromosomes is, the writer has been unable to determine as the preparations lack the intervening stages. The nuclear membrane and nucleolus of each daughter nucleus disappear and the univalent chromosomes become arranged on the homotypic spindles. The position of the spindles with reference to each other is variable. They may be parallel or at right angles to each other just as reported by C. H. Farr (12-15) who finds a variable arrangement of the spindle fibers in Nicotiana, Magnolia, Sisyrinchium and Nelumbo. Figure 21 shows a view in which the homotypic spindles are at right angles to each other. The section is cut through the equatorial region of one of the spindles, through the

chromosomes, and clearly shows the number of chromosomes to be eight. Although no evidence of a split in the chromosomes is seen in this section, they do split very soon after this stage and the components of each univalent chromosome go to the opposite poles. In the anaphase of the homotypic division the chromosomes are at first closely massed together, just as in the heterotypic. They soon become separated and can be counted before the nuclear membrane is formed. A membrane is soon formed around each of the four nuclei and here again the number of chromosomes is seen to be eight. (fig. 22). A nucleolus is formed in each nucleus at this stage. Soon the chromosomes break up into small fragments which are distributed throughout each nucleus. It is interesting to note that in this stage four, and in the similar stage of the heterotypic division, two deeply staining bodies not unlike centrosomes are seen in the cytoplasm (fig. 17, 22). The writer has been unable to ascertain either the origin, function or final disposition of these bodies. The nuclei at the end of the homotypic division are tetrahedrally arranged.

The Wall of the Pollen-Mother-Cell.

The pollen-mother-cells are at first polyhedral in shape (fig. 6) and each surrounded by a thin membrane. During

synizesis a clear homogeneous substance is secreted by the protoplast which is at first seen only at the corners of the mother-cell just as synizesis is initiated (fig. 9). As the development of the nucleus proceeds, the protoplast becomes entirely surrounded by this homogeneous substance (fig. 10), until in the pachynema it becomes quite massive (fig. 11). Microchemical tests clearly show this wall to be composed of practically pure callose. During the secretion of this callose wall the protoplast assumes a spherical form.

The first case in which a wall of this kind was found associated with the pollen grains was noted by Mangin (41). In the tetrads of Carex riparia this wall was found to consist of thickenings which alternate with the pores of the pollen grains. In Juncus silvaticus the wall was observed to form partitions between the microspores of the tetrad. The microchemical reactions given by this substance showed it to be composed of a substance which Mangin called callose. In Gentiana officinalis and Campanula rapunculoides Mangin (42) noted some variations in the chemical nature of this pollen-mother-cell wall. Further evidence of the nature of this wall is given by Beer (1) who demonstrated in Oenothera biennis and Aucuba japonica the presence of a similar pollen-

mother-cell wall which was composed of pure callose. In a later paper (3) he describes in Ipomea purpurea a similar wall which contains callose and pectose. Farr (12) describes and figures in the pollen-mother-cells of Nicotiana, a similar wall which takes a deep orange stain, when orange G is used. He considers this wall to be carbohydrate in nature. By the use of the microchemical tests used by Mangin and by Beer, namely Ruthenium red, lachmoid, aniline blue, corallin soda, congo red, caustic potash, and NaOH, the writer has found this massive wall in Melilotus to be composed of practically pure callose.

Hill (27) believes that in the sieve tubes of Pinus callose may originate directly as the result of protoplasmic activity or indirectly by the transformation of cellulose. Beer (1) thinks there is no doubt that in the case of the walls of pollen-mother-cells the callose originates directly from the activity of the protoplast, for he observes no disappearance of cellulose or pectose to account for its origin. In Melilotus the original thin membrane of the pollen-mother-cell is very poor in cellulose, and the callose wall formed is so massive that there can be no possibility of its having originated by the transformation of cellulose. The writer considers it, therefore, a direct

product of the protoplast of the mother-cell in Melilotus.

The Furrowing Process.

No further change occurs in this callose wall until the resting stage at the end of the homotypic division. Figure 22 shows the condition of the protoplast as the homotypic division nears completion. The spindle fibers are quite conspicuous in this stage, but they soon begin to disappear (fig. 23). When the spindle fibers have almost entirely disappeared, infoldings occur at the periphery of the protoplast equidistant from the nuclei, and at right angles to the former spindles. The subsequent behavior of the protoplast in the formation of the microspores in Melilotus is very unlike that ordinarily observed in microspore formation and a detailed description of the process will therefore be given.

It had long been thought that the quadripartition or simultaneous division of pollen-mother-cells in dicotyledons occurred by means of cell plates. Farr (12-15) has shown that quadripartition in certain dicotyledons, as well as in at least one monocotyledon, occurs by a process of furrowing after the homotypic mitosis is complete, and that there is no evidence of any cell plate formation. He states (12) "It appears that in no instance is the evidence conclusive

that quadripartition of the pollen-mother-cells of any dicotyledons is effected by means of cell plates." In Nicotiana the four nuclei at the end of the homotypic division are tetrahedrally arranged, and a furrow is formed along the equator of each of the spindles, forming four lobes, each of which becomes a microspore. As the furrows proceed from the periphery of the mother-cell toward the center there are four equidistant invaginations of the mother-cell wall which eventually meet in the center of the tetranucleate cell, dividing it into four unicleated protoplasmic masses. Thus the partition walls between the four microspores are continuations of the wall of the pollen-mother-cell. There is no evidence of cell plate formation or of furrowing at the end of the heterotypic division. A similar situation occurs in the other dicotyledons studied, as well as in Sisyrinchium, a monocotyledon. In Magnolia, in which the tetrads are of the bilateral type, he finds that such tetrads are also formed by furrowing. After the heterotypic mitosis, however, a cleavage furrow begins to form but is arrested until the homotypic mitosis is completed, when it resumes its growth toward the center, while at the same time two other furrows subdivide each hemisphere, thus resulting in the formation of four microspores. In

his papers, a very complete review of the literature dealing with the subject of furrowing may be found. Wanda K. Farr (16) reports that in the pollen-mother-cells of Cobaea scandens alba, less dense areas of clear cytoplasm extend across the equators of the homotypic spindles during the process of furrowing, and that as the furrows advance, they work in between the fibers which are in their paths and later surround them.

In Melilotus at the end of the homotypic mitosis the spindle fibers become completely resorbed and the protoplast is seen to contain numerous small refractive vacuoles which are uniformly distributed, and which are apparently filled with cell sap for they were not stained by any of the stains which the writer employed. These vacuoles persist in the cytoplasm even after the four microspores are independent of each other (fig. 24, 26), but disappear as the pollen grains begin to differentiate. Soon less dense areas are recognizable in the cytoplasm, extending midway between the nuclei and reaching from the periphery to the center of the protoplast. Rows of vacuoles, larger than those previously mentioned, and which are formed by fusion of the smaller vacuoles, are seen to extend across these hyaline areas

(fig. 24). While this takes place there is evidently an increase in the density of the cytoplasm in the vicinity of the nucleus, which is due to the movement of cytoplasmic material from the regions of cytoplasm equidistant from the nuclei. That there is such a movement of cytoplasm is evidenced by the appearance of the vacuoles which at this stage are elongated with their ends pointed toward the nuclei (fig. 24) and, furthermore, by the fact that the granules of the cytoplasm between the nuclei often show a linear arrangement and give the appearance of being under tension. The vacuoles as seen in figure 24 soon fuse, forming larger vacuoles which are variable in shape, thus leaving only a few strands of cytoplasm connecting the four protoplasmic masses (fig. 25). Finally the few connecting strands become severed by incoming surface furrows, and the microspores thus become separate protoplasmic masses with a wide furrow between them. The new surfaces at the edge of the protoplasts adjoining the furrow are rough and irregular, caused by the rupture of the strands of cytoplasm which connected the microspore masses. The furrow soon becomes narrower and more regular in outline, due to the turgor of the protoplasts as the newly formed plasma membranes adjoining the furrow approach each other.

This method by which the cleavage of the cytoplasm occurs in Melilotus is not unlike that observed in the Phycomycetes and the Myxomycetes by various workers. Harper (33) found in Synchitrium, that after the single nucleus divides repeatedly forming a large number of nuclei in the vegetative body, the protoplast is eventually divided into a large number of uninucleated masses of protoplasm by narrow intersecting cleavage furrows, which originate at the periphery and progress inward. In Pilobolus he observed a number of small vacuoles arranged in a dome-shaped layer parallel to the periphery of the sporangium. These vacuoles, which are at first spherical, become flattened parallel to the surface of the sporangium and finally fuse edge to edge, forming a furrow which, aided by a cleft starting at the periphery, cuts out the columella. Only a few strands of protoplasm connect the spore plasma with that of the columella as the furrowing process which delimits the columella nears completion. The spore plasma now becomes more vacuolate and surface furrows progress inward, meeting the vacuoles, thus cutting the plasma into irregular multi-nucleated masses. In Sporodinia the process is very similar. A curved region of large vacuoles is formed and these vacuoles gradually flatten, outlining the surface of the colum-

ella. The vacuoles tend to fuse, and some come to lie close to the cell wall and finally rupture the plasma membrane, allowing the cell sap to filter out and evaporate. The two membranes bordering the cleft approach each other and a wall is laid down between them. Swingle (59) found a similar situation in Rhizopus and Phycomyces. In Rhizopus the cytoplasm, nuclei and vacuoles stream toward the periphery of the young sporangium making the outer region denser. A layer of large round vacuoles is formed and these vacuoles are arranged parallel to the surface of the sporangium. Swingle (59) finds that a cleft, formed by the fusion of these vacuoles, is met by a surface furrow coming from the base of the sporangium, thus cutting out the columella. The spores are formed by intersecting surface furrows which push inward, as well as by others which push outward from the columella cleft. In Phycomyces, however, cleavage occurs by furrows which push in various directions from vacuoles in the cytoplasm. Schwarze (53) recently observed a similar behavior in various sporangia, notably in those of Sporodinia grandis and Mucor mucedo. There is much similarity between the method by which the spores are formed in Pilobolus, Sporodinia and Rhizopus, and the method of the formation of the microspores in Melilotus. This is especially

true in the method as described for Pilobolus by Harper as compared with that which the writer has described for Melilotus, for in both cases there are rows of small vacuoles which fuse forming furrows, which are met by furrows that progress inward from the periphery. In both cases also, a few strands of cytoplasm connect the protoplasmic masses after the furrowing process is almost completed.

The mechanics of cleavage and furrowing has received considerable attention by various workers. Bütschli (5) interpreted furrowing and cell division as the result of a higher surface tension at the equator of the cell, caused by the flow of protoplasmic currents toward the centrosomes, and the work of McClendon (38-40) adds credence to this theory, whereas, on the contrary, Robertson (47-49) considers furrowing as due to a decrease in surface tension at the equator, caused by the diffusion of materials from the nuclei toward that region. That Bütschli's observations were probably correct is shown by the work of Spek (54-55), in which, by using droplets of oil and mercury in water he was able to imitate furrowing by lowering the surface tension at the two poles of the droplet, thereby increasing the surface tension at the equator. Moreover, he observed streamings in the droplets and in dividing eggs.

This interpretation is further corroborated by Chambers (6), who found that two semi-solid masses are formed at the poles, and that the elongation of the egg is caused by the growth of these masses. Finally a cleavage furrow develops in the more fluid portion of the egg substance midway between the daughter nuclei. The highest surface tension is, of course, in this more fluid region. In this same connection, Kite (34) from a preliminary study of cell division, concludes that it is very largely the result of "concomitant shrinking and swelling or change in water holding power of different portions of the cytoplasm." Recently, however, Gray (20) has shown that it is unnecessary to assume the occurrence of regions of differential surface tension on the cell surface. Using fertilized animal eggs, as well as two drops of oil in acid and normal sea water, he found that the shape of the dividing cell is the result of an equilibrium between a force inside the cell and surface tension. He attributes cell division to the movement of two asters away from each other, and maintains that the cleavage furrow is due to an equilibrium between the effect of this movement on the protoplasm and the surface tension on the surface of the cell. Farr (12) thinks that the nuclei, after the second division of the mother-cell, behave as though bearing

electrical charges of like sign, thus repelling each other, whereas the plasma membrane bears charges of opposite sign. The resulting attraction of the nuclei for the plasma membrane, the attraction of parts of the membrane for each other and the repulsion of the nuclei for each other all contribute to the furrowing process. This theory is not wholly unlike that held by other investigators, who attribute furrowing to the attraction of the nuclear membrane and the plasma membrane for each other. While this theory seems plausible, it will doubtless lack general acceptance until more experimental evidence is at hand to substantiate it.

The matter has been approached from a different angle by other workers who find that the furrowing process is, in many cases, to be attributed to the fusion of vacuoles. Harper (23) is of the opinion that cleavage might be connected with the loss of water and indicates the similarity of surface furrowing to the cracking of the surface of a drying mass of a colloidal substance. He considers this explanation by itself as inadequate, for the multinucleated mass is segmented with reference to the distribution of the nuclei, since the ultimate masses of protoplasm are uninucleated, and he suggests that the regular segmentation of uninucleated

masses might be attributed to less loss of water in the vicinity of the nuclei than elsewhere. In a later paper, Harper (25) connects this with the effects of alkalis and acids on the imbibition of water by colloids, and suggests that a localized concentration of acid in the sporeplasm, involving a differential water holding power, would determine the orientation of the cleavage furrows, since the cleavage planes would follow those zones containing the least water, thus delimiting the acid-containing areas. If the chemical nature of the nucleus would make it a center of water concentration, uninucleated spores would therefore be produced. Swingle (59) has explained the cleavage process in Rhizopus and Phycomyces on the basis of localized contractions of the cytoplasm and does not consider the nuclei as directly influencing contraction. The explanation is very simple but there is no suggestion as to the origin or cause of these local contractions.

The evidence in Melilotus indicates that the planes of cleavage are predetermined by rather hyaline areas located midway between the nuclei, and extending from the periphery in a manner described by Harper for Pilobolus (23) and for Fuligo (24), in which these hyaline areas extend between the nuclei after the early stages of cleavage have been init-

iated. W. K. Farr (16) found similar hyaline areas extending across the equators of the spindles of the pollen-mother-cells of Cobaea which areas were accentuated by denser areas around the nuclei. In Melilotus, these hyaline areas are apparently due to the movement of the granular material from these regions of the cytoplasm to the vicinity of the nuclei. This is accompanied by the extrusion of liquid into vacuoles which fuse, forming larger vacuoles, thus leaving the cytoplasm of the four masses of protoplasm which are to become microspores, connected by only a few strands of cytoplasm. These strands are soon severed by the pressure exerted by the liquid within the vacuoles, and by surface furrows which originate at the periphery. The nuclei play an important part in determining the planes of cleavage and since the movement of granules toward the nuclei would be initiated at regions equidistant from the nuclei, it is evident that the cleavage planes would be formed at these regions. It is clear that the cleavage furrows are formed almost entirely by the fusion of vacuoles and that the furrows which originate on the surface do not progress centripetally until the vacuolization is well advanced, and that the furrows proceed but a little distance before cutting into the large vacuoles. This formation of the furrows through the cytoplasm until

they meet the vacuoles is doubtless due to higher surface tension in the regions where the vacuoles are seen.

Formation of the Special Wall

Just previous to the appearance of the hyaline areas in the cytoplasm, a denser, more refractive layer of callose is secreted between the border of the protoplast of the pollen-mother-cell and the callose wall which has already been described (fig. 23-25). This is the beginning of the special wall, described by Strasburger (58). As the cleavage furrows are formed by the fusion of vacuoles the special wall assumes a wedge shaped appearance (in section) at the periphery of the protoplast at the outer border of the equatorial zone (fig. 25). As shallow furrows are formed by the invagination of the plasma membrane, due to the higher surface tension of the protoplast in these regions, the wedge-shaped regions of the developing special wall follow the invaginating plasma membrane, which advances but a short distance before it cuts into the large vacuoles at which time it breaks at the innermost point. The inward movement of the surface furrows and of the special wall is delayed until the furrows formed by the vacuoles are almost completed (fig. 25). After the severing of the strands of cyto-

plasm, and the narrowing of the furrow, the protoplasts of the young microspores secrete between them a homogeneous substance which is shown by its microchemical reactions to be callose. The blunt wedges of the special wall, which up to this time have protruded into the furrows only a short distance, become more sharply triangular as seen in section, and move inward from the periphery by the deposition on their inner surface of the callose which is secreted by the protoplasts (fig. 26). These partition walls advance centripetally until they meet in the center, and even when they come in contact at the center, they are still very narrow and somewhat irregular (fig. 26). By the continued secretion of callose this wall becomes uniformly thickened around the four microspores (fig. 27), finally attaining its maximum thickness as shown in fig. 28. This is the mature special wall which surrounds each microspore. In living material, the tetrad of spores can at this stage be easily teased out and studied in the living condition. When mounted in water the original mother-cell wall can easily be distinguished from the special wall. Although both are homogeneous, the special wall is more refractive and more compact. By firm pressure on the cover glass the microspores with the special wall enclosing them can be

freed from the mother-cell wall as it is much more resistant to pressure. The microspores are more or less loosely enclosed within the special wall, and it is frequently possible to see narrow spaces between the special wall and the microspores. This may also be observed in fixed material (fig. 28) and is evident, even before the protoplast of the mother-cell has divided to form the microspores (fig. 23, 24). Figure 29 shows the special wall and the mother-cell wall from which the microspores have been freed by pressure on the cover glass. By applying a few drops of resorcin blue (lachmoid) solution to living groups of tetrads of microspores, the two walls are easily distinguishable. Both walls are stained a brilliant blue, but the special wall becomes more deeply stained and is more refractive than the mother wall. When fixed material is sectioned and stained with safranin, gentian violet, and orange G, the mother wall is stained pale orange, whereas the special wall is much more deeply orange-stained and has the appearance of being more compact. Beer (1) reports that in Oenothera, septa are developed between the cells of the tetrad, forming an extension of the mother-cell wall. In a later paper (3) he describes and figures this special wall around the cells of the tetrads of Ipomoea, and finds

that it is composed of callose and pectose.

The callose mother-cell wall of Melilotus described above, persists for a short time after the special wall is complete. As the walls of the pollen grains form, however, the mother-cell wall gradually disappears, breaking down into a substance, the chemical nature of which has not been fully determined, although some pectic materials are present. The special wall also now begins to disintegrate, starting at the periphery. In living material, stained with resorcin blue, a remnant of the special callose wall is seen between and extending part way around, the microspores, the rest of the special wall having undergone a chemical and physical change. Eventually this remnant disintegrates and the pollen grains are set free in a semi-liquid matrix. As the callose walls disappear there is a noticeable thickening of the exine of the pollen grains which rapidly enlarge, and in doing so, change from a spherical to an elliptical form (fig. 30). The thickening and enlargement continue until the pollen grains are mature. The exine is completely laid down before the intine begins to form. The mature pollen grain has three longitudinal grooves on its surface which are best seen in cross section (fig. 33). On each groove midway between the ends of the

pollen grain is a pore (fig. 31), which is formed as the exine thickens, by the failure of the deposition of any exine material at this point. The exine stains a deep red when safranin and gentian violet are used, whereas the intine stains violet. In mature pollen grains the nucleus has divided and the generative cell is clearly seen (fig. 31).

The tapetal cells are quite large, usually larger than the pollen-mother-cells, are uninucleate and have dense cytoplasm. There is no evident change in the structure or contents of these cells until the pollen grains are rather well developed and the exine is forming. At this point, the deeply staining nucleus gradually loses its contents, and soon appears to be no denser than the cytoplasm. It is only when the pollen grains are nearly mature that the tapetum is disintegrated and is represented only by occasional fragments of the protoplast seen among the pollen grains.

The Biennial Variety

The foregoing description is based on stages found in the annual variety but it serves equally well as a description of the biennial, for in no case has any difference been found between the two varieties from a cytological standpoint.

There is uniformity in number, size and shape of the chromosomes as well as close conformity in all other cytological behavior, at least so far as spermatogenesis is concerned. That the two varieties are morphologically and physiologically distinct from the standpoint of their habit of growth, there is no doubt. Attempts were made to induce the biennial to flower during the first season by bringing plants into the greenhouse near the end of the growing season. The plants grew very little, and although kept in the greenhouse for more than a year, did not flower. However, when they were allowed to remain outdoors until they had been exposed to freezing weather, and were then taken into the greenhouse, they immediately began to grow, and formed new shoots which flowered in two and one-half months. If the biennial is left in the field during the winter, after its first season of growth, it produces new shoots in the spring, and these shoots flower abundantly. Although the annual, if kept under favorable conditions will continue growth and reproduction for a longer period than it normally does, it is killed by allowing it to remain outdoors over winter.

Thus Melilotus alba has two functionally distinct types which are alike cytologically. Cases in which morph-

ological and physiological differences in plants are accompanied by chromosome differences, as well as cases in which structural and functional differences have no corresponding chromosome changes are of frequent occurrence. Numerous examples of both are found in the lists of plants with their chromosome numbers by Tischler (61) and by Ishikawa (28), and of animals by Harvey (26). The mutations in the genus Oenothera, for example, fall into two groups: (1) those accompanied by change in chromosome number, and (2) those in which no chromosome alteration occurs. These cases are summarized by Overeem (45) for Oe. biennis and by De Vries and Boedijn (10) for Oe. Lamarckiana. Most investigators who have studied the species and varieties in this genus regard them as mutations which are accompanied by and are often the result of a change in chromosome number, or as the result of factor changes. Täckholm (60-61) investigated 157 distinct forms of the genus Rosa. The section Caninae is characterized by the remarkably constant appearance of seven gemini and 14 univalent chromosomes, or 14 gemini and seven univalents, or seven gemini and 28 univalents in the pollen-mother-cells. All other sections of the genus have 7, 14, 21, or 28 gem-

ini and no unpaired chromosomes in meiosis of the species and species hybrids. It is especially interesting to note that in his work, species and related varieties were often found to have different chromosome numbers, whereas other species belonging to widely separated sections were frequently found to have the same chromosome number. In this same connection, Rosenberg (51) working on the genus Crepis found four species with three, eight with four, four with five, one with eight, one with nine and one with twenty chromosomes. Studies on the cytology of different species of wheat show that Triticum monococcum has 14, T. diococcum, T. polonicum, T. durum, and T. turgidum have 28, and T. spelta, T. vulgare and T. compactum have 42 chromosomes. Especially interesting are the results of Collins and Mann (9), who found that a cross between Crepis setosa, which has four pairs of chromosomes, and C. biennis which has twenty pairs, produced vigorous F_1 individuals which show much less irregularity of meiosis and in pollen formation than do the F_1 hybrids of a cross between C. setosa which has four pairs of chromosomes and C. capillaris, which has three pairs. They conclude that normality of reduction does not depend upon similarity of chromosome number, but rather upon likeness of internal composition of chromosomes.

A similar conclusion was reached by Gregory (22), who, as a result of a study of two giant races of Primula sinensis, one of which had twelve pairs of chromosomes, and the other, 24 pairs, is of the opinion that the results obtained throw no light on the relationship between the factors and the chromosomes. Moreover, Jorgensen (31) recently observed that in Callitriche stagnalis, some plants have a haploid number of five whereas other plants have ten as a haploid number, although the two kinds of plants have only very slight morphological differences. These plants show various irregularities in the heterotypic division. Although he considers this species as a composite of more than one species on a cytological basis, it is clear that even so large a difference in chromosome number may not always be associated with corresponding conspicuous morphological differences.

From the consideration of morphological behavior as related to chromosome behavior it is evident that morphological changes may occur without visible changes in chromosomes. Likewise, there may be variability in chromosomes without corresponding morphological changes. The two varieties of Melilotus alba exemplify functional differences with no visible corresponding cytological change, at least

so far as spermatogenesis is concerned, and it seems that any explanation of the morphological difference must go beyond the chromosome as an entity. There is evidently a difference in factors between the two plants and these factors are beyond the realm of cytological investigation.

Abnormal Pollen.

In material which was collected from a field of plants of the annual variety, numerous pollen grains were found, the volume of which was from five to six times that of the normal pollen in the same anther. The abnormal grains have less dense vacuolate cytoplasm and pores are absent on the exine (fig. 37). The pollen-mother-cells which give rise to the abnormal grains are easily identified. At an early stage they stain much less densely than do normal mother cells (fig. 33). This is particularly noticeable in the nucleus, which although of normal size, seems to be greatly lacking in chromatin for the reticulum stains a very dull gray, whereas normal nuclei in the same section are stained deeply. The nucleolus, however, is deeply stained. When diakinesis is reached the chromosomes are stained very feebly, and frequently all that is seen of the chromosome is a membrane like edge with a few feebly stained

granules on the interior. In this, as well as succeeding stages, the protoplast is normally spherical in shape but here the protoplast has failed to round up, and is usually the same shape as that of the mother-cell when in the resting stage, that is, polyhedral (fig. 34). The writer's material shows no mitotic figures of either the heterotypic or the homotypic division. The next stage observed was after the homotypic division had taken place and was represented by numerous mother-cells, each with four nuclei (fig. 34). The appearance of the mother cell at this stage is very different from that of a normal mother cell for the protoplast is still irregular in shape and the nuclei are variously arranged. Rogers (50) studying the biennial variety, reports that one of the four nuclei enlarges and the other three degenerate. The writer's material does not show this condition, for some of the oldest pollen grains in the preparations show four large well organized nuclei (fig. 37), and in no case have any degenerating nuclei been found. Pollen-mother-cells have been found, however, in which several of the nuclei had fused (fig. 39). Occasionally giant pollen grains are found which show the presence of a generative cell (fig. 38). In anthers containing both normal and abnormal pollen, num-

erous normal grains have a generative cell, whereas in all other material which contained only normal grains, the appearance of the generative cell was extremely rare.

Thus, each pollen-mother-cell usually forms only one pollen grain which is very large. Occasionally, however, cross walls are seen at various stages of completion, varying from no cross walls to cases in which complete cross walls are formed. Some mother-cells were seen in which only one cross wall was formed (fig. 36). In others no wall was formed but the elongated pollen grain became somewhat constricted in the middle. In other cases, both cross walls were formed, but the four microspores thus formed remained joined together (fig. 35). Whether or not these large pollen grains are capable of germination, has not been determined.

The occurrence of abnormal pollen grains is not uncommon, especially in hybrids. The cases reported naturally fall into two groups, those in which more than four grains are formed from a single mother-cell, and others in which less than four are formed. Of the former, cases are rather numerous. Wille (66) summarized the work of earlier workers, especially Hofmeister, Tangl, Wimmel, and Tschistiakoff, and added the results of his own extensive invest-

igations. Five microspores were found in Funkia ovata, Ficaria ranunculoides, Stellaria glauca, Scleranthus annus, Prunus cerasus, Rumex patientia, Azalea indica, Lonicera coerulea, Syringa persica, and Symphytum officinale. Six microspores were reported in Hemerocallis fulva, Ficaria ranunculoides, Elatine hexandra, Cornus sanguinea, Lonicera coerulea, and Fuchsia sp. Seven microspores were observed in Fuchsia sp., and a doubtful case of fourteen is reported in the same genus. Azalea indica showed eight, and eight to twelve were found in Lonicera coerulea although their origin in some cases was doubtful. Strasburger (57) found nine coming from a single mother-cell in Hemerocallis fulva, and Juel (32) and Fullmer (17) reported six to eight for the same species. Miss Lyon (37) reports five or six microspores of equal size produced by a single mother-cell in Euphorbia corollata. More recently Beer (3) reinvestigated Fuchsia and found six to ten microspores from a single mother-cell to be of frequent occurrence. The small pollen grains formed are well organized. His study of the nuclear divisions led him to conclude that the numbers are the result of irregularities in chromosome distribution during the anaphase.

Tischler (63) made a careful investigation of three races of Musa sapientum, the edible banana, and found that each of the three races has a different haploid chromosome number, 8, 16, and 24. He found a close correlation between chromosome number and the volume of the pollen grain for, whereas the chromosome numbers ran 8, 16, and 24, the volume of the pollen grains is in the ratio 1:2:3. In pollen-mother-cells having the increased numbers there were irregularities in pollen formation, to the extent that as many as eight pollen grains were sometimes formed from a single mother-cell. Jeffrey, Longley, and Penland (29) investigating known hybrids or species, concluded that polyploidy is a common result of incompatible species crosses. This is frequently accompanied by the formation of four normal and several small abortive pollen grains from a single mother-cell, due to the irregular behavior of the chromosomes during meiosis. Very recently Longley (36) carefully studied the genus Rubus cytologically. The diploid species are regular in meiosis and in pollen formation, which fact he considers as evidence of pure species. The polyploid species show much irregularity in chromosome distribution and pollen formation frequently resulting in polyspory. Since these

forms show such similarity in irregularity to those found in known hybrids, he considers the group to be made up of hybrid species and forms. Jorgensen (31) investigated the genus Callitriche and observed that in C. stagnalis, the heterotypic division is irregular to the extent that micronuclei are sometimes formed, resulting eventually in the formation of some small pollen grains which disintegrate at an early stage. As many as eight pollen grains may be formed from a single mother-cell.

Cases in which less than the regular number of pollen grains is formed from a mother-cell are fewer. Wille (67) reports the occasional occurrence of two from a mother-cell in Convallaria multiflora, Asparagus officinalis, Aconitum napellus, Euphorbia lathyrus, Begonia sp., Saxifraga caespitosa, Azalea indica and Syringa vulgaris. Three microspores were reported for Saxifraga caespitosa, Azalea indica, and Lonicera coerulea. Collins and Mann (9) found that in F_1 plants of a cross between Crepis setosa which has four pairs of chromosomes and was used as the pistillate parent, and C. capillaris which has three pairs of chromosomes, various irregularities occur in pollen formation. A large number of the tetrads are normal although in many cases only two or three micros-

pores are formed. In a smaller number of cases, five or six microspores are formed from a mother-cell, the size of the microspores corresponding to the size of the enclosed nucleus. Gates (18) noted erratic behavior in the formation of the pollen grains in Oenothera gigas, a mutant, in which accessory lobes are sometimes formed on the pollen grains. Sterility of the pollen was quite common.

Instances in which only one microspore results from a mother-cell are very rare. Elfving (11), Wille (67) and Strasburger (56) found in several species of the Cyperaceae that only one functioning microspore is formed from a mother cell as the other three members of the tetrad disintegrate. Juel (33) observed in Carex acuta that in meiosis, each division is followed by the formation of a cell plate. These plates are resorbed and the four nuclei lie free within the mother-cell wall, which becomes the microspore wall. Three of the four nuclei then degenerate.

The formation of abnormal pollen is often associated with hybridity, although hybridity cannot be considered as the sole cause of pollen abnormality or sterility. In Melilotus it seems as though the giant pollen grains are the result of hybridity, and the writer is inclined to

think that it is due to hybridity between the annual and biennial varieties, for plants have been found in fields of the annual variety which had the appearance of being natural hybrids, for they possessed both annual and biennial characters. In some of the flowers of these plants giant pollen grains have been found and this occurrence of giant pollen grains in material which seems to be hybrid between the two varieties suggests the explanation of the cause of the giant pollen grains.

Discussion.

That the two varieties of Melilotus alba are distinct is evidenced by their different functional activity which results in their different habit of growth. That the two varieties are distinct is shown by the fact that it is impossible to force the biennial to flower by providing conditions suitable for its continued growth at the end of the growing season. The annual variety, as all other annuals, continues normal growth and reproduction if suitable conditions are provided as the unfavorable season approaches.

Attention has already been called to the fact that from an examination of published lists of chromosomes in plants and animals it is evident that the number of chromo-

somes shown by the species of a genus is often variable. The chromosomes are often in multiples, but occasionally species differ by only one or two pairs of chromosomes. On the other hand, these chromosome lists show that there are species of a genus which, although morphologically distinct, have an identical chromosome number. In Oenothera which has already been mentioned, although some species and varieties show different chromosome numbers, there are others which have an identical number. It is evident, therefore, that there are two categories of behavior with reference to chromosomes and morphology. In the first, morphological difference is associated with chromosome difference. In the second, morphological difference in species or varieties is unaccompanied by visible chromosome variation. Since the chromosomes are regarded as the structures in which plant or animal character determining factors reside, and since no chromosome differences are evident in many cases where morphological differences exist, it is logical to conclude that these morphological differences are brought about and are accompanied by changes within the chromosome itself. The two varieties of Melilotus alba would differ, therefore, not only in

functional characters, but in character determining factors within the chromosomes. Jeffrey (30) is of the opinion that the origin of new species is to be attirbuted to hybridization between species, although he says that it is "impossible to regard hybridization as the universal and sole cause of the appearance of new species." Other workers, of whom the most outstanding is De Vries (64), regard mutation as the cause of new species. It is generally conceded that the annual variety of Melilotus alba has originated from the biennial variety, but as to whether its origin is to be attributed to hybridization of the biennial variety with another species or to mutation within the biennial itself, the writer's investigations have thus far not produced evidence to draw any conclusions.

The furrowing process in Melilotus is especially noteworthy for the formation of the furrows by vacuolization has not been previously described in the formation of microspores. From a careful study of the process it seems evident that there is a movement of granular cytoplasmic material toward the nuclei from the regions of the protoplast, equidistant between the nuclei. Thus, hyaline

areas are formed and there is a higher surface tension in the vicinity of these areas than around the nuclei. There is an extrusion of liquid into vacuoles which soon fuse, eventually forming rows of large vacuoles which would be continuous furrows, but for the few strands of cytoplasm between them. The furrows which progress inward from the periphery until they meet the vacuoles, are the result of the higher surface tension in these regions. The furrowing process may not be due to the same causes in all organisms, but from the study of the writer's material and from a study of the careful work of Bütschli, McClendon, Spek, Chambers, and Kite, the writer would conclude that the furrows which proceed from the periphery of the protoplast are to be attributed to the higher surface tension in the equatorial regions, whereas the cleavage of the cytoplasm in the deeper regions of the cell is caused by the extrusion of liquid into vacuoles which fuse forming furrows.

The occasional formation of but a single pollen grain from each mother-cell is quite unusual, and the only cases reported are those for the Cyperaceae which have been mentioned. Melilotus differs from Carex, described by Juel, in that a new wall is formed around the single microspore whereas in Carex the old mother-cell wall becomes the mic-

rospore wall directly. Strasburger (56) and Juel (32) found in Hemerocallis fulva that during the heterotypic division one or more chromosomes failed to pass to either pole and that these chromosomes gave rise to small supernumerary microspores, although Fullmer (17) considers these small microspores as due to the division of the members of the tetrads. The writer has not observed the meiotic division in Melilotus but both divisions occur, for in the abnormal pollen grains four nuclei may be seen. Although there may be irregularities in the heterotypic or homotypic divisions in the mother-cell, irregularities certainly exist previous to these divisions, as evidenced by the appearance of the mother-cells while in the resting stage, for they are then very abnormal in appearance, particularly so in being almost devoid of chromatin. Abnormality occurs, therefore, further back in the life history of the plant than the meiotic division. The writer is inclined to regard this abnormality as due to hybridity between the annual and the biennial varieties. Further evidence that irregularity in pollen grain formation is due to hybridity has been given by Jeffrey (30) for a number of plants in which it seems that pollen abnormalities are unquestionably due to hybridity, particularly in species

of Potamogeton, Rubus, and Ranunculus, and Longley (36), from his investigations on the genus Rubus, concludes that "multiplication of species in this genus has taken place by hybridization in their natural habitats."

Conclusions.

The evidence secured from a careful study of the cytological phenomena in spermatogenesis in the annual and biennial varieties of Melilotus alba indicates that the two varieties are identical cytologically, although they are functionally distinct. The evidence at hand warrants no conclusion as to the exact method of origin of the annual variety.

The development of the anther is similar to the usual method of anther development, with the exception that the pollen-mother-cells in each anther lobe are derived from a single row of cells of the primary sporogenous layer rather than from the whole layer.

In both varieties the haploid number of chromosomes is eight, the diploid sixteen.

The daughter nuclei at the end of the heterotypic division are completely reorganized before the homotypic division is initiated.

Quadripartition of the pollen-mother-cell is effected by means of furrows which are formed largely by a system

of vacuoles which are met by ingrowing surface furrows, advancing centripetally only a short distance before cutting into the vacuoles. The process is not unlike that described for certain fungi. There is at no time any evidence of the formation of a cell plate across the equator of the spindles.

During the early stages of nuclear development a massive callose wall is secreted by the protoplast of the pollen-mother-cell. As cleavage is initiated, the <u>special wall</u>, composed of dense refractive callose, is secreted around the protoplast just inside of the first callose wall. As the cleavage furrows are nearing completion, the <u>special wall</u> advances centripetally with the furrows which soon cut into the vacuoles. Partitions are formed between the young microspores which are continuations of the incoming callose <u>special wall</u>. These partitions are formed by the deposition of a callose secretion of the protoplasts on the surface of this incoming wall.

Giant pollen grains each containing four nuclei, are found in both varieties, each pollen grain being the entire product of a single mother-cell in which walls have failed to come in to form the tetrad.

The writer wishes to express his appreciation to Dr.

J. N. Martin, under whose direction the work was carried on, and to whom he is greatly indebted for advise and criticism, and to Dr. E. J. Lindstrom for valuable suggestions during the course of the investigation.

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EXPLANATION OF FIGURES.

All figures highly magnified but not drawn to the same scale.

Fig. 1.- Transverse section of anther lobe showing the archesporium. x 450.

Fig. 2.- Transverse section of anther lobe showing the primary parietal and the primary sporogenous layer. x 450.

Fig. 3.- Transverse section of anther lobe. The sporogenous cell, which is to give rise to the pollen-mother-cells, is easily distinguished. x 450.

Fig. 4.- Longitudinal view of the sporogenous row, a single cell of which is seen in fig. 3. x 450.

Fig. 5.- Transverse view of anther lobe. The sporogenous cell seen in fig. 3. has divided. x 450.

Fig. 6.- Group of pollen-mother-cells in anther lobe. Transverse view. x 750.

Fig. 7.- Median section of nucleus in resting stage. x 1500.

Fig. 8.- Leptonema stage - median section. Nucleolus differentiated into two regions. x 1500.

Fig. 9.- Synizesis stage. Chromatin massed at one

side of the nucleus. Nucleolus shows a bud being constricted. Callose mother-cell wall being secreted at corners of the protoplast. x 1400.

Fig. 10.- The anastomosing threads are seen as the nucleus comes out of synizesis. The callose mother-cell partly formed. x 1400.

Fig. 11.- Pachynema stage. Threads much thicker. Nucleus is still seen. Callose mother-cell wall fully formed. x 1400.

Fig. 12.- Chromatin thread has broken up into a number of irregular pieces. x 1400.

Fig. 13.- Median view of nucleus at diakinesis stage, showing eight bivalent chromosomes and the nucleolus which is differentiated into the two regions. x 1400.

Fig. 14.- Side view of heterotypic metaphase, showing the chromosomes. x 1500.

Fig. 15.- Polar view of the heterotypic metaphase, showing the eight bivalent chromosomes, which are easily seen to be double. x 1300.

Fig. 16.- Side view of heterotypic anaphase. Chromosomes closely massed together. Part of the callose mother-cell wall is shown. x 1400.

Fig. 17.- Side view of heterotypic telophase. Chromosomes distinct and some are seen to be partly split. Eight univalent chromosomes are easily seen in the lower nucleus. Two centrosome-like bodies are seen. x 1500.

Fig. 18.- Nucleus of late heterotypic telophase. Chromosomes have fused. x 1600.

Fig. 19.- Resting stage of nucleus at end of heterotypic division. Nucleus completely reorganized. x 1700.

Fig. 20.- Median section of nucleus just previous to the initiation of the homotypic division. The chromosomes are split at the ends. Nucleolus is still evident. x 1700.

Fig. 21.- Median section of homotypic metaphase. Polar view of one spindle shows eight chromosomes. Spindles are nearly at right angles. x 1500.

Fig. 22.- Telophase of homotypic division. Three of the four nuclei are seen. In one nucleus the eight chromosomes can easily be counted. Three of the four centrosome-like bodies are shown. x 1500.

Fig. 23.- Nuclei in resting stage. Spindles have almost disappeared. Protoplast has begun to invaginate at the periphery, equidistant from the nuclei. First evid-

ence of special wall shown around the protoplast. Outside of this a part of the mother-cell wall is shown. x 1500.

Fig. 24.- Vacuoles have been formed in rows equidistant from the nuclei by the fusion of smaller vacuoles. Shallow furrow evident at top of figure. Many of the original small vacuoles are seen. x 1500.

Fig. 25.- Large vacuoles formed by fusion of smaller ones. Only a few strands of cytoplasm connect the future microspores. Cytoplasm has become denser around the nuclei. Special wall has thickened slightly. x 1500.

Fig. 26.- The few connecting strands of cytoplasm seen in fig. 25 have been severed, forming continuous furrows. Special wall has advanced into the furrows. Many small vacuoles are seen. x 1500.

Fig. 27.- Special walls have met in center, forming partition walls between the microspores. Special wall thickens. Mother-cell wall shown in part. x 1500.

Fig. 28.- Special wall complete. Cytoplasm of microspores more compact. Part of mother-cell wall shown. x 1500.

Fig. 29.- View of callose mother-cell wall and special wall drawn from living material. x 400.

Fig. 30.- Young pollen grain. Exine just forming. Two pores are shown. x 1200.

Fig. 31.- Mature pollen grain showing thick exine and generative cell. x 1000.

Fig. 32.- Transverse section of mature pollen grain. The three grooves are evident. Intine also seen. x 1000.

Figures 33-39 are not drawn in proportion to actual size as compared with the other figures. These figures represent abnormal stages.

Fig. 33.- Abnormal pollen-mother-cell which is to give rise to a giant pollen grain. Scant amount of chromatin in nucleus. x 800.

Fig. 34.- Abnormal pollen-mother-cell at end of homotypic division. Three of the four nuclei shown. Protoplast has failed to round up. x 700.

Fig. 35.- Tetrad from abnormal mother-cell. Microspores have failed to separate. The wall between them is exine. x 300.

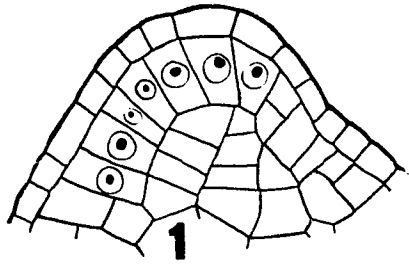
Fig. 36.- Pollen-mother-cell has formed only two microspores by a single cross wall. x 300.

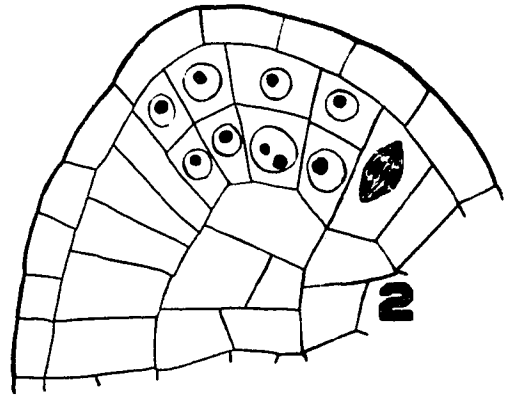
Fig. 37.- Giant pollen grain containing four nuclei. No pores in exine. x 300.

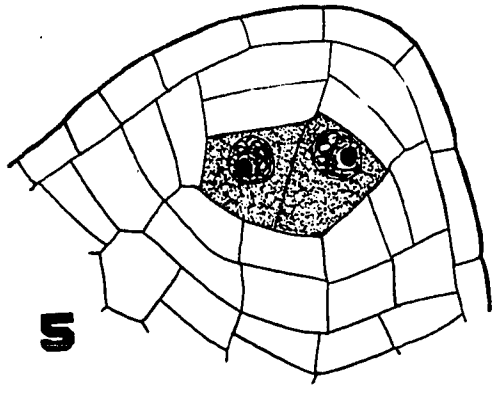
Fig. 38.- Giant pollen grain with generative cell. x 300.

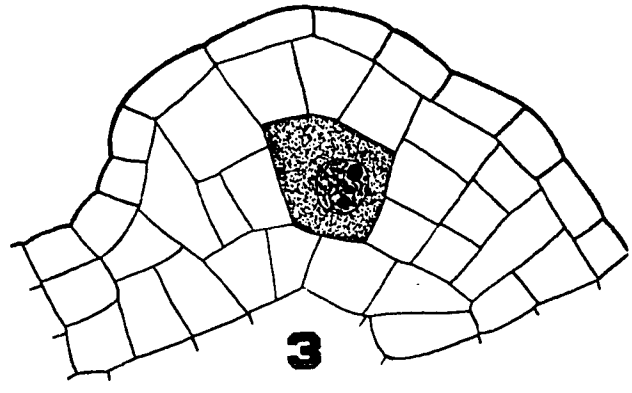
Fig. 39.- Giant pollen grain in which the four nuclei have fused in pairs. x 300.

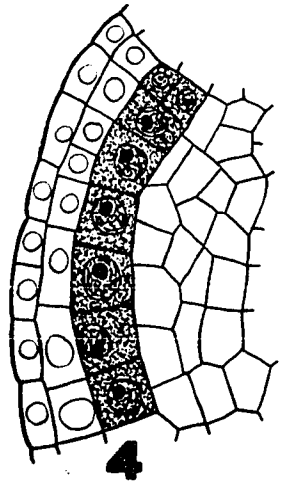
PLATE I.

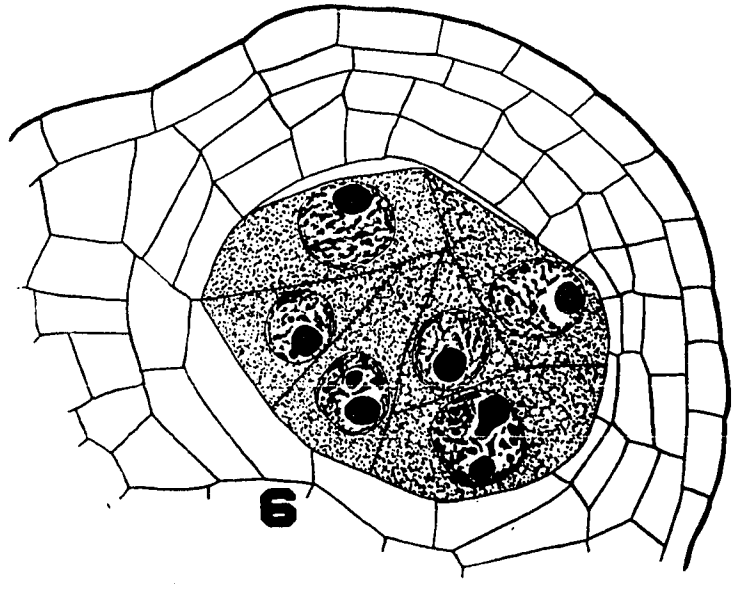












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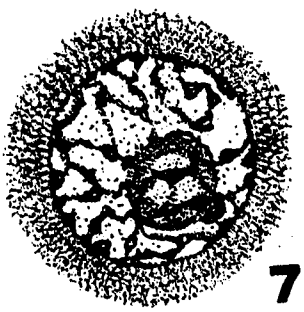
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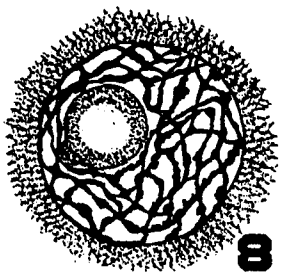
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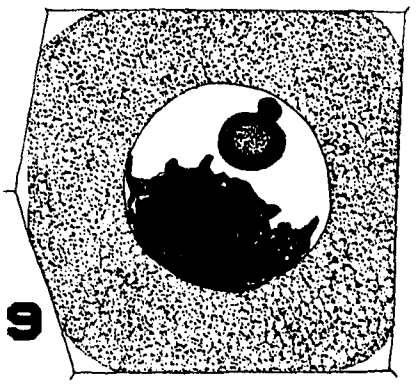
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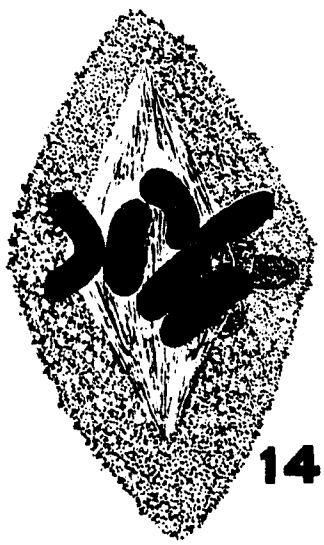
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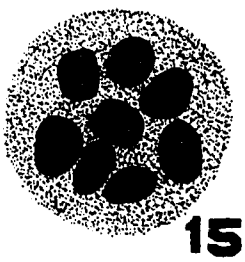
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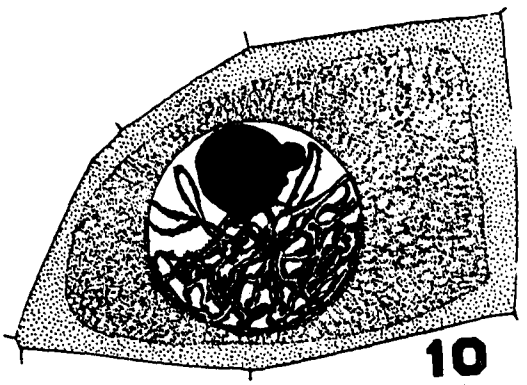
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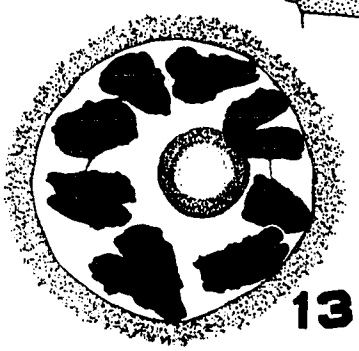
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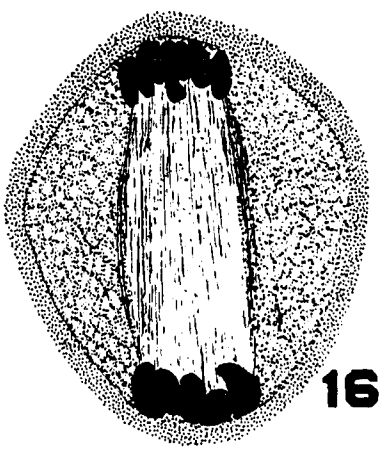
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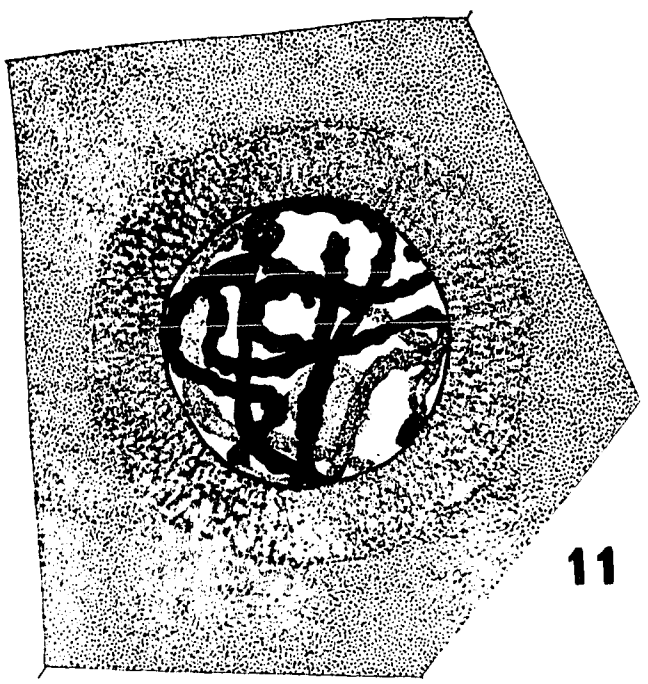
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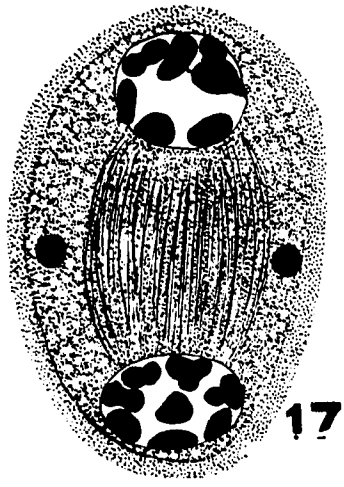
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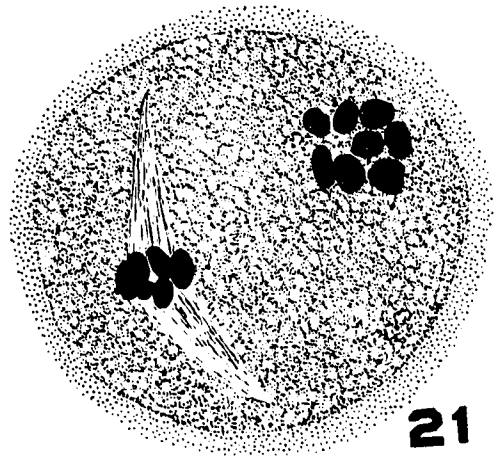
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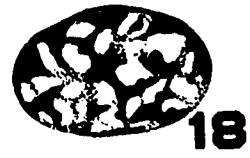
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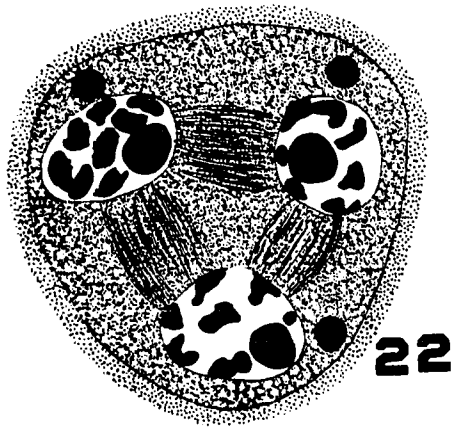
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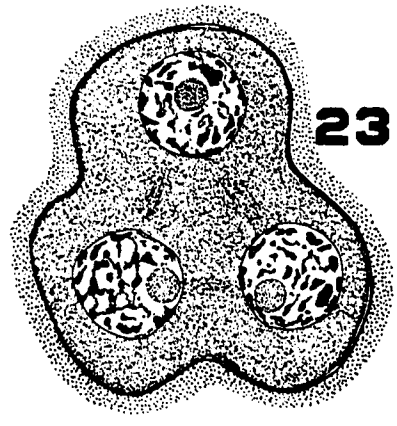
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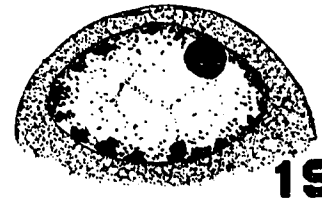
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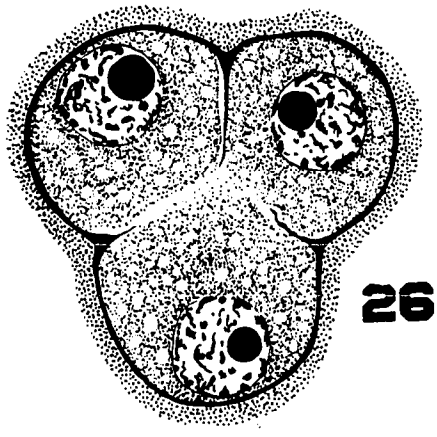
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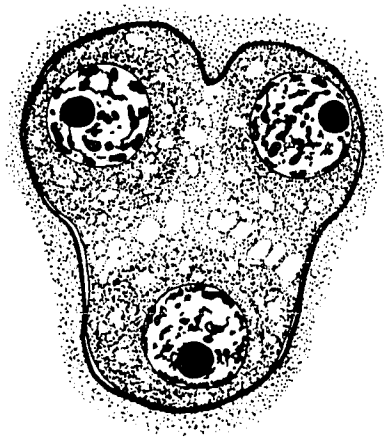
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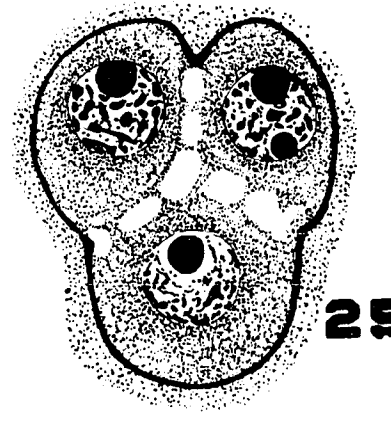
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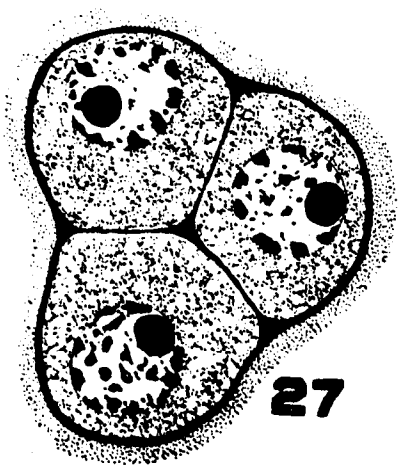
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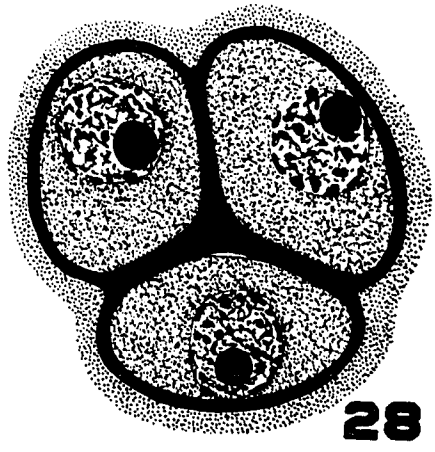
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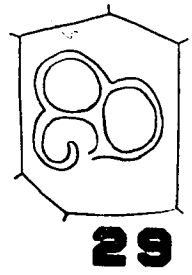
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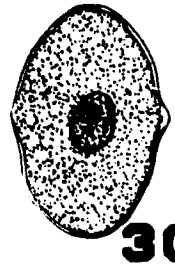
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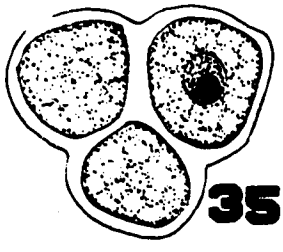
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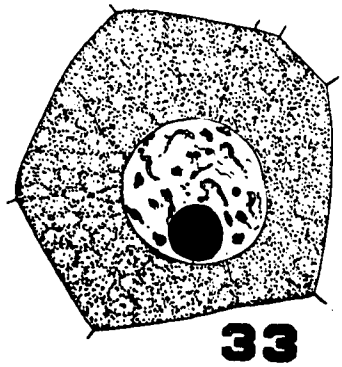
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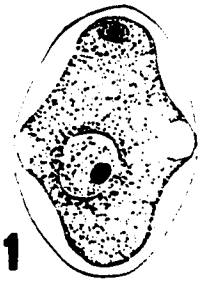
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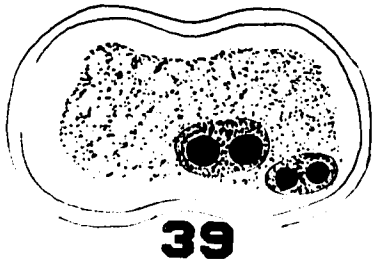
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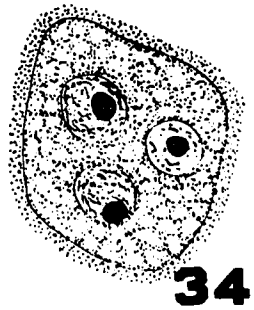
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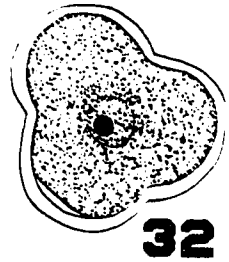
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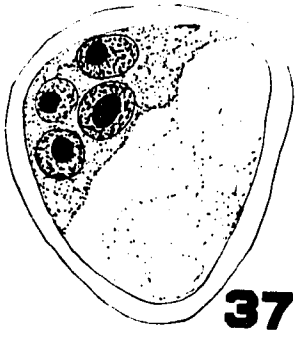
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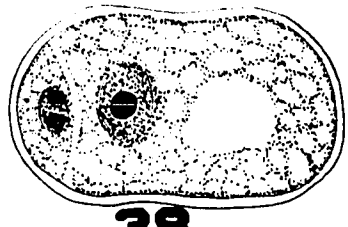
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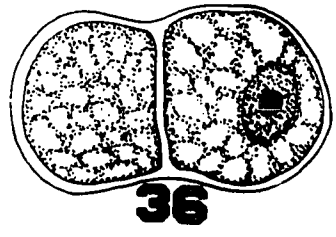
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