# A CYTOLOGICAL STUDY OF SPORORMIA OBLIQUISEPTATA SPEG.

by

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# INTRODUCTION

In recent years, with the use of the more advanced techniques employed in studying the nuclear cytology of higher organisms, knowledge of nuclear behaviour in the fungi has increased rapidly. Not only are such studies of value in providing adequate knowledge of the nuclear structure and chromosome behaviour of the nuclei in these organisms, which may be of importance to taxonomy, but also such studies are of invaluable aid to the numerous investigators of genetical problems within this group.

The Ascomycetes, in particular, have provided exceptionally good experimental material for cytological and genetical investigation, and recently a number of these species have been studied intensively. The results of these investigations are providing an explanation for various perplexing phenomena. For example, certain aspects of sexuality, particularly the varying manifestations of homothallism and heterothallism, as well as variability within a species and other genetical phenomena can now be explained in greater detail.

A major factor in the success of these studies in the Ascomycetes has been the suitability for cytological investigation of the large fusion nucleus in the ascus and its series of divisions, which can be followed in detail to the formation of the eight ascospores. The fact that the fundamental laws of inheritance of higher organisms are applicable to the fungi studied makes the ascospores important tools in genetic research.

Some of the better known examples of fungi studied both cytologically and genetically occur in the genus Neurospora, belonging to the Sordariaceae, and first investigated by Dodge (1927). The recent works of

McClintock (1945) and Singleton (1953) give an impressive account of nuclear divisions and chromosome morphology in N. crassa Shear and Dodge.

McClintock (1945) and Singleton (1953) made a detailed study of pachytene chromosomes in the primary nucleus and were able to attempt the construction of a chromomere map of individual chromosomes so that each chromosome and its segments might be recognized. They also determined the position of spindles during nuclear division in the ascus, since relative position of ascospores within the ascus is determined largely by spindle orientation. Such cytological information was necessary for the genetical studies of the fungus by workers such as Beadle and Tatum (1945), who are concerned with inducing and detecting mutations in this fungus. It is possible that persistent study along these lines will result in the locating of segments in the chromosomes associated with certain cultural characteristics of the fungus.

Another species of this family has been a useful source of information. The genetical work of Ames (1934) explained the homothallism of the binucleate ascospores of Sordaria anserina (Ces.) Wint. By culturing the two uninucleate ascospores which are occasionally found in place of the single binucleate one, he was able to show that the homothallism of this fungus was due to two hermaphroditic strains, which are self-sterile but which are reciprocally fertile.

Hansen and Snyder (1943), (1946) made some interesting genetical studies on Hypomyces solani f. cucurbitae S. and H. which belongs to the Hypocreaceae and is pathogenic on squash. This fungus was found to have male and female, hermaphroditic and neuter thalli, and these were used in crosses. They concluded from their results that the factors for female and male were

not alleles, but were located at different loci in homologous chromosomes. Crossing over between these two loci would account for the origin of hermaphrodites and neuters. Hirsch (1949) made a cytological study of the fungus and concluded from her results that the various sex differences were due to differences in chromosome numbers. The hermaphrodites and neuters arise from the cross, female by male, as a result of occasional non-disjunction of the two sex chromosomes. This explanation by Hirsch was rejected by El-Ani (1954) since it could not account for certain genetical ratios which he obtained in his crosses with the fungus. He suggested that the previous explanation given by Snyder and Hansen (1946) should be re-considered and the cytology of the fungus be reinvestigated.

Members of the Pseudosphaeriaceae have also been found amenable to cytological studies. Jones (1926) gave some excellent figures of nuclear divisions in the asci of Ophiobolus graminis Sacc.; Backus and Keitt (1940) have given details of the cytology of Endostigme inaequalis (Cke.) Syd. (= Venturia inaequalis (Cke.) Wint.); and Lucas (1946) and Wheeler et al. (1948) showed details of nuclear division and determined the chromosome number in Glomerella. The variability and genetical behaviour of the latter two fungi is now undergoing extensive study.

Sporormia obliquiseptata Speg. is another member of this family, which has proved to be a valuable source of experimental material, and it is proposed in this investigation to give details of crozier development and the various nuclear divisions which accompany the development of the ascus and the production of ascospores.

## MATERIALS AND METHODS

### Cultural Methods

The isolate of Sporormia obliquiseptata used in this study was from horse dung collected in Montreal in the fall of 1953. Large numbers of beaked pseudothecia were found partially embedded in the straw contained in the dung in the manner characteristic of this genus.

Before a cytological investigation could be attempted, it was necessary to reestablish the fungus on a suitable substratum so that an ample amount of young fruit bodies would be available. A small portion of the dung containing the fructifications of Sporormia was transferred to moist, sterilized horse dung kept in a moist chamber, in an attempt to get a fresh culture of the fungus growing. Because of the various contaminants, such as Sordaria and fast-growing Phycomycetes, present in the inoculum, the Sporormia failed to get established.

Only bacterial colonies grew when squashed pseudothecia were washed in water and planted on dung agar. At this time it was not known that washing the fruit bodies in dilute sodium hypochlorite solution (commercial "Javex", etc.) would have killed any bacteria present. This solution also has the effect of promoting germination which will be described under the section "Germination".

The difficulty in getting a culture of this species to grow was finally overcome when discharged ascospores of Sporormia were found on the lid of a Petri dish in which a small portion of dung was kept moist. These spores would not germinate when planted on malt agar but a few germinated when treated chemically. This treatment presumably softens the wall of the ascospore and allows water to penetrate the spore.

Germination

Ascospores were soaked in a 0.5% to 2% solution of commercial "Javex" (Beadle and Tatum, 1945) for one to two minutes. The strength of this commercial product is determined by a titration method using sodium thiosulphate. The weaker solution killed the bacteria, while the stronger solution both killed the bacteria and stimulated germination in some of the spores. Two other methods were found successful in promoting germination but failed to control bacterial contamination. These were allowing the spores to soak for ten minutes in 0.5% sodium hydroxide, and one to several hours in 0.5% - 1% solution of sodium acetate. The first method was recommended by Miss Chuan-Chang Yu (1954) for spores of Ascobolus, and the second was based on the method recommended by Bretzloff (1954) for spores of Sordaria. Washing the spores in a weak solution of sodium hypochlorite, and then soaking them for several days in water also improved germination.

The percentage of spore germination in this species of Sporormia was found to be exceedingly small, not more than one or two per cent using the methods described above, with the exception of the last method in which percentage germination was not determined.

Culture media

Unlike Sordaria fimicola (Bretzloff, 1954) which requires a special synthetic media with added biotin, Sporormia obliquiseptata fruits well on malt extract, potato dextrose and oatmeal agars, and probably on other media which are similar to these. Most of the fruit bodies used in this study were grown on oatmeal agar, since on this substrate the fungus grows rapidly and produces a great abundance of large fruiting

bodies in a compact layer over the agar. Sterilized horse dung inoculated with mycelium from an agar culture also furnished good cytological material. Cultures from both multiple and single spore isolations were used in this study.

Cytological Methods:

The stain used in this investigation was 65% acetocarmine. It was made by refluxing a mixture of 65 cc. acetic acid, 35 cc. water and an excess of one gram of carmine stain for one hour. To darken the stain, an iron needle was added to the reflux mixture thereby introducing a black precipitate, which darkens the chromosomes appreciably.

Fixation

Since this stain has fixing as well as staining properties, it was used alone at first. However, it was found that prefixation with a three to one mixture of 95% ethyl alcohol and glacial acetic acid at 60°C. for fifteen minutes gave better results in the stained material. The chromosomes stained more quickly and with greater density and also the karyolymph inside the nuclear membrane did not take up the stain so readily and remained clear for some time. As the stained material aged, however, the stain tended to penetrate this clear area and loss of differentiation resulted. Another advantage of fixation was that it helped to distinguish spindle fibers, centrioles and asters.

Pseudothecia were transferred from the culture to a large drop of fixative on a clean slide, and a cover glass placed over them. More of the fixative was added under the cover glass with an eye dropper, and the contents of the pseudothecia were expressed by applying pressure to the cover glass with a firm needle. These bodies with their contents

still adhering were picked up with a fine needle (a dental broach was used) and transferred quickly to a glass vial containing fixative. The material remaining on the slide was prevented from drying out by frequent additions of fixative to the pseudothecia on the slide.

The vial was corked and suspended in water at 60°C. for 15 min. with precautions being taken to prevent any water seeping into the vial. The hot fixative was then poured off quickly and replaced with cold fixative. The material was stained immediately or left in the fixative for several days. In fact, the cytoplasm of the material left in the fixative over this period of time seemed to be less granular than that freshly-fixed.

Mordanting

Toward the completion of this investigation, a method of mordanting with 4% iron alum to give a darker stain in the chromosomes was tried (Fincham, 1949) using the iron-aceto-carmine stain. The fixed material was placed for 10 min. in a vial containing mordant, the mordant was then poured off and replaced quickly with distilled water, and staining followed. This procedure was found to give good results, especially of the late prophase chromosomes of the fusion nucleus, which had been difficult to stain.

Staining

Staining was accomplished in the following manner. The fixed, or fixed and mordanted, pseudothecia were picked up with a dental broach and placed in a large drop of stain on a clean slide. The expressed contents were freed from the remnants of the pseudothecia and the latter discarded. A cover slip was placed over the drop of stain carefully to

prevent the material from escaping from under the cover slip. The slide was then placed on a heated Hamly microscope lamp to warm. This type of lamp was found suitable to this purpose because of its flat top, and because the heat generated by it after a few minutes was found to be the right amount for activation of the stain. The slide was heated thus for several minutes during which time it was necessary to wipe away the dried stain from the edges of the cover slip and introduce fresh stain under it. The slide was then pressed between layers of absorbent paper to spread the material and remove excess stain. A paraffin-gum mastic preparation was used to seal the edges of the cover slip.

Some stages of division were seen almost immediately after staining, but in others the intensification of the stain improved with standing for several days.

Microscopy

A Spencer microscope fitted with N.A. 130 condenser and 90x, N.A. 130 apochromatic objective, and both 10x and 15 oculars was used. A No. 58 Wratten filter helped to increase contrast in some of the photographs. Photographs were taken on Eastman Contrast Process Panchromatic film using an E. Leitz Wetzlar "Makam" camera. The film was developed in Eastman D 11 developer and printed on Velox and Kodabromide papers.

The nomenclature of the fungi mentioned in this study was taken from the classification given by Gäumann (1952).

## OBSERVATIONS

### Cultural Observations

On oatmeal agar and sterilized dung, mature pseudothecia of S. obliquiseptata developed in about three weeks' time. They are globose, black in colour, with smooth walls. Ordinarily each pseudothecium has a single, short neck possessing an ostiole through which the ascospores are forcibly discharged when ripe. Quite commonly, however, several necks are present especially when produced on oatmeal agar.

The spores when mature are cylindrical-clavate, four-celled, deeply constricted, black in colour and with a gelatinous sheath. Eight are produced in each ascus, and they are either uniseriate or biseriate.

As the fructifications on oatmeal agar mature in more or less radial progression, it is possible to select a fruit body at the desired stage from its position on the plate.

The earliest stage of fructification seen was simply a row of enlarged hyphal cells as illustrated for Sporormia leporina Niessl. by Arnold (1928) and for S. bipartis Cain by Page (1939). The next stage on which observations were made was the crozier stage in the young fruit body. These croziers were quite numerous and were scattered throughout the variously shaped hyphal cells found in the interior of the fruit body. From the croziers, asci were formed in the usual way, and in cultures two to three weeks old, delimitation of the ascospore initials was taking place. Most fruit bodies at three weeks old contained all stages from croziers to mature ascospores. These fruit bodies were the most suitable

cytological material. Pseudothecia examined from cultures four weeks and older were found to contain mostly mature spores.

Monosporous cultures

The four segments of the ascospore were easily separable and it was found that each segment germinated independently to produce a fertile mycelium. S. obliquiseptata is therefore homothallic. The same condition was found in S. bipartis and S. intermedia by Miss Page (1939), who described S. bipartis as having an 8-celled ascospore with two nuclei to each cell, and S. intermedia having four cells in the ascospore, with four nuclei per cell. At various times in this study, the cells of S. obliquiseptata were seen to contain four nuclei, and it is possible that the cells of the mature spores always contain this number. The difficulty encountered here is that at this stage the walls are too dark to reveal clearly the cytological details within the spore.

In one culture recently examined, minute conidia were found which were produced on slender phialides. The phialides were not numerous and could not be found in a later examination of the culture. Arnold (1928) reported spermagonia and spermatia in S. leporina. The spermagonia were described as perithecium-like arising on rhizomorphs, and the spermatia as small and developing in a gelatinous mass in the interior of the spermagonium. From his description it would appear that the mode of formation of the spermatia (or conidia) are not the same in S. leporina and S. obliquiseptata.

Cytological Observations

The various stages in the formation of the crozier can be followed with almost diagrammatic clarity in this species of Sporormia. The hook formation is very conspicuous, the nuclei stain well at this stage and the septations which follow the second nuclear division are clearly seen.

In Fig. 1, the resting stage of the nuclei in the hook are seen. In Fig. 2, the two sets of chromosomes present in the hook are presumably at early metaphase. The chromosomes are difficult to count at this stage because of their small size and rather compact arrangement. From this two-nucleate stage, there follows the conjugate division, in which both nuclei undergo division simultaneously to give the four-nucleate stage seen in Fig. 3. In Fig. 4, only three of the nuclei are in focus, and the chromosomes are seen in the arc arrangement commonly seen at conjugate division. A similar configuration to this was often seen in the smaller nuclei of the later divisions of the ascospores.

A septum appears cutting off the tip cell with its single nucleus (Fig. 5). A second septum forms cutting off a cell at the bend of the hook, called the penultimate cell. This septum is clearly seen in Fig. 6. Two nuclei are now present in the penultimate cell, and one in the basal cell below the septum. The tip cell bends around and fuses with the basal cell, to complete crozier formation (Fig. 6).

At this time, the penultimate cell is beginning to elongate upward as shown in Figs. 7 and 9, the extension thus formed to become

the ascus, or to be the early stage of another crozier. Crozier proliferation takes place frequently in this species of Sporomia (Fig. 7).

Nucleoli again form after the completion of the crozier (Figs. 7 and 9). In one case the nuclei of the crozier were found at different stages. The two basal cell nuclei were at metaphase and the two in the young ascus had nucleoli present (Fig. 8). It is thought that at this stage the basal cell was preparing to proliferate a second crozier laterally.

Fusion takes place sometimes in the penultimate cell before ascus elongation, and sometimes in the young ascus. In Fig. 10 is shown a young ascus before fusion has taken place. In Fig. 11 on the right can be seen the fusion nucleus which is identified by its size, lying at the base of the ascus which has attained a fair length. In Fig. 12 can be seen an ascus of similar size containing a fusion nucleus of the same proportions. Both Figs. 11 and 12 were taken from the same fruit body, whereas Fig. 10 with its large nucleoli was from other material. It was also found that croziers could be quite different in size although at the same stage.

It is thought that in Sporormia obliquiseptata the separate nucleoli disappear and a new fusion nucleolus forms. A few stages were seen in which the chromosomes were arranged in a network and were much thickened, and at such times no nucleolus was seen. Fig. 13 shows such a configuration, although here fusion has apparently been greatly delayed since the ascus has reached fairly large proportions. For the most part such a stage was seen about the time fusion might be expected to occur.

Nuclear enlargement accompanies elongation of the ascus. A typical nucleus in a fairly young ascus can be seen clearly in Fig. 14. It is a well defined structure, more or less globose in shape. In the centre of the nucleus is a conspicuous round nucleolus which usually looks homogeneous and darkly stained. Scattered around the nucleolus in a clearly defined karyolymph is a network of chromatin material. In a more mature ascus, the nucleolus occupies about half the width of the ascus (Fig. 15). Occasionally, the nucleolus has a doughnut-shaped appearance due to the fact that the outer part of the nucleolus stains more deeply than the interior (Fig. 16, ascus to the right). This effect has been seen in the nucleoli of the young as well as in the mature asci, and has also been seen in the nucleoli of the ascospores.

The nucleolar chromosome may sometimes be seen attached to the nucleolus (Figs. 16 and 17). The small, darkly stained area seen to one side of the nucleolus and attached to a chromosome is believed to be the nucleolus organizer.

Before the ascus reaches its full size the nucleus, which is usually situated about the centre of the ascus, begins to undergo noticeable alterations. The nucleolus becomes smaller and finally breaks up into one to several fragments before it disappears altogether (Figs. 18 to 22). At the stage where several nucleolar fragments are present, only one of them is found to be attached to a chromosomal strand.

During nucleolar disintegration, the chromosomes become attenuated (Figs. 16 to 19). Pairing of chromosomes is evident in Figs. 19 to

22. Chiasmata can then be seen (Fig. 22). The chromosomes thereafter become shorter and thicker, and stain unevenly (Fig. 23). Distinct chromatic granules, the significance of which is not understood, are present along the chromosomes in Fig. 24. This stage is very rare and was seen only twice at first division. A similar stage was seen during the second division, but the granules were farther apart. In Fig. 25, the chromosomes have lost much of their sinuous nature and become almost rod-shaped. The double nature of the chromosomes is not apparent. Fig. 26 probably represents a stage of metaphase prior to the chromosomes assembling on the equatorial plate. Counts taken at these stages indicate that there are about seven or eight bivalents.

The metaphase stage shown in Fig. 27 was seen on only one occasion and therefore the chromosomes must pass through this stage very rapidly. The chromosomes which look rod-shaped, with some a little longer than others, are lined up along the equatorial plate. The two centrioles are very conspicuous and have a characteristic outline in this species. They are in the shape of a fine straight line extending across the poles of the spindle, and are in length somewhat more than half the distance of the spindle at its widest part. At the right hand corner of the upper centriole, a part appears to be turned downward. An aster can be seen above the upper centriole and appears to mark the cytoplasm quite strongly. The spindle is not visible with aceto-carmine stain at metaphase, but shows up clearly at telophase (Fig. 41).

Figs. 28 and 29 are different views in one nucleus of early anaphase. Centrioles can be seen at the poles. Fig. 30 gives another grouping of early anaphase chromosomes (about 14 can be counted). The centrioles are out of focus in this picture. A comparison of size and number of the chromosomes in this figure with those in Fig. 26 gives fairly good evidence that the large bivalent chromosomes have split into two. Fig. 31 shows another view of early anaphase, and this is apparently a side view since the chromosomes are again rod-shaped. Centrioles are strongly marked. In Fig. 32, anaphase is complete, and centrioles are still present at this stage. In Sporormia obliquiseptata the first meiotic division was longitudinal in the ascus.

In Fig. 33, the chromosomes are still in the crowded arrangement of telophase and the spindle fibers and centrosomes have disappeared. Just above the lower group of chromosomes a fine thread-like chromosome is seen extending out from the mass of chromosomes. At the end of this thread is a tiny dot. From this and later stages seen but not photographed, the fine chromosome is the nucleolar chromosome becoming attenuated prior to the interphase stage, and the dot at the end of it is the first appearance of the nucleolus. In Fig. 34, the interphase stage is well advanced, with the nucleolus clearly seen at one side of the network of fine chromosomes.

The chromosomes in Fig. 35 are probably at late prophase of the second division judging by their size and number and the fact that no centrioles are present. The telophase of the second division is shown in Fig. 36. This division, like the first, has likewise only been

found to take place longitudinally in the ascus. Subsequent to telophase stage, the nuclei again go through an interphase stage as in the first division. As seen in the second lowest nucleus in Fig. 37, the chromosomes are beginning to lengthen prior to interphase. Heterochromatic dots are seen at the end of these fine chromosomes, one of which will become the nucleolus.

Rod-shaped chromosomes again are found (Figs. 38 and 39), and centrioles can be seen in some of the nuclei. It is evident that the position of the centrioles will determine the orientation of the resultant eight nuclei of the ascus. In Fig. 39, the position of the centriole lying beside the top set of chromosomes indicates that this division will be transverse. On the other hand, the position of the centriole in the nucleus second from the bottom indicates a longitudinal division. Hence, in the third division both types of division can take place, and this is borne out in Fig. 40 where transverse division is taking place, and in Fig. 42 where longitudinal division is completed. Following this stage all eight nuclei pass into interphase stage.

Eight round masses are present in the ascus in Fig. 43, indicative of the initial stage in spore formation. Much more indicative of the delimitation of ascospores are the events taking place in the stained material shown in Fig. 44. Eight nuclei at interphase were present in this ascus, and three centrioles were plainly visible. It was not possible to show the entire ascus in the photograph, so that only one centriole is shown. Adjacent to the centriole seen at the right edge of this material, rifts in the cytoplasm are appearing. These rifts were

seen to curve around cytoplasmic masses containing the nuclei, following the direction much the same as the spindle would take. These cleavages also appeared between daughter nuclei. It appears that the centrosomes of the third division persist (or are reformed) and are instrumental in the delimitation of the ascospores, and account for the lines of cleavage.

In Figs. 45 and 46, the central rift is quite apparent, and it is obvious that such a cleavage will result in a biseriate arrangement of ascospores. In this arrangement, the peripheral position of the ascospores was probably the result of the third division being transverse.

In Fig. 47, the eight ascospores are arranged in a single median line throughout the ascus. The type of septation which follows this arrangement can be seen in Fig. 48, which when complete will show the uniseriate condition. This is obviously the result of the third division being longitudinal.

In all stages of division in the ascus so far described, divisions have always taken place simultaneously and each of the nuclei passed through the same cycle at identical times. After the ascospores have been cut out from the cytoplasm, divisions of the nuclei in the ascospores take place more or less randomly, but the spores in each ascus eventually mature at about the same time.

The eight segments of the ascus each contain a single nucleus as shown in Fig. 48. Quite frequently the divisions in the lower ascospores of the ascus take place in advance of the upper spores in the same ascus. In Fig. 50, the nuclei in the ascospores at the base of the ascus are at telophase, while those in the adjoining ascospores

were at early anaphase (only one of these is shown in the photograph).

A series of four mitotic divisions of the single nucleus in the ascospore takes place, so that the ascospore eventually contains sixteen nuclei. Between these divisions, the septa are laid down, three to each ascospore. In Figs. 49, 50 and 51, the two-nucleate condition of ascospore is shown. The first median septum is just beginning to appear in Fig. 52. It is more pronounced when the nuclei are at the stage shown in Fig. 53. A spindle is also present between the two upper nuclei in this photograph. In Fig. 54, the nuclei are at interphase and only the large round nucleoli can be seen in this photograph. Some of them show the doughnut-shaped staining effect already described.

In Fig. 55 a rather typical resting stage of nucleus is seen, round in outline, with a network of fine chromatin threads and a small central nucleolus. It is believed that here the nucleus is in an early prophase stage. Following this stage, the nucleolus disappears and the individual chromosomes become more distinct (Fig. 56). In Fig. 57, the chromosomes are probably at metaphase, since they are very short and thick. Each of these nuclei divide to give two nuclei per cell (Fig. 58). Following the telophase stage seen in Fig. 58, the nuclei become the resting type again (Fig. 59). Later, after division of each of these nuclei, the cells contain four nuclei (Fig. 60). This statement should be qualified to the extent, already mentioned elsewhere in this paper, that because the spores at this stage are beginning to darken, one cannot be certain that all the cells contain four nuclei but it at least seems very likely. In Fig. 61, the second cell from the left can be seen to contain four nuclei though pigment is present in the walls and tends to obscure them.

## DISCUSSION

A study of the cytology of Sporormia obliquiseptata was undertaken to determine the sequence of nuclear changes in the ascus. For this purpose, the material proved to be eminently suited. The methods used and found successful were the same as those employed in the study of higher plants and animals. They consisted mainly of the use of the squash technique with iron-aceto-carmine stain. This stain alone was found effective at all stages of nuclear divisions, but with fixation and sometimes mordating, the division figures showed up more clearly.

The morphological details of the fusion nucleus bear a striking resemblance to the typical diploid nucleus of higher organisms, and its behavour during meiotic division also suggests a similarity. Centrioles, asters, and spindles were all present in the material studied although only the centrioles were frequently seen. Asters and spindles were more readily observed directly after rapid fixation followed by quick staining methods.

The croziers in this fungus are very prominent and are found in fruiting bodies of various ages. Because these structures are so numerous and so typical in appearance, Sporormia would be excellent classroom material for demonstrating crozier development. It is also felt that, inasmuch as the croziers are so distinct and can be seen readily in the very young fruit body, this fungus might yield satisfactory results in a study of the origin of ascogenous hyphae and hook formation, about which very little is known in Ascomycetes generally.

Fusion of the haploid nuclei could not be followed at all stages, but certain figures could be interpreted to mean that in place of nucleolar fusion, as reported by Jones (1926) in Ophiobolus, these nucleoli disappear and, after the two sets of chromosomes have combined, an enlarged fusion nucleolus forms. It is suggested that pairing of homologous chromosomes might also take place at this stage.

In one figure, it was clearly observed that the chromosomes of each haploid nucleus appeared as a bundle of fine threads extending out from one side of each nucleolus so that the two sets of chromosomes crossed one another with the nucleoli remote. This may have been the initial stage in fusion, with the chromosomes greatly elongated and about to pair.

On several occasions, in a slightly later stage of ascus development, a meshwork of much thicker, darkly-staining chromosomes was present. In such figures the nucleoli were not seen. It is this stage which led to the suggestion that fusion had taken place with the simultaneous disappearance of the nucleoli. The drawings by Cleveland (1949) of the fusion stage in certain protozoa indicate that such a series of events evidently takes place in these organisms.

The fusion nucleolus was of conspicuous proportions, especially in the almost mature ascus, in which it sometimes filled more than half the ascus diameter. Because the nucleolus stained well with carmine, several of its characteristics were clearly evident, the

most noticeable of which was its gradual disappearance throughout prophase and its entire absence during metaphase and anaphase. It reappeared in late telephase of every subsequent division in the entire nuclear cycle of the ascus, and probably also of the ascospores, but in size was then usually relatively small. The nucleolar chromosome was seen attached to the large fusion nucleolus on several occasions, and the area of attachment seemed to be very similar to a typical nucleolus organizer, for example such as that illustrated in maize.

The nucleolus was often seen in a ring- or doughnut-shaped form, in which the outer region for some reason stained darker than its interior. This effect was not associated with any special stage of the ascus, but was seen alike in very young and mature asci, and also in the ascospores. It is not known what significance, if any, can be attached to this ring-like appearance of the nucleolus, although it might be suggested that it is related to its metabolism.

The arrangement of spores within the ascus depends on the position of the spindles at the end of the third division. Thus, a transverse division will result in the biseriate condition, and a longitudinal division in the uniseriate condition. It seems evident that in this species, at least, no taxonomic significance can be attached to this character of the orientation of ascospores within the ascus.

In the past, some disagreement existed as to the means of spore initiation in the ascus. The interpretation of spore delimitation

given by Jones (1926) for Ophiobolus graminis Sacc. and by Jenkins (1934) for Cordyceps agariciformia (Bolt.) Seaver differs from that given by Dodge (1927) and Singleton (1953) for Neurospora. Jones considered that spores were initiated by the formation of long, narrow vacuoles, but felt it was possible that assistance was given to the process by the later appearance of astral rays, since at one extremity of each of the spore initials a centrosome with its astral rays was visible. Singleton (1953) found that in Neurospora a group of fibrils radiates out from each centriole cutting out a mass of cytoplasm containing a nucleus which becomes the spore initial. Harper (1905) was the first to recognize this function of the centriole-astral rays in his studies of the powdery mildews.

There seems little doubt that in Sporormia too, ascospore development is initiated by such a centriole-astral ray mechanism, although this belief is based on a single observation. But, since the centrioles were seen to persist (or perhaps were reformed) after the third division while the spindle disappeared and the daughter nuclei reorganized, it seems very unlikely that these centrioles would be functionless. The shape and prominence of the centriole in this fungus also suggests its suitability for this purpose. With this explanation, no difficulty is encountered in accounting for the position of the cleavage lines in the ascus, whether leading to the uniseriate or biseriate condition, for these lines appear to coincide with the expected position of centriole and astral rays, or with the separation between daughter nuclei.

## SUMMARY

1. Sporormia obliquiseptata, which is normally coprophilous in habit fruits well on standard agar media.
2. The ascospores are four-celled and separate easily into segments, each of which will germinate. The fungus is homothallic.
3. The squash method was used in the examination of material for this study.
4. Iron-aceto-carmine stain was used throughout this study. Material that was fixed first in alcohol acetic gave clearer pictures, since the karyolymph remained unstained for some time, and mordanting with iron alum also improved the staining.
5. The stages in the formation of croziers are easy to follow in this fungus and for this reason it would provide useful classroom material.
6. It is thought that a further study of this fungus might reveal the origin of its ascogenous hyphae and hook formation.
7. Fusion takes place sometimes in the penultimate cell and sometimes in the young ascus, and it is believed that at fusion the nucleoli of the fusion nuclei disappear and after the chromosomes have combined in the fusion nucleus a large, new fusion nucleolus is formed.
8. The large fusion nucleus passes through the usual changes in the course of meiotic division to give the haploid number of chromosomes, believed to be seven or eight.
9. A distinct linear centriole is present, and an aster and spindle fibers were seen on several occasions.

10. Evidence that the centriole is instrumental in the cutting out of the eight ascospores in the ascus was obtained.

11. It was found that when the third division was longitudinal within the ascus, a uniseriate condition of the ascospores was the result, whereas when this division was transverse, the spores were biseriate.

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## PLATES AND FIGURES

## PLATE 1

Fig. 1 A crozier with two nucleoli. (x2700)

Fig. 2 A crozier with two nuclei in which the chromosomes appear to be at metaphase. (x2700)

Fig. 3 A crozier just after conjugate division; the four sets of chromosomes are at telophase. (x2700)

Fig. 4 Another crozier just after conjugate division, showing arc arrangement of chromosomes. (x2700)

Fig. 5 Crozier showing the two septa which appear after conjugate division to cut off the tip cell and separate the basal cell from the penultimate cell. (x2700)

Fig. 6 Completed crozier, showing four nuclei, two septa and tip and basal cell fused. The upper darkened body in the basal cell is the nucleus. (x2700)

Fig. 7 Proliferating croziers with interphase nuclei, shown at upper left. (x1350)

Fig. 8 A crozier in which two nucleoli are present in the young ascus, and two sets of metaphase chromosomes are present at the base of the crozier. (x2700)

Fig. 9 Typical crozier with protruding ascus, one of the nuclei of the penultimate cell having migrated into the ascus where fusion will take place. (x1350)

Fig. 10 Two nuclei are now present in young ascus, where fusion will take place. The nucleoli are larger than usual. (x1350)

Fig. 11 Fusion has taken place in penultimate cell of crozier while young ascus has grown out. The nucleolus of the fusion nucleus is prominent. (x1350)

Fig. 12 Fusion nucleus is present in young ascus. Nucleolus is prominent. (x1350)

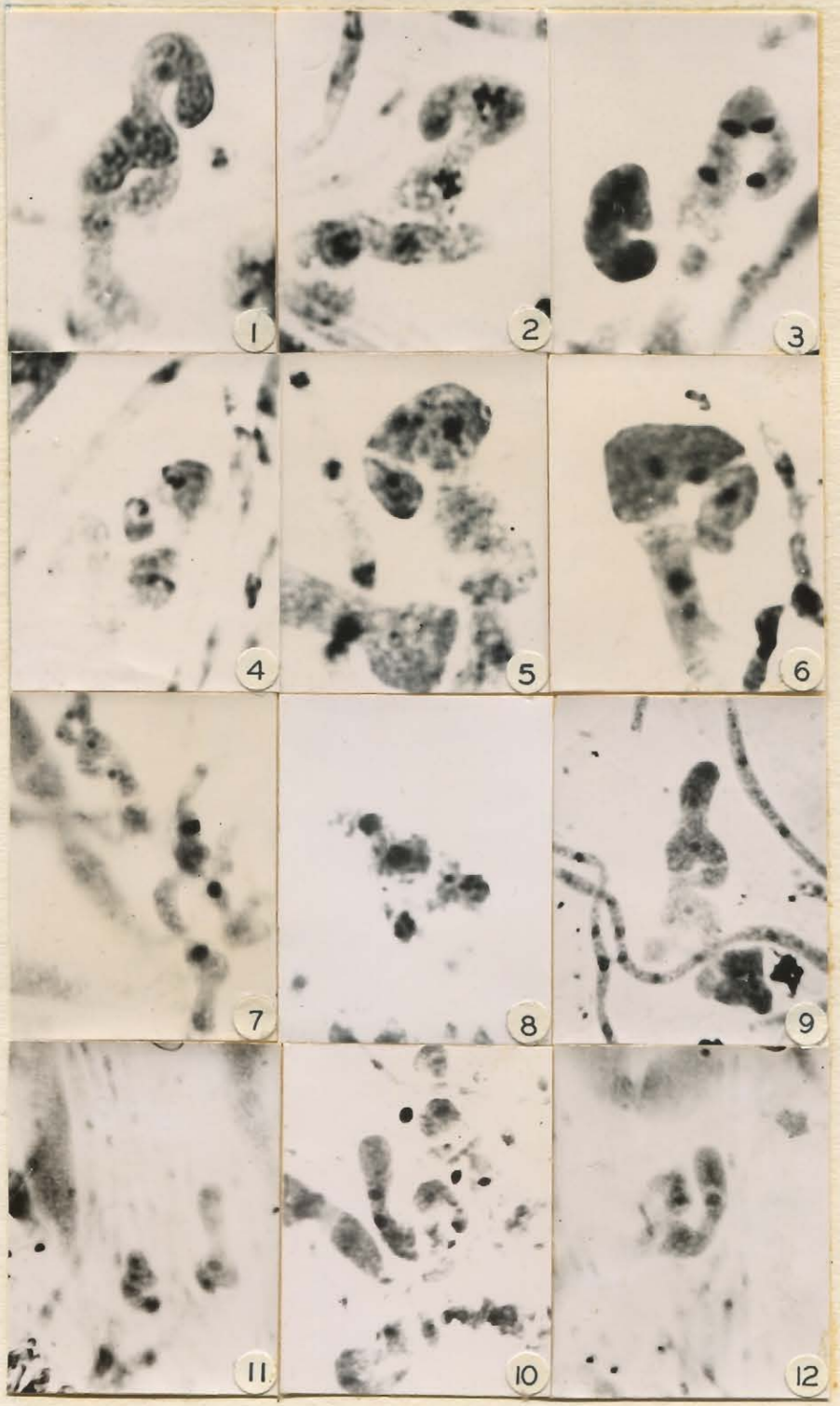


PLATE I

## PLATE II

Fig. 13 Immature ascus showing the thickened network of chromosomes and nucleolus absent, believed to be the stage of fusion of the two haploid nuclei. (Fusion has been delayed in this ascus). (x2700)

Fig. 14 Typical diploid nucleus of an immature ascus, showing large central nucleolus and chromatin network suspended in clear karyolymph. (x2700)

Fig. 15 Two large asci, the one on the right shows the relative size of the nucleolus prior to disintegration, the ascus on the left shows the appearance of the nucleolus at a late stage of prophase. (x1350)

Fig. 16 The ascus at the left shows the round nucleolus and at its lower surface the triangular-shaped nucleolus organizer with nucleolar chromosome attached. The ascus at the right shows the ring-shaped appearance of the nucleolus due to unequal staining. (x2700)

Fig. 17 Another view of the nucleolus organizer and attached chromosome. (x2700)

Fig. 18 An ascus with nucleus at mid-prophase showing long, slender chromosomes and two remaining fragments of the nucleolus. (x2700)

Fig. 19 Same ascus as in Fig. 18 but at a different focus. Chromosomal threads are paired in this view. (x2700)

Fig. 20 Another nucleus at mid-prophase showing pairing of chromosome threads and the much reduced nucleolus. (x2700)

Fig. 21 Same stage as in Fig. 20, showing loops in the chromosome pairs. Material for this photograph was unmordanted, whereas that in Figs. 18-20 was mordanted. (x2700)

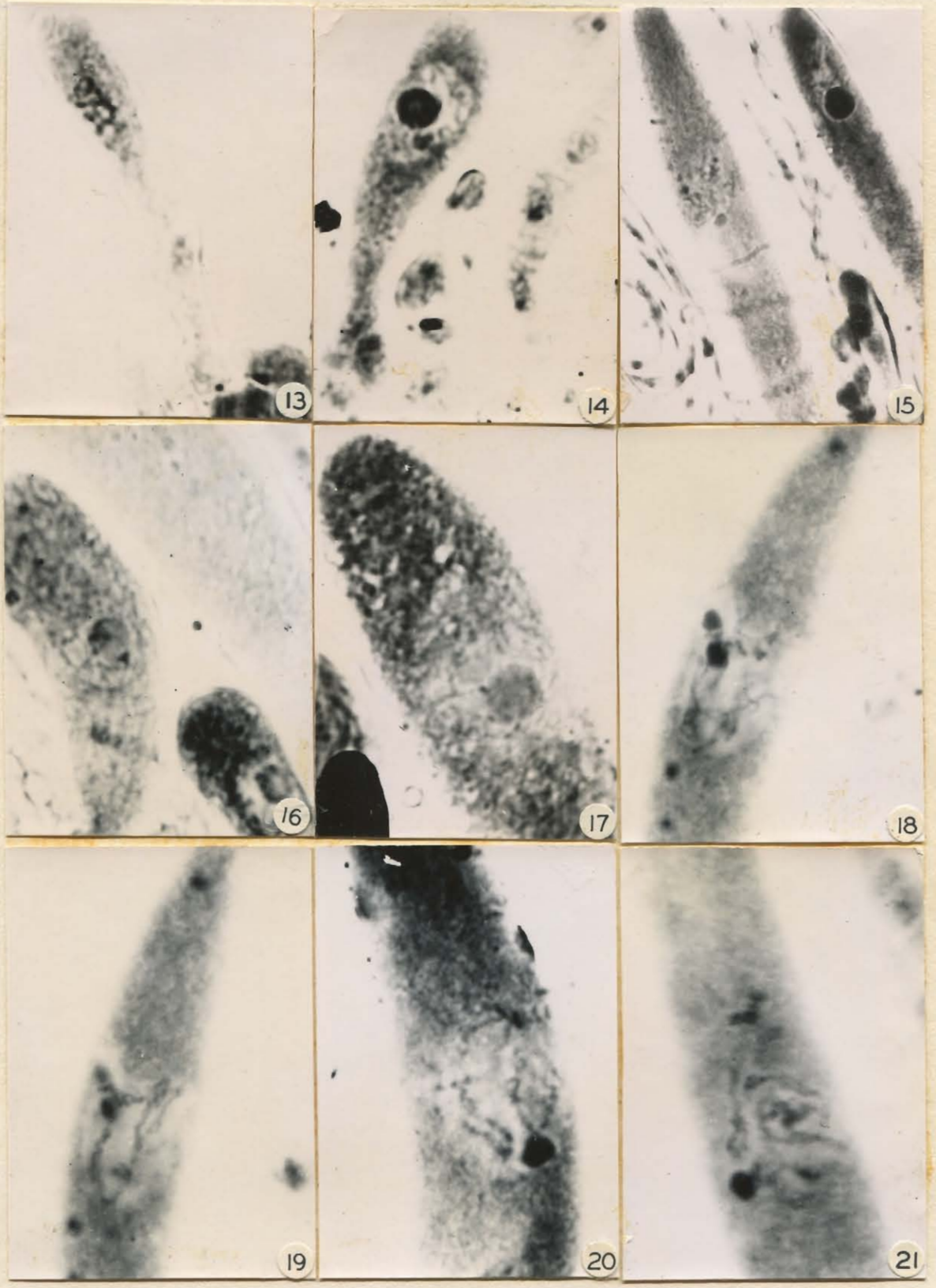


PLATE II

## PLATE III

Fig. 22 One chromosome pair show chiasmata at this stage of prophase. (x1900)

Fig. 23 Chromosomes are becoming heterochromatic. Nucleolus has almost disappeared. (x2700)

Fig. 24 Chromosomes have shortened and distinct chromatic granules are present along their length. (x2700)

Fig. 25 Chromosomes are becoming rod-shaped and the nucleolus has disappeared. (x2700)

Fig. 26 Bivalent chromosomes just prior to metaphase. (x2700)

Fig. 27 Metaphase stage. Rod-shaped chromosomes are lined up on equatorial plate, two linear centrioles are present at the poles and an aster shows above upper centriole. (x2700)

Fig. 28 Early anaphase. (x1900)

Fig. 29 Early anaphase at a different focus from than in Fig. 28. (x1900)

Fig. 30 Early anaphase, chromosomes seen from end view. (x2700)

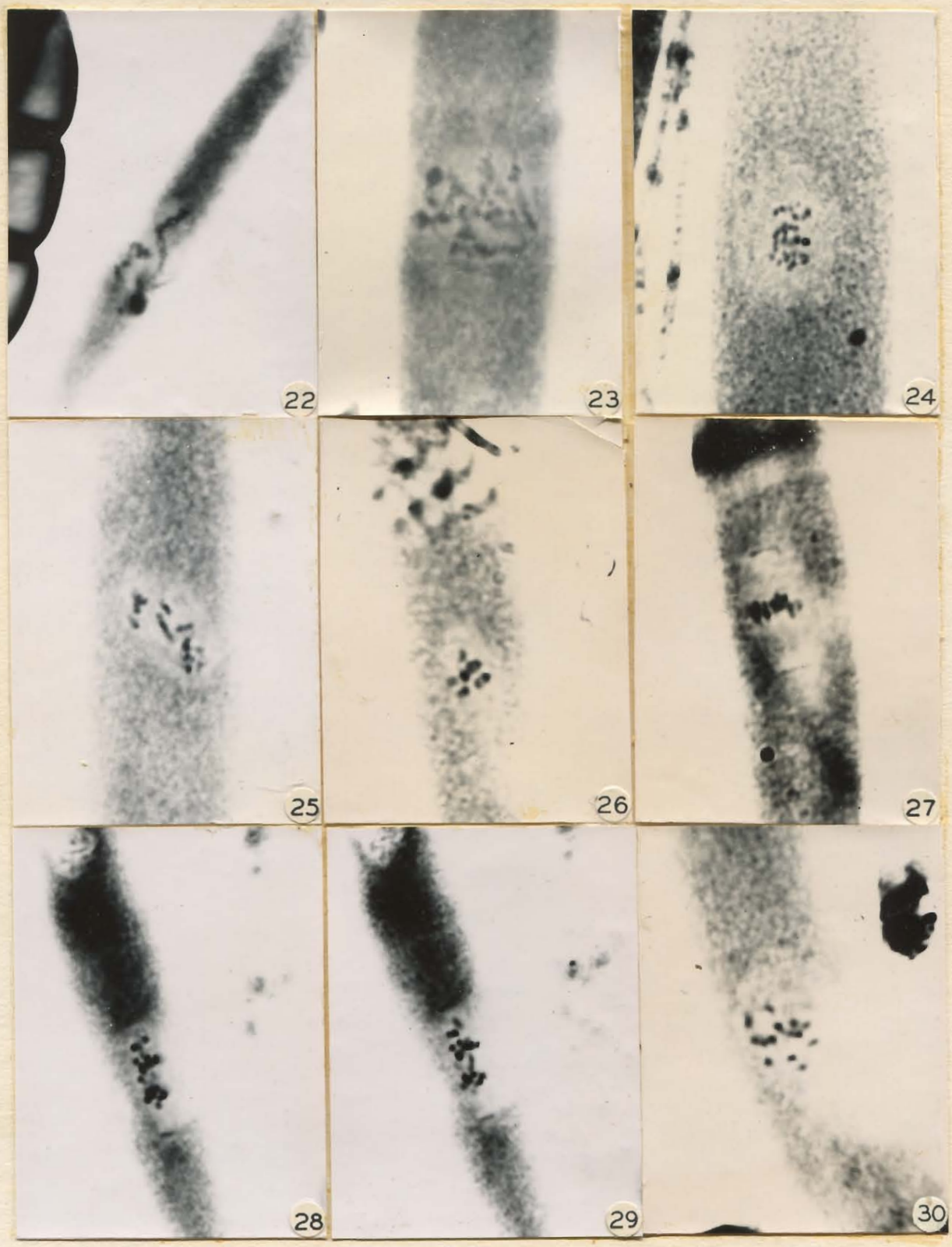


PLATE III

## PLATE IV

Fig. 31 Another group of chromosomes at early anaphase, seen from side view. (x2700)

Fig. 32 Anaphase stage. At each pole there are about seven or eight univalents, all of which are not in focus. (x1900)

Fig. 33 Early interphase. Lower group of compact chromosomes has a slender chromosomal thread extending from it, at the tip of which can be seen a tiny dot believed to be the incipient nucleolus. (x1350)

Fig. 34 Interphase nuclei after first division. (x1350)

Fig. 35 Metaphase stage prior to second division. (x1350)

Fig. 36 Telophase stage of second division. (x1350)

Fig. 37 Early interphase. (x1350)

Fig. 38 Metaphase of third division. (x1350)

Fig. 39 Four nuclei at metaphase. Upper nucleus has a centriole to the left indicating that division will be transverse. Nucleus second from bottom has centriole above it, so that division when it occurs will be longitudinal. (x1350)

Fig. 40 Anaphase of third division. (x1350)

Fig. 41 Upper two nuclei are at anaphase of third division, with spindle fibers still present. Lower two nuclei are at telophase. (x1800)

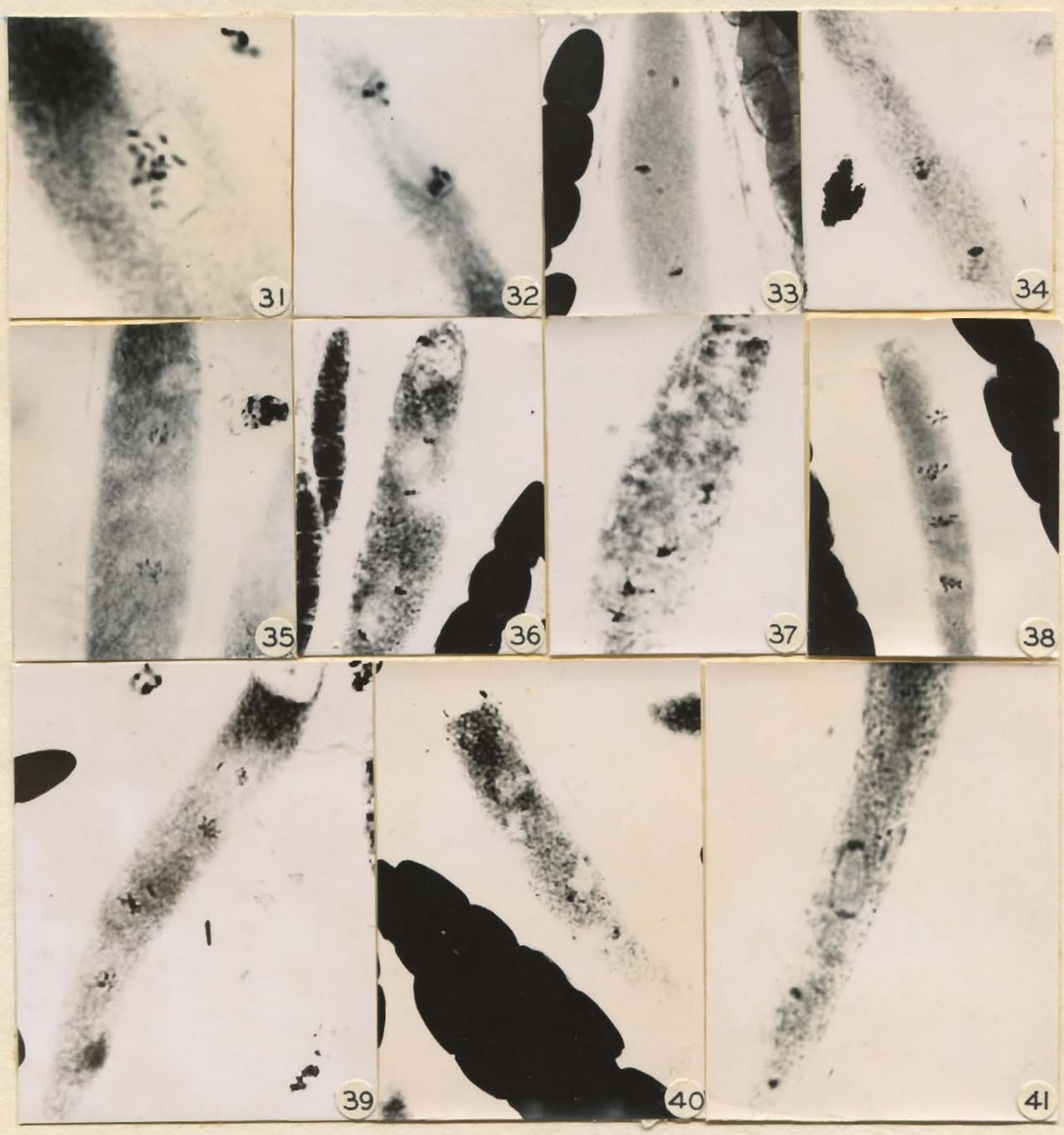


PLATE IV

## PLATE V

Fig. 42 Telophase nuclei of third division showing linear arrangement which will give uniseriate ascospores. (x1350)

Fig. 43 Round areas in ascus may indicate initial stage of spore delimitation. (x1350)

Fig. 44 Cytoplasmic masses being cut out around interphase nuclei. A centriole can be seen at the edge of the ascus on the right. (x2700)

Fig. 45 Eight nuclei alternately placed at periphery of ascus, indicating mature spores will be biseriate. (x1350)

Fig. 46 Same stage as Fig. 45 - central rift shows clearly. (x1350)

Fig. 47 Nuclei in linear arrangement with some indication of spore formation at top of ascus. (x1350)

Fig. 48 Uniseriate arrangement of spore initials. (x1350)

Fig. 49 Two-nucleate ascospore initials. (x1350)

Fig. 50 Two lowest ascospore initials contain nuclei at telophase of first mitotic division. In adjacent spore initial, chromosomes are at early anaphase of first mitotic division. (x2700)

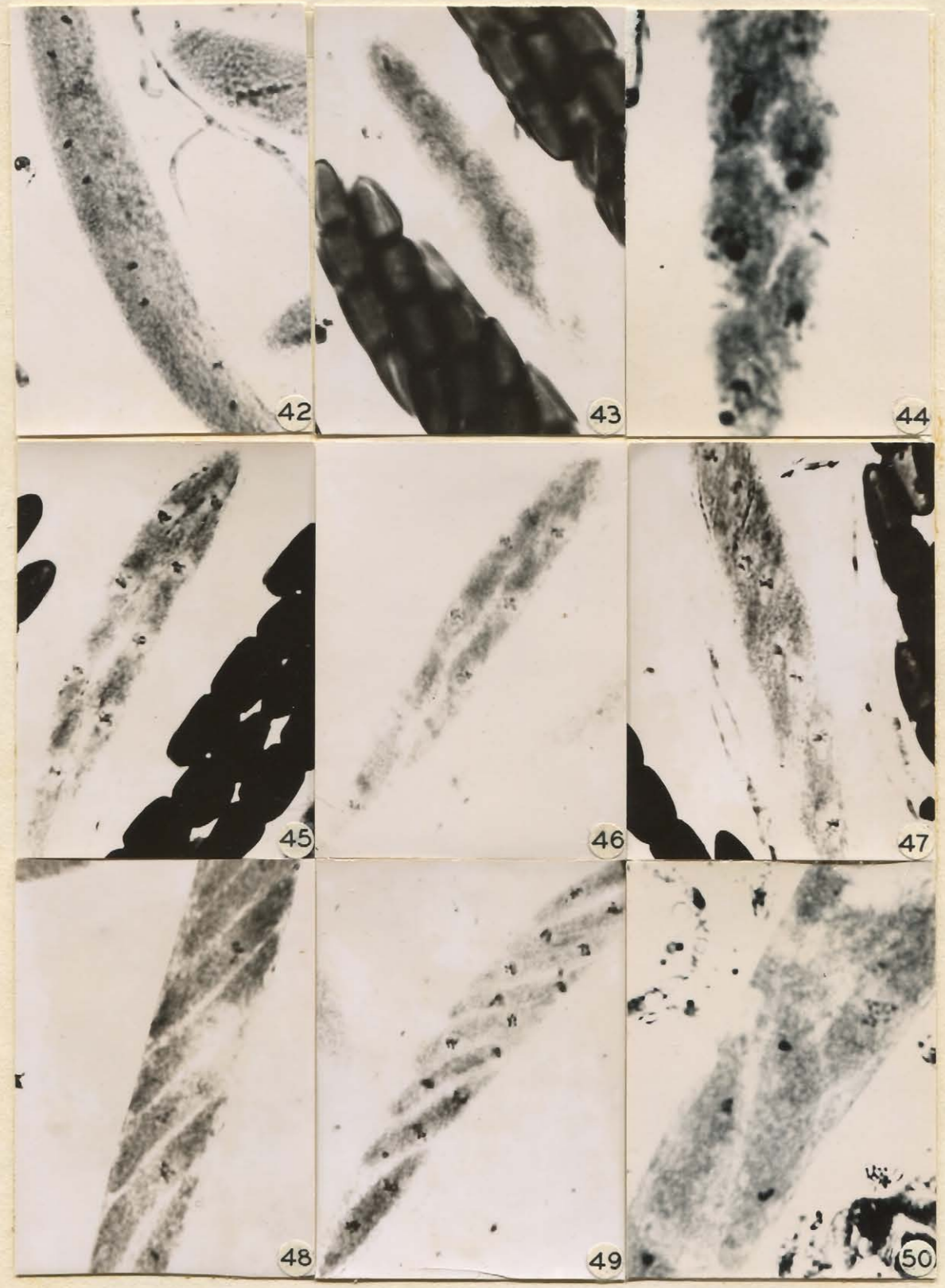


PLATE V

## PLATE VI

Fig. 51 Young spore with two nuclei. (x2800)

Fig. 52 Young spore with one of the two nuclei undergoing a further mitotic division. Central septum as yet is indistinct. (x2800)

Fig. 53 Four nuclei present in youngspore, central septum now distinct and also spindle fibers are clearly seen in upper cell. (x2700)

Fig. 54 Four-nucleate stage of ascospore, with nucleoli present. (x2700)

Fig. 55 Nuclei are at interphase. Small nucleoli may be seen. (X2700)

Fig. 56 Nuclei are at late prophase. (x2700)

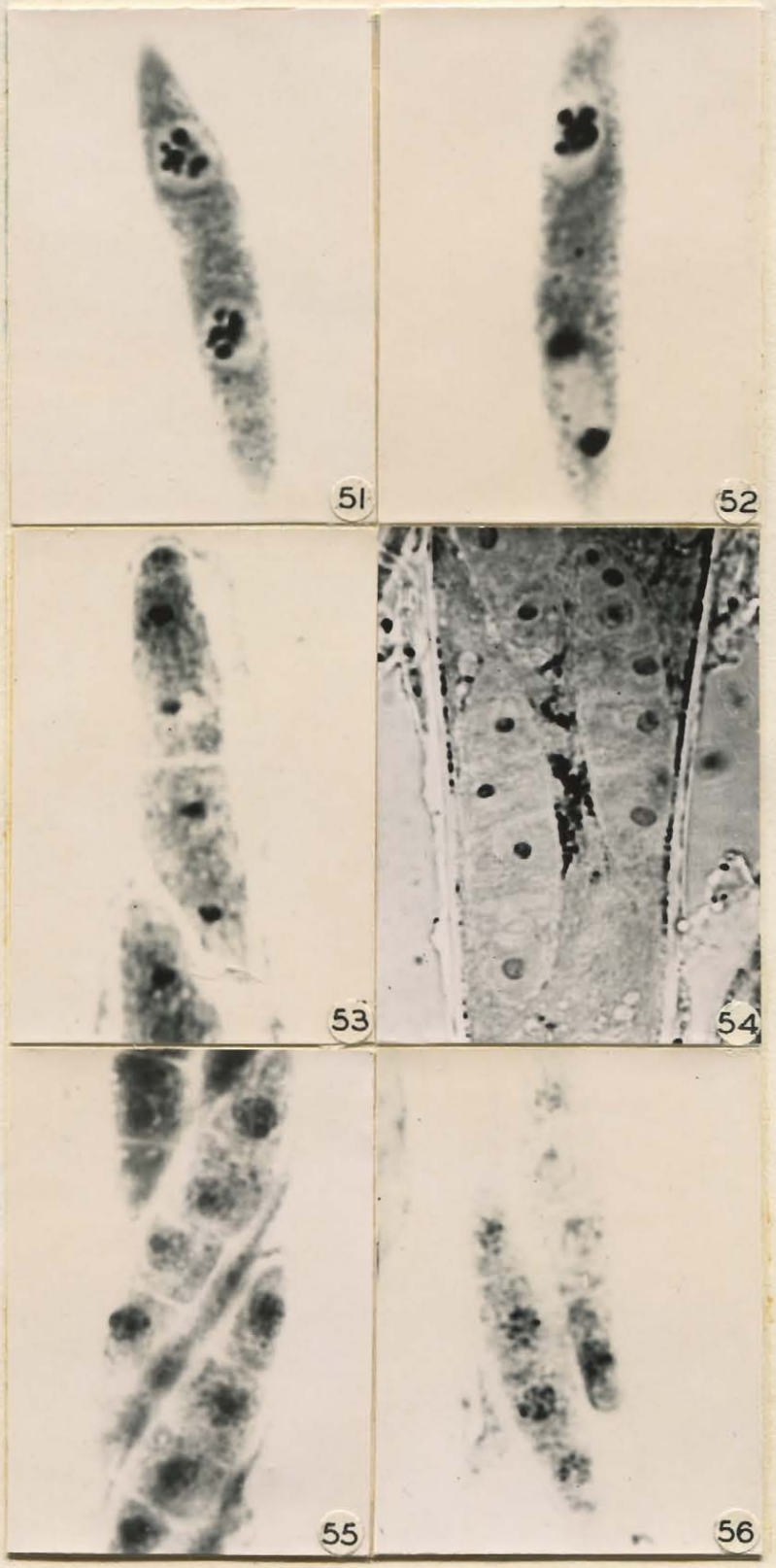


PLATE VI

## PLATE VII

Fig. 57 Nuclei are at metaphase. (x2700)

Fig. 58 Two nuclei at telophase stage are present in each segment. (x1350)

Fig. 59 Two nuclei are at interphase in each segment. (x2700)

Fig. 60 Four nuclei are present in each segment. (x1350)

Fig. 61 Four nuclei can be seen in second segment from left in maturing ascospore. (x1350)

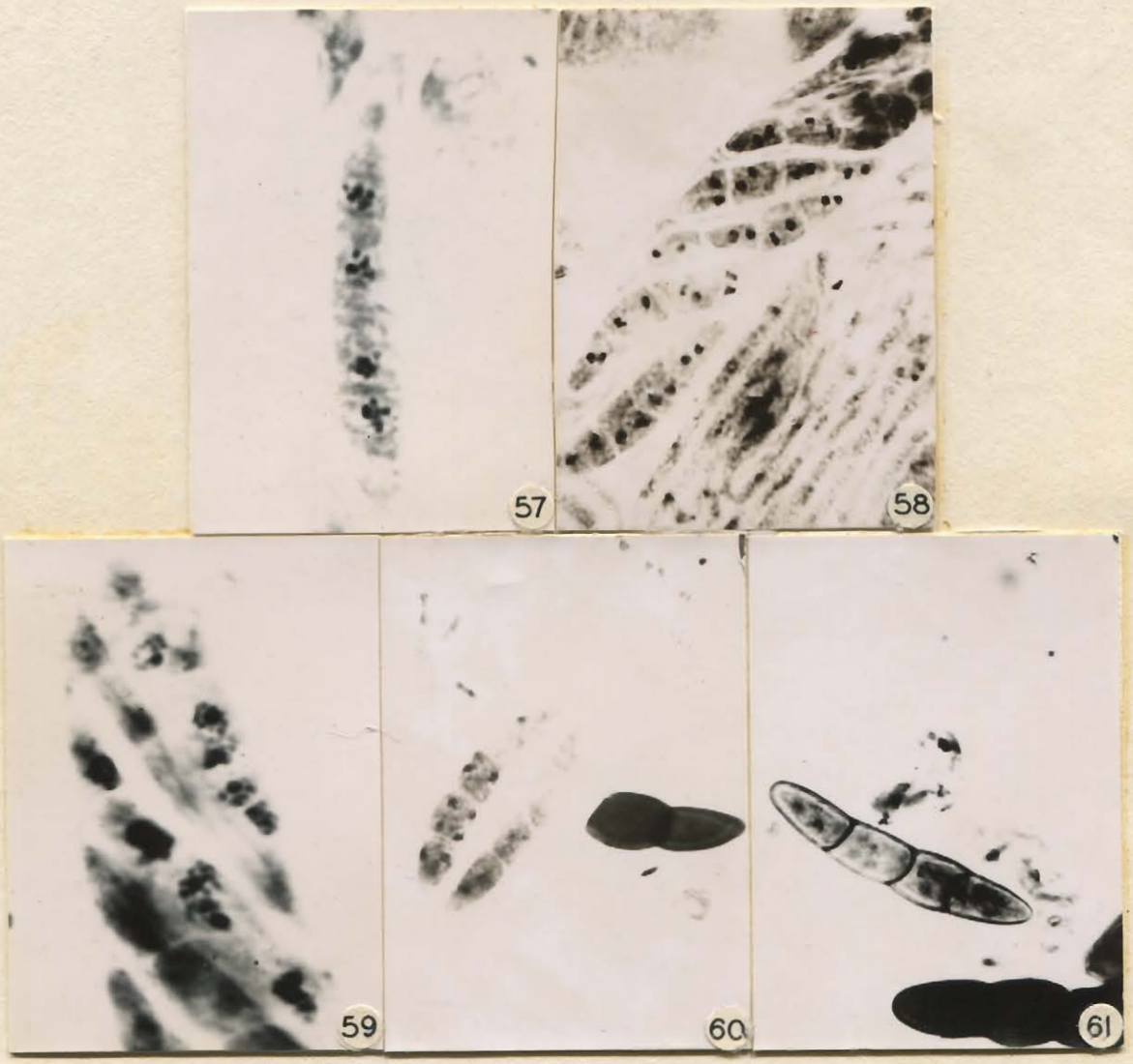


PLATE VII