THE CHROMOSOMES OF *NEUROSPORA CRASSA*[1]

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(With Plates XI and XII and Three Text-figures)

Introduction

In *Neurospora crassa* the zygote nucleus is formed in the young ascus by the fusion of two minute, apparently structureless, haploid nuclei. The zygote nucleus increases rapidly in size and separate chromosomes become visible, each one heavily coated with chromatin. Before synapsis these chromosomes uncoil to form the leptotene threads. The number of separate uncoiled threads and the number of chromomeres on each thread can be estimated fairly accurately.

Technique

Young perithecia of *N. crassa* were flooded with Flemming's or Navaschin's fluid and crushed between two slides. A drop of Mayer's albumin was smeared over the surface of each of two thin cover-glasses. A dozen or more clusters of asci were picked up with a pipette and placed on one cover-slip. Most of the fixing fluid was removed with a pipette; a second smeared cover-slip was placed over the first and the two cover-glasses were pressed together to entangle the asci in the albumin. After about an hour, the cover-glasses were separated and the fixative washed out. The chromosomes were stained, using Belling's brazilin (1928) or iron haemotoxylin.

In analysing the tangled leptotene strands, photographs were made on Eastman 35 mm. panatomic film and developed with Champlain No. 15 fine-grain developer. A complete series of optical cross-sections was made of each nucleus on a single roll of film. Beginning at high focus, after each exposure, the objective was lowered $\frac{1}{2}\mu$., as indicated on the calibration of the knurled knob regulating the fine adjustment. To draw the figures the roll of film was placed in a Focomat enlarger and projected on a piece of drawing paper at 4·75 ×. Thus the outlines of the different optical sections were superimposed over each other. Exact superimposition was obtained by setting the adjustable table so that the

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shadow of the opening of the camera frame exactly coincided in each successive optical section. This technique has a definite advantage over camera lucida drawing because so much more light can be obtained on the pencil. A green safety light helps direct the pencil. To determine whether or not a particular object has been outlined a loose sheet of paper can be placed in the field. Any shadow obscured by the lines already drawn is immediately visible. The pencil outlines are subsequently inked in while the slide is studied.

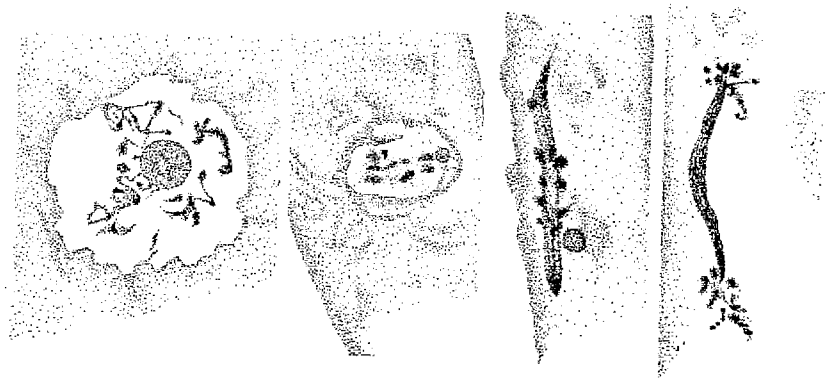
Observations

Mitosis in the ascus

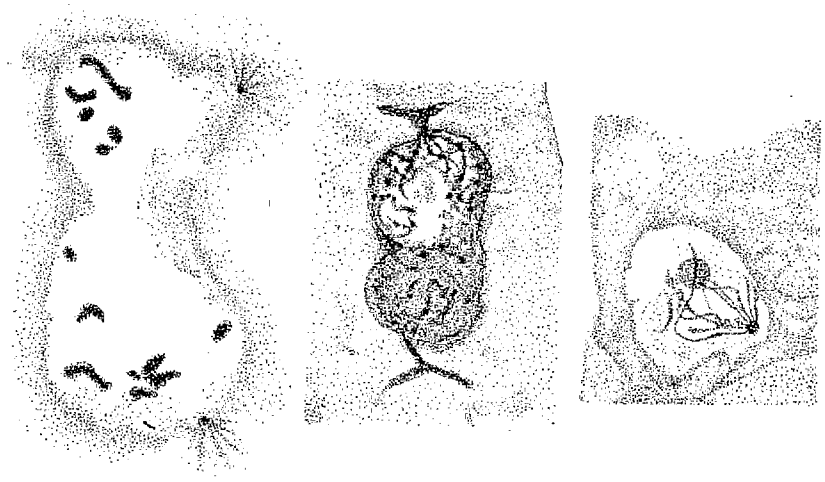
Three mitoses occur in the ascus, producing eight nuclei. A spore wall is produced surrounding each of these eight nuclei (Dodge, 1927; Lindegren & Scott, 1937), and a fourth nuclear division occurs within the spore wall producing two nuclei in each spore. In Text-fig. 1, the different division stages are drawn on the same scale. The ascus is growing as the divisions occur, and the corresponding nuclei and spindles of each of the four divisions are approximately the same size. Although the drawings in Text-fig. 1 are made at the same magnification, they are selected from different divisions. The four mitoses are indicated by Roman numerals: "early prophase" is taken from the second mitosis, "late prophase" from the fourth, etc.

In early prophase the nucleolus is the most conspicuous feature of the nucleus. The fine threads of the chromosomes are beginning to shrink down. At late prophase the chromosomes have contracted in anticipation of the formation of the spindle. Usually eight to ten bodies can be counted in each nucleus; one of these is the nucleolus. At metaphase the chromosomes are very small, and it is not possible to distinguish split pairs from unsplit pairs. In anaphase a cluster of chromosomes appears at each pole. In late anaphase the chromosomes enlarge and the nuclear membrane invaginates to form two new nuclei. At early telophase, before the two nuclei have entirely separated, the central body has produced beaked process extending into the cytoplasm, and the chromosomes have unwound until the nucleus is full of slender threads carrying prominent chromomeres. The tangle of chromonemata is too dense to unravel, but the chromosomes are all attached to the central body and the central body is in turn attached to the beak which seems to function in separating the two daughter nuclei. These pear-shaped nuclei have been described by Dodge (1927), but he has not described the chromosomal threads visible within them. The beak disappears in late telophase, but the

chromosomes are still attached to the central body by their spindle attachments. These mitoses are all intranuclear, but Navaschin's fixative and brazilin stain often obscure the nuclear membrane.



Early prophase II Late prophase IV Metaphase I Early anaphase II



Late anaphase II Early telophase II Late telophase III

Text-fig. 1. Phases of nuclear division in the ascus of *N. crassa*.

The nucleolus

At early prophase the nucleolus attains its maximum size and is probably free in the nuclear sap. At metaphase the nucleolus occupies a position at the periphery of the nuclear membrane, while the rest of the chromosomes are tightly clustered together at the metaphase plate. Dodge (1927, 1937) has described many spindles in *Neurospora* and the

closely related *Gelasinospora tetrasperma* showing the repelled nucleolus. The spindle shown in Pl. XII, fig. 18, is so high above the focus of the nucleolus that the nucleolus could not be shown in the same optical section. It is shown in the drawing.

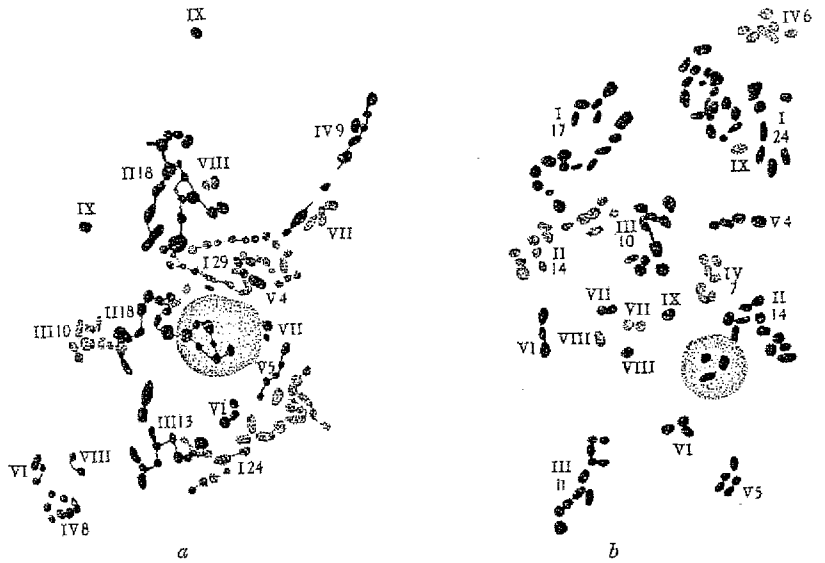
The nucleolus does not divide and usually disintegrates at early anaphase, but is sometimes detectable at this stage. More often it has already disintegrated. A new nucleolus reappears at telophase obviously attached to one of the chromosomes from which it has probably originated in the usual manner.

The formation of the leptotene strands

The haploid conidial nuclei, 1·2μ in diameter, are shown in a conidium (Pl. XI, fig. 1). Each conidium is a coencytium and contains a variable number of nuclei. About a dozen nuclei are present in the conidium shown, but only seven are visible in the optical section photographed. In the young zygote nucleus (Pl. XI, fig. 2) the two chromosomal clusters from each parent begin to enlarge and become heavily encrusted with chromatin before spinning out the leptotene strands. Since each zygote contains two genomes comparable with those contained in the conidial nuclei, the amount of chromatin has increased more than tenfold when compared with the conidial nuclei. Dodge (1927) has described "extranuclear" substance about the periphery of the zygote nucleus. According to the present interpretation these extranuclear bodies are probably chromosomes within the nuclear membrane which have not yet spun out into leptophase. The zygote nucleolus is now much larger than the entire conidial nucleus. Harper (1905) showed that the first act in the zygote nuclei of the Erysiphaceae was the fusion of the two gametic nucleoli.

As the chromonemata begin to uncoil, the chromosomes become beaded with large coarse beads (Pl. XI, figs. 3, 4, 5). These figures represent three foci at different levels through a nucleus; fig. 3 is in high, fig. 4 in median, and fig. 5 in low focus. As the chromonemata continue to untwist and become longer and thinner, the beads become smaller and more evenly distributed (Pl. XI, figs. 6, 7, 8, 9; fig. 6 is in top focus and fig. 9 is in bottom focus). The single nucleolus is quite prominent and some of the chromosomes which are slow in spinning out are still shown heavily stained on the nuclear membrane. Before synapsis the chromonemata must become untwisted (Pl. XI, figs. 10–13 and figs. 14–17). In Text-figs. 2*a* and 2*b* all the chromomeres visible in all the foci through the nuclei are represented in a single plane. Although the chromosomes are not entirely untwisted these figures represent the most advanced stages thus

far found. It is possible to get a clear idea of the approximate size of each chromosome by counting the chromomeres. However, the difference in size in the chromomeres indicates that each chromosome is probably not completely untwisted. In chromosome I with twenty-nine chromomeres (Text-fig. 2 *a*) the thin thread of eight fine chromomeres which shows up



Text-fig. 2. *a*, presynaptic leptophase. Nucleus showing the numbers of chromomeres in the different chromosomes. The Roman numeral indicates the number of the chromosome, the Arabic numeral indicates the number of chromomeres. Contiguous chromosomes are indicated by stippling. In Pl. XI, figs. 10–13 are photographs of this nucleus. *b*, presynaptic leptophase nucleus photographed in Pl. XI, figs. 14–17.

clearly in the photograph (Pl. XI, fig. 11) is probably untwisted and stretched out to a stage in which the ultimate chromomeres are revealed. Analysis of the drawings shows that there are probably eighteen clusters of chromomeres in each nucleus which can be arranged into nine presynaptic pairs. The estimated number of beads on each chromosome is shown in the tabulation below:

	a	*b*		*a*	*b*
I	29	24	VI	3	3
I	24	17	VI	3	3
II	18	14	VII	3	2
II	18	14	VII	2	2
III	13	11	VIII	2	1
III	10	10	VIII	2	1
IV	9	7	IX	1	1
IV	8	6	IX	1	1
V	5	5			
V	4	4			

These tangles are rather difficult to follow through, and different observers might not agree with the writers' interpretation, but there is a general agreement in these two and the several other figures analysed. Further study may result in revision and will certainly give a larger number of chromomeres when each chromosome is untwisted and stretched to show the ultimate chromomeres. *Neurospora* contains a small total number of chromomeres as compared with other more complex forms such as *Lilium* (Belling, 1928) and *Drosophila* (Bridges, 1935). However, there are about twenty times more chromomeres in this form than in the simpler bacterium described by Lindegren (1936*a*).

The number of chromosomes

If the analysis of leptophase can be trusted (it is very difficult to follow the connexions), there are nine haploid chromosomes in *Neurospora crassa*. But three of these are so tiny that they are possibly insignificant. At late prophase eight or nine chromatic bodies exclusive of the nucleolus can usually be found. The metaphase plate is too crowded to make an

a *b*

Text-fig. 3. Two late anaphase chromosome clusters after the third division (Pl. XII, figs. 19–22). In *a* is shown the upper cluster and the lower one is shown in *b*.

accurate count possible but at late anaphase the chromosomes enlarge and are not yet too tangled to be indistinguishable. Text-fig. 3 is a drawing of the two nuclei shown in Pl. XII, figs. 19–22. Six chromosomes are visible in each nucleus. Evidence of the possible formation of trabants in these chromosomes is given by the constrictions visible. It is possible that the larger number of particles present at late prophase is due to the fact that the chromosomes have not completely condensed. The particles visible at the poles in early anaphase show connexions which may mean that two or three of them possibly represent a single chromosome. The best estimate of the number of chromosomes is probably six, taken from Text-fig. 3, at late anaphase.

DISCUSSION

The haploid somatic nuclei found in the mycelia, conidia, and perithecial walls are minute densely staining bodies. Reticulate structure, nucleoli, and nuclear membranes are only found in stages having an obvious relation to sexual reproduction, i.e. in the perithecium or shortly before its formation. The writers have never found spindles except in the ascus. Somatic haploid nuclei are to all intents and purposes merely dense tiny globules containing chromatin. Harper (1905) has described division figures with spindles in the much larger somatic nuclei of the Erysiphaceae, but the division was not unmistakably mitotic. Any of the numerous writers who have described amitotic spindles in the so-called "primitive" nuclei would label the division drawn by Harper as an amitosis. In the ascus mitoses, Harper had no difficulty in accurately counting eight chromosomes. In asexual tissue of many ascomycetes, the nuclei do not appear to be nucleolate, reticulate, or vesicular nor is it possible to find evidence for the view that they divide by mitosis. Darlington (1937) has defined a nucleus as a body which divides by mitosis and with Belar (1928) he shares the view that all nuclei in the "germ track" divide by mitosis. Spearing (1937), in a study of the nuclei of the blue-green algae, distinguishes between true nuclei which are nucleolate, reticulate, and vesicular and central bodies which are not. Spearing presents evidence indicating that "primitive" nuclei divide by amitosis, and challenges the view expressed by Belar that primitive nuclei do not exist. In contrast to morphological definitions of the nucleus, Lindegren (1936*a*) (in discussing the nuclei of bacteria) has defined a nucleus on purely genetical grounds as consisting of one or more gene strings normally present in multiples of the haploid number. Text-fig. 1 shows clearly that the ascus nuclei of *N. crassa* are true nuclei. But these true nuclei are produced by the fusion of two structures which Spearing would classify as central bodies rather than nuclei if he had only the asexual mycelia to study. The asexual nuclei probably contain not less than two to three hundred genes each, judging from the count of the leptotene chromomeres. Genetical analysis of the crosses originating from conidia prove that these nuclei must divide by mitosis because the gene complex is delivered intact to the zygote. The development of unquestionable nuclei from zygotes produced by the fusion of two apparently structureless bodies proves that they are true nuclei. This places the burden of proof squarely on the shoulders of those who attempt to defend the thesis that similar undifferentiated structures

in other forms are primitive nuclei. From a genetical viewpoint the concept of amitosis is completely indefensible. Division by strangulation or constriction in bacteria or blue-green algae cannot be interpreted as failure of the gene strings to split longitudinally. When a longitudinal split divides the two cylindrical chromosomes of a higher plant, the gene strings in the splitting chromosomes are not arranged in two straight lines but in two coils each of which is imbedded in chromatin and enclosed in a membrane, and the apparent longitudinal split is merely the final act of separation which has been preceded by a microscopically undetectable separation of two subsequently coiled gene strings. Amitosis is an artefact just as the earlier view that the hereditary factors were arranged in a straight line in each of the two longitudinally splitting chromosomes was artefactual. Those who refuse to accept the simple interpretation that amitosis is the final act of separation of gene strings which have already been split longitudinally in some microscopically undetectable earlier stage, are faced with the enormously complex problem of formulating a plausible hypothesis concerning the constancy with which the hereditary characters are transmitted.

Summary

An estimate was made of the number of chromosomes in the presynaptic leptotene stage of the zygote nucleus of *N. crassa*. There appeared to be 9 haploid chromosomes, 2 large, 2 medium, 2 small, and 3 very small pairs. A maximum of 155 chromomeres were counted, but this is possibly less than half the ultimate number since all the chromosomes were not completely uncoiled. Six chromosomes were visible in the best anaphase figures discovered, which probably indicates that some of the smaller bodies found in leptophase have shrunken down to invisibility at this stage. At any rate the smaller bodies are probably insignificant genetically.

REFERENCES

Belar, K. (1928). "Die cytologischen Grundlagen der Vererbung." *Handb. Vererbungsw.*

Belling, John (1928). "The ultimate chromomeres of *Lilium* and *Aloë* with regard to the numbers of genes." *Univ. Calif. Publ. Bot.* **14**, 307–18.

Bridges, C. B. (1935). "Salivary chromosome maps, with a key to the banding of the chromosomes of *Drosophila melanogaster*." *J. Hered.* **26**, 60–4.

Darlington, C. (1937). *Recent Advances in Cytology*. London.

DODGE, B. O. (1927). "Nuclear phenomena associated with heterothallism and homothallism in the Ascomycete *Neurospora*." *J. Agric. Res.* **35**, 289–305.

—— (1937). "Spindle orientation and spore delimitation in *Gelasinospora tetrasperma*." *Cytologia*, Fujii Jubilee vol. pp. 877–87.

HARPER, R. A. (1905). "Sexual reproduction and organization of the nucleus in certain mildews." *Publ. Carneg. Instn*, No. 37.

LINDEGREN, CARL C. (1936*a*). "Genetical studies of bacteria. III. Origin of *G*-type colonies by transgenation." *Zbl. Bakt.* II, **93**, 389–400.

—— (1936*b*). "A six-point map of the sex-chromosome of *Neurospora crassa*." *J. Genet.* **32**, 243–56.

—— (1936*c*). "The structure of the sex-chromosomes of *Neurospora crassa*." *J. Hered.* **27**, 250–9.

LINDEGREN, CARL C. & SCOTT, MARY ALLAN (1937). "Formation of the ascospore wall in *Neurospora*." *Cellule*, **45**, 361–71.

SPEARING, JOHN K. (1937). "Cytological studies of the Myxophyceae." *Arch. Protistenk.* **89**, 209–78.

EXPLANATION OF PLATES XI AND XII

PLATE XI

All the photographs (figs. 1–33) are taken at the magnification indicated by the photograph of the stage micrometer.

Fig. 1. Conidium containing nuclei. These nuclei are metabolically quiescent and it is at this stage in the life cycle that the nuclei of *N. crassa* are smallest (diameter 1·2μ.).

Fig. 2. Young zygote nucleus. The finger-like structures extending from the clusters are chromosomes that are rapidly increasing in size. The nucleolus has already attained a diameter more than twice that of the whole conidial nucleus.

Figs. 3–5. Optical sections of a zygote nucleus containing thickly beaded expanding chromosomes and large nucleolus.

Figs. 6–9. Most of the chromosomes are almost completely unwound, but about four are visible on the nuclear membrane as dark pycnotic structures.

Figs. 10–13. Continued enlargement of the young ascus has been accompanied with increasing extension of the chromonemata. Probably the only chromosome in which any ultimate chromomeres are present is No. 1, above the nucleolus in Fig. 11 (see Text-fig. 2*a*).

Figs. 14–17. Zygote nucleus in presynaptic leptophase (see Text-fig. 2*b*).

PLATE XII

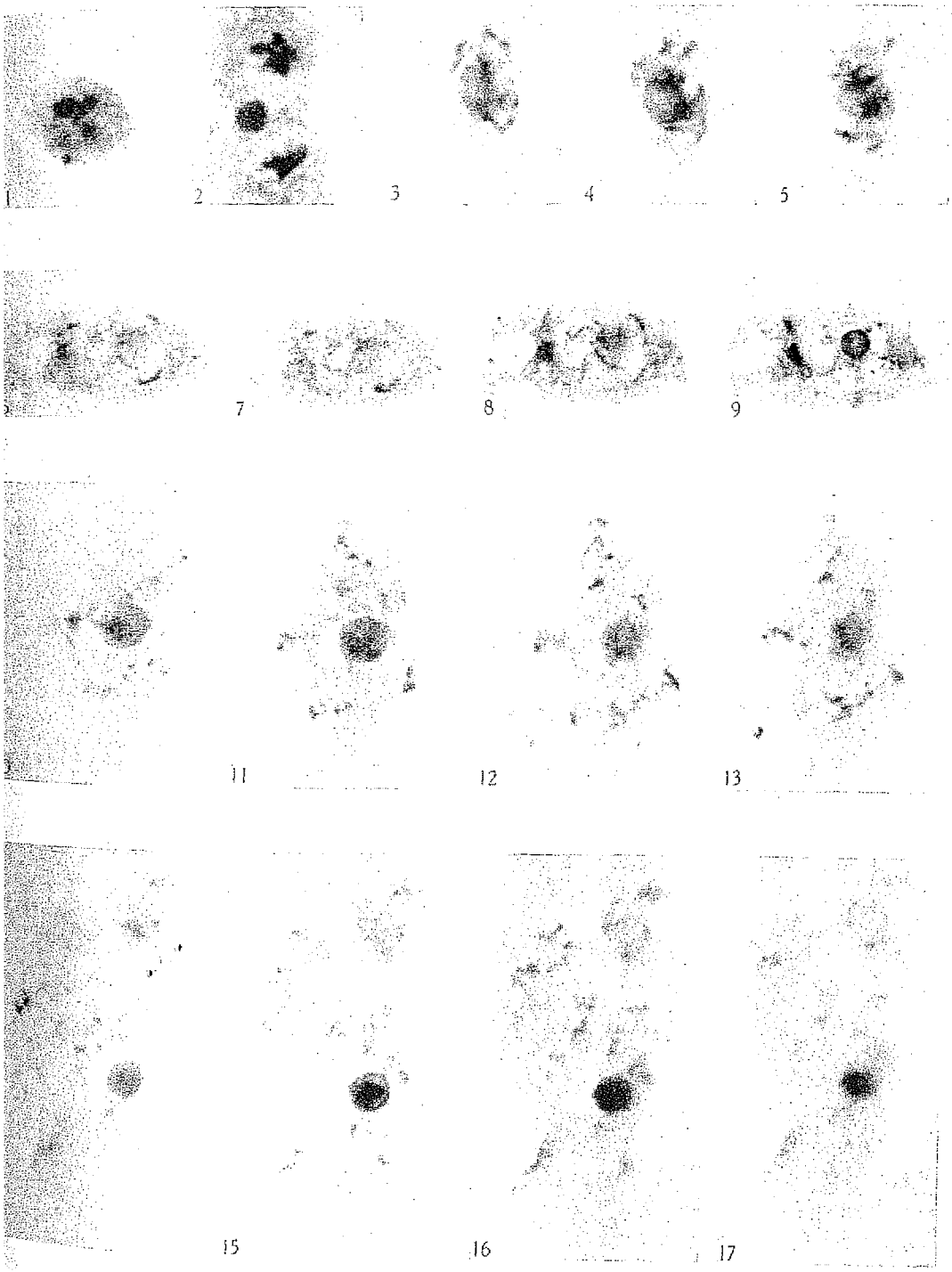
Fig. 18. One optical section of the intranuclear spindle at the first division containing nine chromosomes. The nucleolus is repelled from the spindle and is in low focus.

Figs. 19–22. Series of optical sections through two nuclei of an ascus after the third division. The chromosomes are at late anaphase. The long axis of the ascus and the photographs coincide. The spindles are arranged across the long axis of the ascus so the two nuclei shown are not sisters but each has arisen from the division of different nuclei.

Figs. 23–27. Optical sections through two nuclei at late telophase after the third division. These nuclei have just lost their "beaks". The outlines of both the nuclear membrane and the spore membrane are visible. The chromosomes are attached to the central body and the nucleolus in the upper nucleus is attached to one of the chromosomes (fig. 23). The central body is visible in the lower nucleus (fig. 24).

Figs. 28, 29. Two nuclei in the same ascus at early prophase after the first division. The strands of the contracting chromosomes and the grossly enlarged nucleoli are clearly visible in the nuclear sap. The nuclear membrane is outlined by the cytoplasm.

Figs. 30–33. Optical sections through a nucleus at late prophase after the third division. The uppermost body in *Fig.* 30 is the nucleolus. About eight or nine other structures are visible.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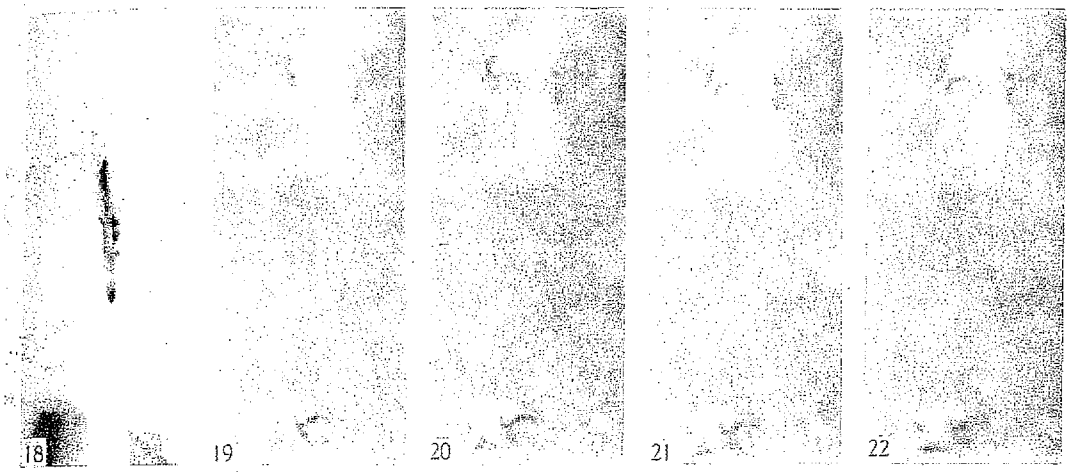

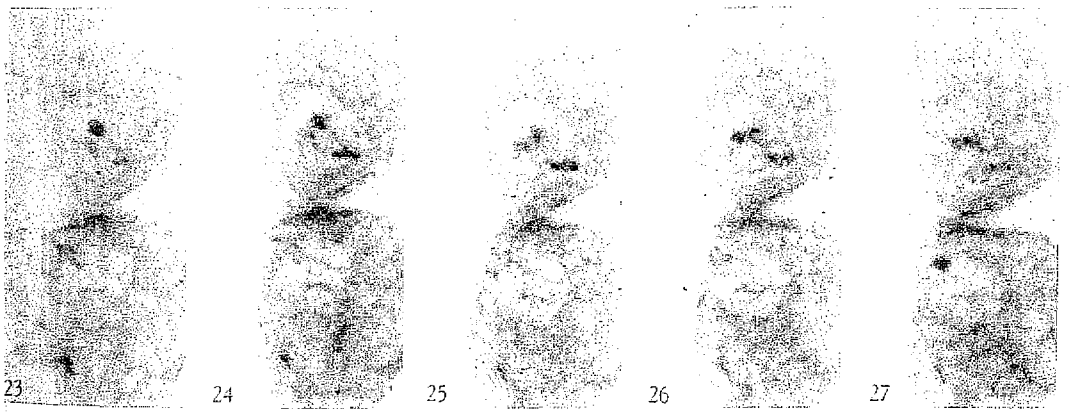

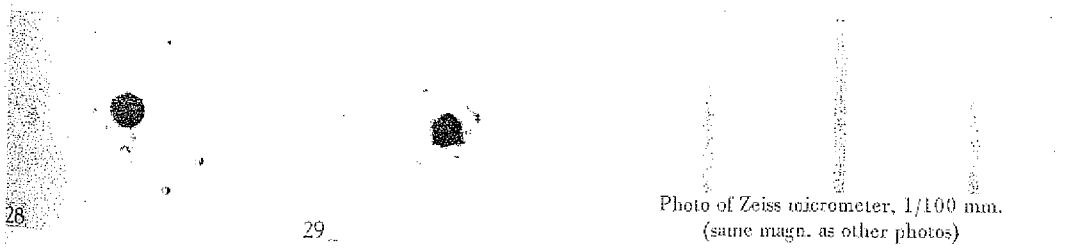

Photo of Zeiss micrometer, 1/100 mm.
(same magn. as other photos)