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The cytology of *Aspergillus nidulans*

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INTRODUCTION

In recent years, the cytology of a number of ascomycetes has been studied, using modern staining methods and squash preparations: e.g. *Neurospora crassa* (McClintock, 1945; Singleton, 1953), *Glomerella cingulata* (Wheeler, Olive, Ernest & Edgerton, 1948), *Patella melaloma* (Olive, 1950), *Pyronema confluens* (Wilson, 1952), *Venturia inaequalis* (Day, Boone & Keitt, 1956), *Hypomyces solani* f. *curcubitae* (El-Ani, 1956), *Sordaria fimicola* (Carr & Olive, 1958) and *Nectria peziza* (El-Ani, 1959). This list, though far from complete, reflects the attention that has been paid to species with linear or clavate asci, and particularly to the pyrenomycetes, to the apparent exclusion of the plectomycetes. *Aspergillus nidulans*, the subject of the present investigation, is a plectomycete with a non-linear ascus.

Both haploid and diploid strains of *Aspergillus nidulans* have been investigated. Haploid strains are those in which, as usual in fungi, the nuclei of the vegetative hyphae are all haploid. Diploid strains are heterozygotes made by Roper's (1952) technique; the nuclei of their hyphae are mainly diploid (Pontecorvo & Roper, in Pontecorvo, 1953). The main object of this work was to study the behaviour of the nuclei in asci of diploid strains, but it was necessary to have as a basis for interpretation that in haploid strains. In the primordium of the ascus in haploids, there are two nuclei which fuse to give the zygote nucleus, the only diploid nucleus of the life cycle; it immediately undergoes meiosis. It was desired to find out whether in diploid strains fusion of two diploid nuclei occurs in the ascus primordium, followed by a tetraploid meiosis.

The account of the cytology of the ascus in haploid strains is based mainly on *wild type*. Development of asci has been studied in detail in two diploids. Diploid A, one of the first to be synthesized by Pontecorvo and Roper, has the genotype $\frac{+\ +}{ad2\ y}\ \frac{w1}{+}\ \frac{lys1}{+}$. The genotype of Diploid B is $\frac{ad14\ +\ +\ +\ bi1}{+\ pro1\ paba1\ y\ +}\ \frac{w3}{+}$. (For symbols etc., see Käfer, 1958.)

CYTOLOGICAL TECHNIQUES

Material was grown at 37° C. in slants of minimal medium (or for auxotrophs, of supplemented minimal) in 6 × 1 inch tubes. The slants were inoculated either with conidia, or in the case of heterokaryons with a block of agar cut from the growing

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front of the heterokaryotic mycelium. First metaphase is observed in wild type 4 days after inoculation of conidia, and in diploids after 5 days.

A block of agar with perithecia (or more strictly, cleistocarps) of the age required for cytological examination was cut out of the slant and fixed in freshly mixed 1 : 3 acetic alcohol at room temperature. The material was left in the fixative and stored in a refrigerator. The best preparations were made from freshly fixed material, but satisfactory preparations have been made from material stored for 6 weeks.

The only staining procedure found to be consistently satisfactory was as follows. A piece of agar with the perithecia on it was removed from the fixative and hydrolysed for 5 minutes in normal hydrochloric acid at 60° C. Perithecia were then scraped off the agar block and placed in a drop of aceto-orcein on a slide. A 1% solution of Gurr's synthetic orcein in 50% acetic acid was used. The contents of the perithecia were pressed out with a needle, and the wall debris removed. The material was spread by tapping the cover slip, and the preparation squashed and sealed.

Most of the photographs were taken using a Cooke Troughton and Simms 80 × apochromatic objective and a Leica camera with 'Mikas' attachment. The film was Ilford 'Microneg pan', and an Ilford 'micro-green' filter was used. Some were taken with a Bausch and Lomb 90 × apochromat and a Leitz 'Makam' on Kodak contrast process panchromatic sheet film. All the drawings have been made from photographs. The magnification in all illustrations is × 2500, unless otherwise stated.

HAPLOID STRAINS

Asci in haploid strains arise from croziers. The two nuclei of the terminal cell of an ascogenous hypha divide simultaneously (Figs. 28–29) and a uninucleate tip cell, a binucleate penultimate cell and a uninucleate basal cell are formed (Figs. 4, 30). The penultimate cell enlarges to form the ascus; its two nuclei fuse to give the diploid zygote, which immediately undergoes meiosis (Figs. 4, 30, 5, 25, 31). The tip and basal cells fuse to give a binucleate cell, which forms a second crozier (Fig. 1). The process may be further repeated.

In prophase of the first meiotic division in haploid strains, the chromosomes form too dense a mass for the details of their structure and pairing behaviour to be made out. In early prophase fine threads are seen extending free from the mass. In pachytene a large knob is often conspicuous (Figs. 7, 31). By restaining the preparation with propiono-carmine, so that the nucleolus, which is unstained in the orcein preparations, could be seen, it was shown that this knob is the satellite of the nucleolus-organiser chromosome. Many of the chromosomes have prominent terminal chromomeres situated against the nuclear membrane.

While it appears that in pachytene the chromosomes are fully paired throughout their length, by early diakinesis the two chromosomes of the bivalent are often widely separated; generally they are in contact only at one end, forming a V (Figs. 8, 2). During diakinesis they again pair, so that in late diakinesis the

bivalent appears as two rods lying parallel to each other without any evidence of chiasmata (Fig. 2).

At diakinesis eight bivalents are seen. They differ somewhat in length, and are

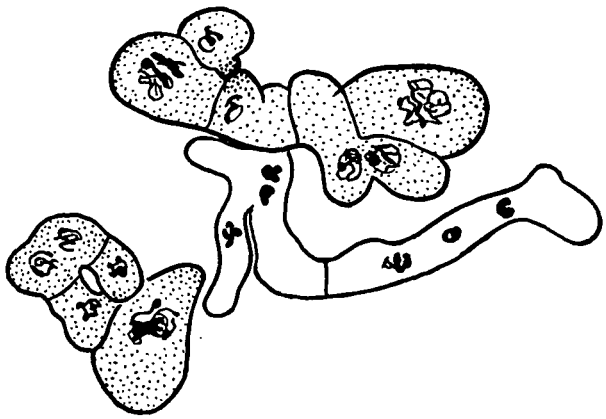


Fig. 1. *Wild type.* × 2000. Three asci with stages in the proliferation of croziers. At *top left*, the tip and basal cells of the crozier are beginning to fuse. *Top right*, binucleate cell resulting from fusion of tip and basal cell. *Bottom left*, secondary crozier with uninucleate tip cell, binucleate penultimate cell and uninucleate basal cell, still attached to ascus formed by primary crozier.

either bent or show a constriction at a characteristic position, which may indicate the position of the centromere (see Figs. 3, 35*a*).

Chromosome 1, the longest chromosome, is frequently curved or bent near the middle in mid-diakinesis.

Chromosome 2 is about the same size as Chromosome 1, and has a satellite about

Fig. 2. Diagram showing appearance of a bivalent in early diakinesis, late diakinesis and first metaphase.

one-fifth of the length of the whole chromosome at late diakinesis. It is frequently bent in mid-diakinesis at the satellite constriction.

Chromosome 3 is slightly smaller than Chromosomes 1 and 2, and is bent at mid-diakinesis in a sub-median position.

Chromosomes 4 and 5 are rather smaller than Chromosomes 1 to 3. Both have sub-terminal constrictions.

Chromosomes 6 and 7 are smaller than Chromosomes 4 and 5. One at least has a sub-terminal constriction.

Chromosome 8, the smallest chromosome, is rather smaller than Chromosomes 6 and 7.

In general, the chromosomes may be classified at first metaphase as three large,

two medium sized and three small. Often, however, the size differences are not very apparent at first metaphase. They are generally much more distinct at late diakinesis (Figs. 34, 35, 35*a*, 3). The eight bivalents at first metaphase are shown in Figs. 10–13, 36–37.

The first metaphase spindle is narrow with flattened ends (Fig. 42). The bivalents are attached to the outside of the spindle, forming a ring in polar view (Fig. 36). Their centric regions are drawn out, converting the parallel rods of diakinesis into a round or diamond shaped figure in side view (Figs. 12, 2).

First anaphase is shown in Figs. 38–39. The three smallest chromosomes may disjoin early; the difference in size between Chromosome 8 and Chromosomes 6 and

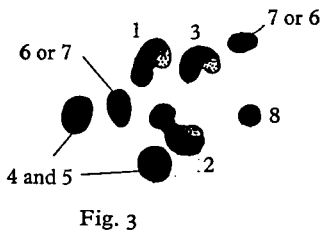


Fig. 3

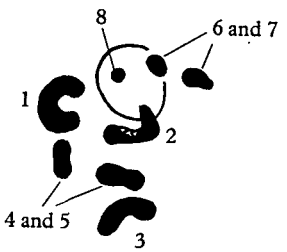


Fig. 35*a*

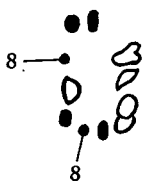


Fig. 15*a*

Fig. 3. *Wild type.* × 6000. Mid-diakinesis, showing differences in size between the eight chromosomes.

Fig. 35*a*. *pro*1 *paba*1 *y* × *ad*14 *bi*1; *w*3. × 5000. Mid-diakinesis. Drawing of cell shown in Fig. 35. In the photograph Chromosome 4 (or 5) appears to be touching Chromosome 1.

Fig. 15*a*. *Wild type.* Drawing of cell shown in Fig. 15. Univalents (early anaphase) in black, bivalents in outline. The bottom right univalent is partly obscured by one of the bivalents in the photograph.

7 is then apparent (Figs. 15, 15*a*). In the second meiotic division, and in the mitotic division which follows meiosis (third ascus division), the chromosomes are very small, and nothing can be seen of their morphology; indeed, they can hardly ever be counted even subsequent to first anaphase. The interphase nuclei are however quite large (Figs. 18, 22).

At late anaphase of all three divisions, two rings are seen in which the individual chromosomes cannot be distinguished (Figs. 16, 17, 21), and a thread, presumably representing the spindle, often connects them (Figs. 16, 24). The division of the nuclei of an ascus is synchronized (Figs. 17, 19–21, 23–24).

Centrioles cannot be demonstrated by the staining methods used. During ascospore formation the cytoplasm appears to be divided by cleavage about each

EXPLANATION OF PLATES

Plate I, Figs. 4–16. *Wild type.*

Fig. 4. Ascus with nucleus in early meiotic prophase; and secondary crozier with uninucleate, binucleate and uninucleate cells.

Fig. 5. Three asci showing progressive stages from fusion nucleus to pachytene, the ascus enlarging meanwhile.

Fig. 6. Pachytene.

Fig. 7. Pachytene, with conspicuous knob at 10 o'clock (*arrow*).

Fig. 8. Early diakinesis. Note V-shaped appearance of two bivalents at right of nucleus (*arrows*).

Fig. 9. Mid-diakinesis.

Figs. 10–13. First metaphase; early stages with eight bivalents spread out along the length of spindle.

Fig. 14. First metaphase; late stage with bivalents arranged at equator of spindle.

Fig. 15. Early first anaphase, with the three smallest pairs of chromosomes already disjoined. See drawing, Fig. 15*a*.

Fig. 16. Late first anaphase, showing chromosomes clumped into two rings joined by a thread (spindle).

Plate II, Figs. 17–27. *Wild type.*

Fig. 17. Late first anaphase (*left* ascus) and late second anaphase (*right*).

Fig. 18. First interphase.

Fig. 19. Second prophase (*left*); early first prophase (*right*).

Fig. 20. Second metaphase.

Fig. 21. Late second anaphase.

Fig. 22. Second interphase.

Figs. 23–24. Third anaphase.

Fig. 25. *Top left:* third prophase. *Centre:* early stage in formation of ascospores. *Bottom:* third telophase. *Top right:* ascus with nucleus in early first prophase and to left of it crozier with fusion nucleus in penultimate cell.

Fig. 26. Ascus (ruptured) with uninucleate spores with well developed walls.

Fig. 27. Mature binucleate spores.

Plate III, Figs. 28–43. (Figs. 28–41. *pro*1 *paba*1 $y \times ad14$ *bi*1; *w*3)

Fig. 28. Prophase of division of two nuclei of young crozier.

Fig. 29. Late anaphase of division of nuclei in young crozier.

Fig. 30. *Right:* ascus with nucleus in early first prophase. *Left:* secondary crozier with uninucleate, binucleate and uninucleate cells.

Fig. 31. *Right:* pachytene, showing knob at 3 o'clock (*arrow*). *Left:* crozier with uninucleate tip cell, penultimate cell with fusion nucleus, and uninucleate basal cell.

Fig. 32. Diplotene

Fig. 33. Mid-diakinesis.

Figs. 34–35. Diakinesis, showing differences in size between chromosomes. See drawing, Fig. 35*a*.

Fig. 36. First metaphase in polar view.

Fig. 37. First metaphase in side view.

Fig. 38. Early first anaphase; chromosomes disjoining simultaneously.

Fig. 39. Mid-first anaphase. Eight chromosomes could be counted in upper figure.

Fig. 40. Third metaphase.

Fig. 41. Delimitation of ascospores, each potential spore with single nucleus.

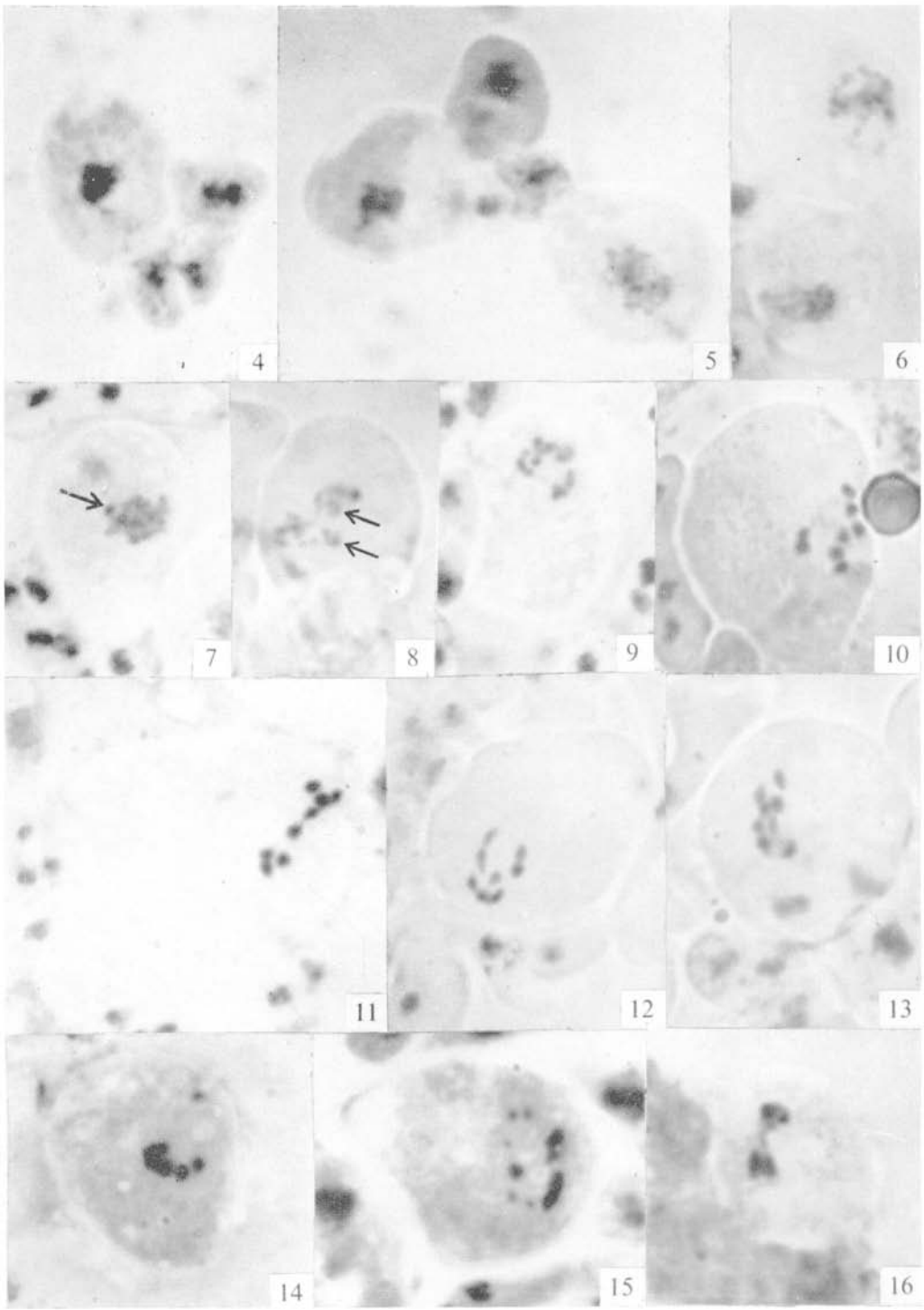
Fig. 42. Diploid A. First metaphase, showing spindle.

Fig. 43. *Wild type.* Ascus with a 'blob,' presumably the result of clumping of chromosomes at first metaphase.

Plate IV, Figs. 44 and 45.

Fig. 44. *Wild type.* Squashed perithecium. × 800.

Fig. 45. Diploid A. Squashed perithecium. × 800.



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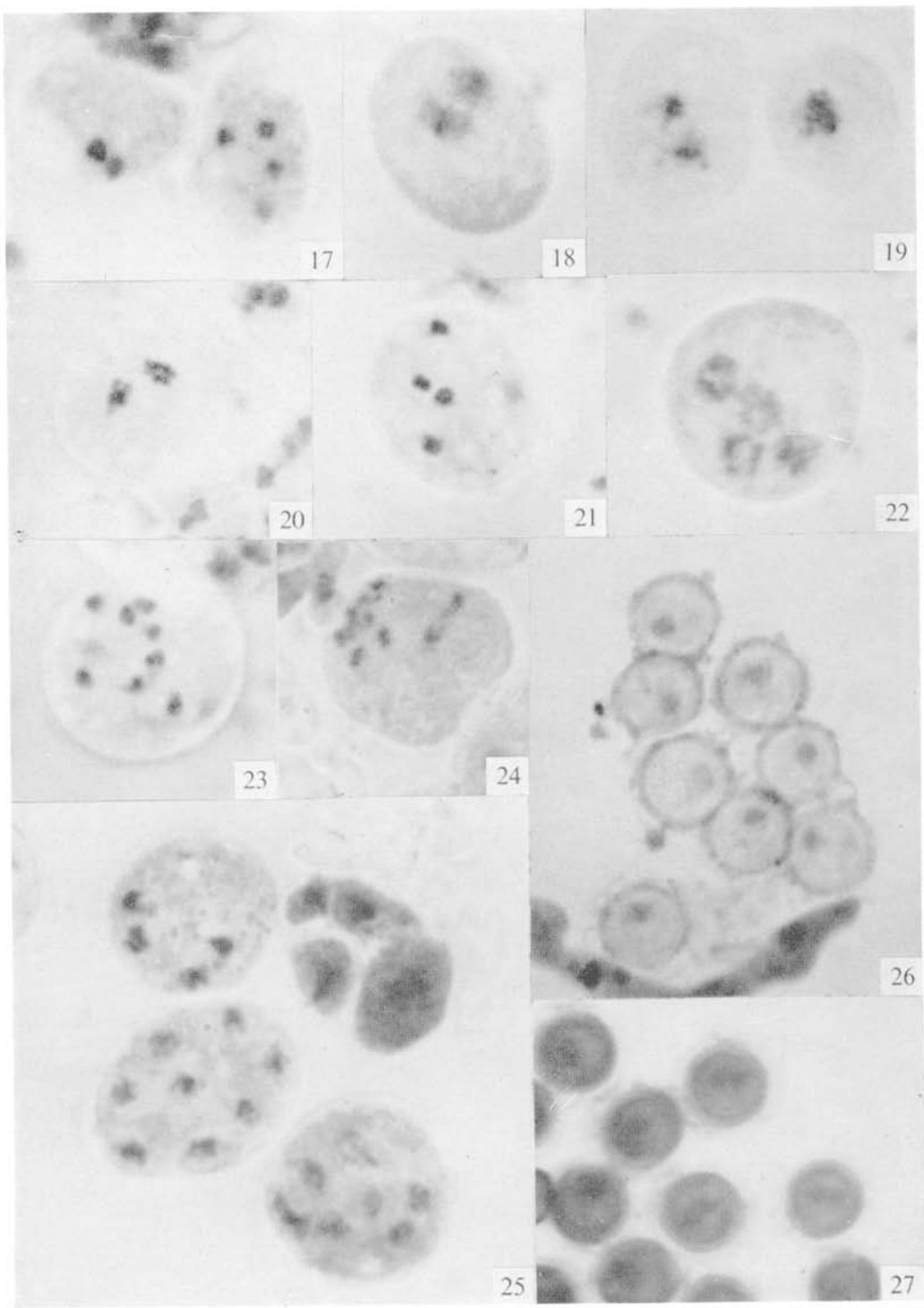

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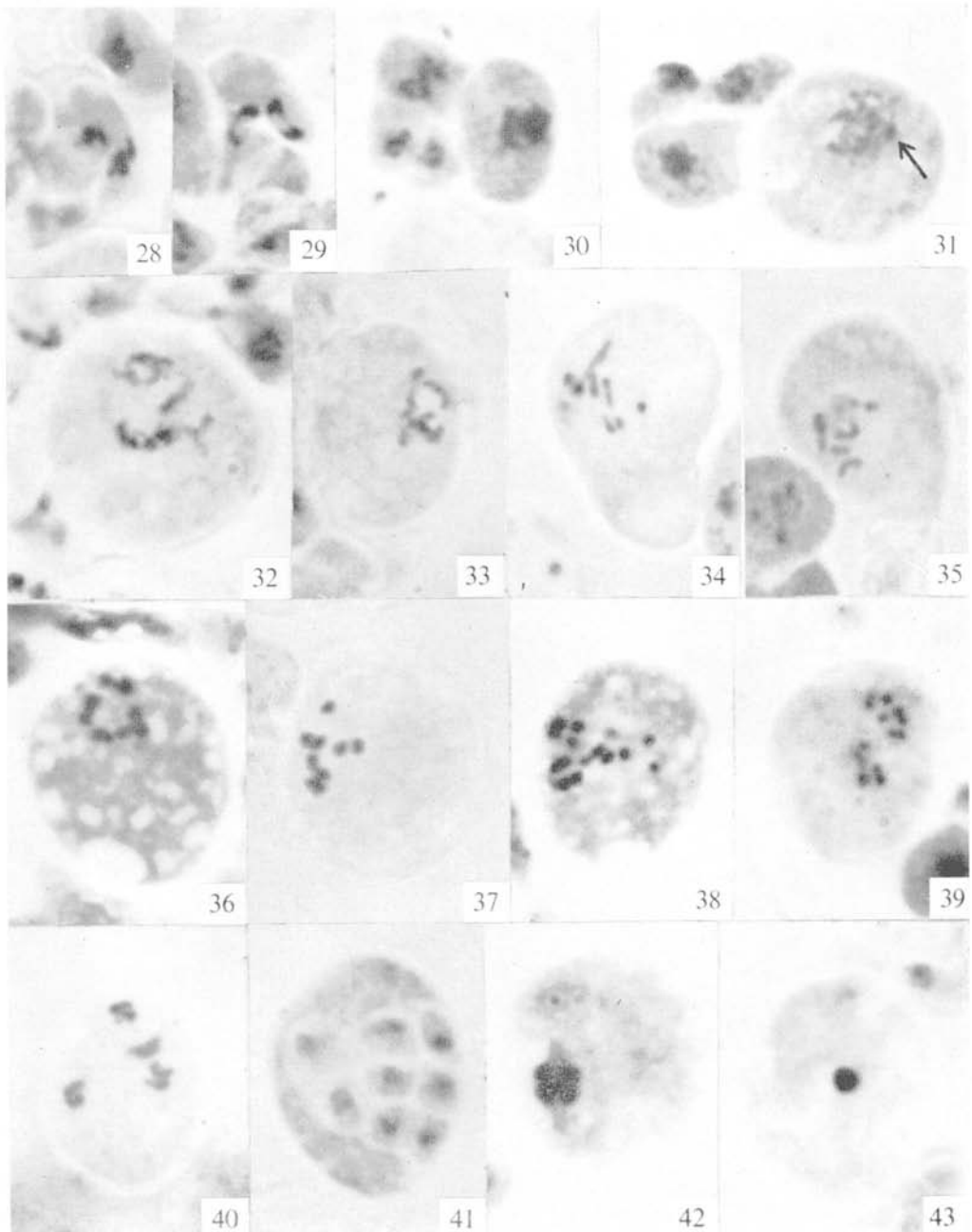

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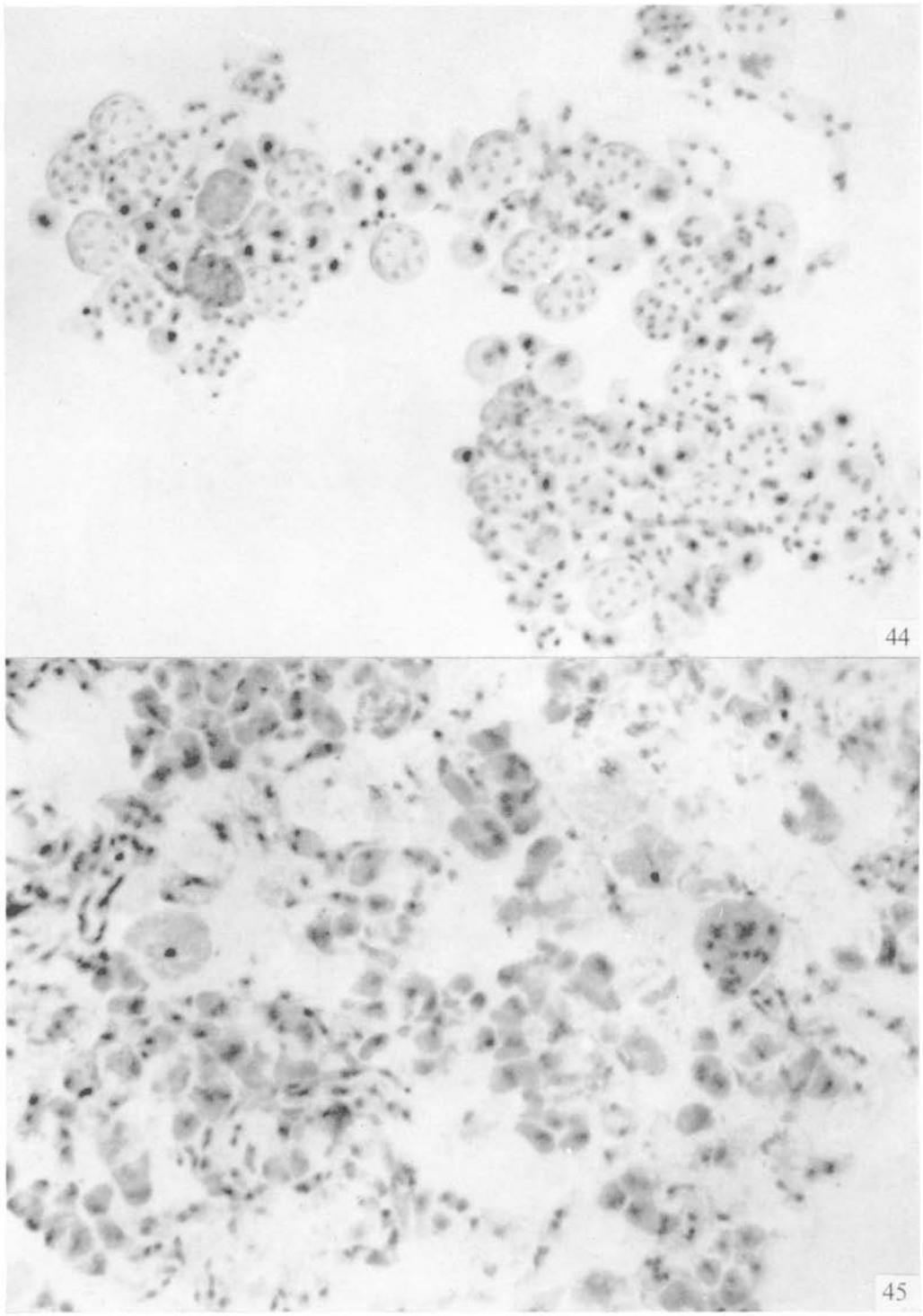

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of the eight nuclei, forming eight uninucleate spores. Generally not all the cytoplasm is included in the spores (Fig. 41). As the sculpturing of the wall of the ascospore develops, the contents are obscured, but later a single nucleus may again be seen inside (Fig. 26). Some time afterwards a further mitosis takes place, so that the spores are binucleate when mature (Adam, in Pontecorvo, 1953) (Fig. 27).

The above account refers principally to *wild type* (Figs. 4–27). The heterokaryon between *pro*1 *paba*1 *y* and *ad*14 *bi*1; *w*3, the two haploid strains from which Diploid B was synthesized (Figs. 28–41), differs in no essential cytological details from wild type, but there may be a greater number of sterile hyphae.

DIPLOID STRAINS

The general appearance of a preparation of the contents of a perithecium(cleistocarp) of a haploid strain, at the stage when the perithecial wall is beginning to turn dark in colour, is shown in Fig. 44. There are many asci in all stages of development from early meiotic prophase to the commencement of ascospore formation,

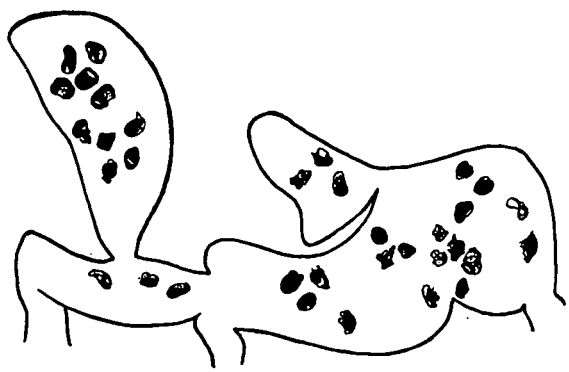
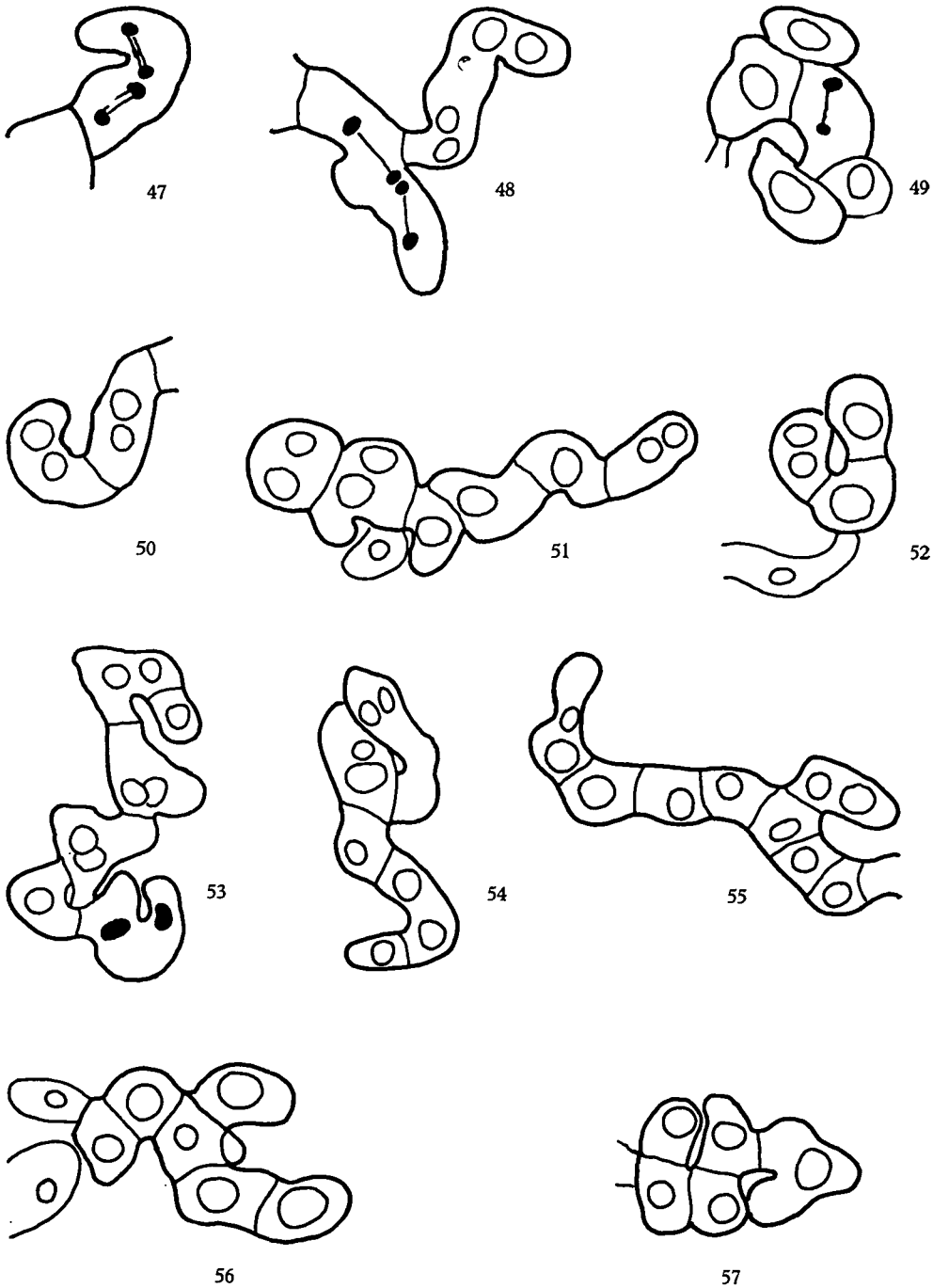


Fig. 46. Diploid A. Inflated sac of sterile hypha. × 1500.

and very few sterile hyphae. A preparation of the perithecial contents of a diploid strain at the same stage, as judged by the colour of the perithecium wall, is strikingly different, as shown in Fig. 45. There are many more sterile hyphae. These have little cytoplasm, small dense nuclei, and are irregular in shape; they may be inflated into large multinucleate sacs (Fig. 46). There are relatively few asci, and those present contain nuclei in pachytene or diakinesis and first metaphase, or have a single darkly staining blob. Typical stages later than first metaphase are very infrequent, and asci with spores are rarely seen. There are also coiled hyphae with densely staining cytoplasm and large diffuse nuclei with a large nucleolus (Figs. 58–59)—quite unlike anything in haploid strains.

In young perithecia, before any asci are recognizable, these coiled hyphae with dense cytoplasm may be distinguished from the sterile hyphae, and at this time they have both binucleate and uninucleate cells. In older perithecia, their cells are mostly uninucleate. The nuclei of both binucleate and uninucleate cells may divide (Figs. 47–49, 53). The terminal cell and penultimate cell of a hypha may



Figs. 47–57. Diploid A. 'Ascogenous hyphae' from a young perithecium. × 1800. Anaphase of nuclear division in Figs. 47–49; prophase in Fig. 53.

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both be binucleate (Fig. 50); these are evidently derived from the quadrinucleate state following nuclear division in a binucleate cell (Fig. 48). The terminal cell may be binucleate and the penultimate uninucleate (Figs. 51, 55), or the terminal cell may be uninucleate and the penultimate binucleate (Figs. 53, 54). Even when a hypha is curved at the tip and has uninucleate, binucleate and uninucleate cells in sequence, it does not look like the crozier of the haploid strains. The asci of diploid strains, it appears, do not develop from croziers, but from some of the uninucleate cells of the coiled hyphae.

That the ascus develops from a cell with a single (diploid) nucleus is indicated by observations on later stages. *The chromosome configurations seen at diakinesis and first metaphase in diploid strains are the same as those in haploids.* In Diploid B, eight normal bivalents are seen (Figs. 64–66). Diploid A has a fragment (see below), and the usual configurations are eight bivalents and a fragment either free or associated with one of the bivalents (Figs. 68–71)—the same as seen in the cross between the haploid strains from which the diploid was synthesized.

The bivalent structure of the chromosomes in diploid strains is clearly evident at diakinesis and first metaphase; indeed their appearance at diakinesis as two parallel rods is often more conspicuous in diploids than in haploids. At pachytene also the chromosomes are often much more clearly seen than in haploid strains at that stage. This may be due to the size of the asci in diploid strains; they are larger and more rounded in outline than those of haploids (Table 1; compare Figs. 8–15 and 33–39 with Figs. 64–66 and 68–72, which are all at the same magnification). The chromosomes themselves are not, however, noticeably larger in diploid than in haploid strains.

Table 1. *Dimensions of squashed asci at diakinesis–first metaphase*

		Mean diameter (μ)			Ratio length/breadth		
	No. of cells	Range	Mean	Standard deviation	Range	Mean	Standard deviation
Haploids	60	9–15	11·6	1·31	1·00–1·85	1·28	0·175
Diploids	65	10–16	13·9	1·34	1·00–1·56	1·14	0·108

The stages most frequently seen are pachytene and diakinesis–first metaphase. Asci with two or four nuclei, or showing the second and third nuclear divisions, or containing ascospores are rare; much less frequent, even in older perithecia, than would be expected from the number of first metaphases. This suggests that many asci degenerate at first metaphase. It is presumed that Fig. 61 (a normal first metaphase in polar view), and Figs. 62 and 63 represent three stages of degeneration. Meiosis proceeds normally up to first metaphase (though possibly more slowly than in haploid strains), but first anaphase frequently fails to occur, and the chromosomes clump together to form a darkly staining 'blob' (Fig. 63). These blobs are a conspicuous feature of older perithecia. They are not seen in young perithecia with only a few asci, and they are seen only in large asci, as large as or larger than asci at diakinesis and first metaphase.

These dense blobs are occasionally seen in haploids (Fig. 43). One is illustrated by Pontecorvo (1953, Fig. 4*b*), who considered it to be the zygote nucleus. In his figure, the ascus is too large for this to be the correct interpretation. Blobs may be more frequent in some haploid auxotrophic strains than in wild type, but even so are much rarer than in diploids where they are extremely common.

Soon after any spores formed are mature, the contents of other asci and of the coiled hyphae degenerate, and one then finds some straight hyphae, very like those of vegetative mycelium, in the perithecium, together with a few ascospores and a lot of detritus.

In the first diploids synthesized, asci with 16 spores were frequently seen; 8-spored asci were found with them in the same perithecium (Pontecorvo & Roper, in Pontecorvo, 1953). I have found 16-spored asci to be somewhat rarer than is suggested by Pontecorvo and Roper. The majority of asci in Diploid A were 8-spored; and in Diploid B I have seen no 16-spored asci at all. Nothing definite has been discovered about their origin.

EXPLANATION OF PLATES

Plate V, Figs. 58–66.

Figs. 58–59. Diploid A. Overstained preparation showing coiled hyphae with dense cytoplasm and sterile hyphae with little cytoplasm. × 1800.

Fig. 60. Diploid A. Cells of coiled hyphae enlarging and becoming asci.

Fig. 61. Diploid B. First metaphase in polar view.

Fig. 62. Diploid A. Chromosomes at first metaphase clumping into a ring.

Fig. 63. Diploid B. 'Blob' of clumped chromosomes.

Figs. 64–66. Diploid B. First metaphase showing eight bivalents.

Plate VI, Figs. 67–80. (Figs. 67–72. Diploid A).

Fig. 67. Diakinesis.

Fig. 68. First metaphase, showing single free fragment towards one pole of the spindle.

Figs. 69–70.* Late diakinesis, showing single fragment associated with Chromosome 6 and seven other bivalents.

Fig. 71.* Single fragment associated with Chromosome 6 (*centre bottom*), six other bivalents and (*top*) pair of Chromosome 8 univalents.

Fig. 72.* Two fragments, one associated with Chromosome 6 and one free (at 4 o'clock); seven other bivalents.

Figs. 73–74.* *ad*2 *y*. Diakinesis with nine bivalents.

Figs. 75–80. *ad* 2 *y* × *ribo*1 *bi*1.

Fig. 75.* First metaphase, showing single fragment associated with Chromosome 6.

Fig. 76. Ascus with four normal ascospores at early stage of wall formation and four nuclei (in two pairs) not in normal spores.

Fig. 77. Mature ascus with four normal spores and two small degenerate spores.

Fig. 78. Mature ascus with three normal spores and four small degenerate spores.

Fig. 79. Mature ascus with one normal spore and five degenerate spores.

Fig. 80. Ascus with two normal anaphase figures and a scattered group of chromosomes.

* See drawings, Figures 69*a*–72*a*, 73*a*–74*a*, 75*a*.

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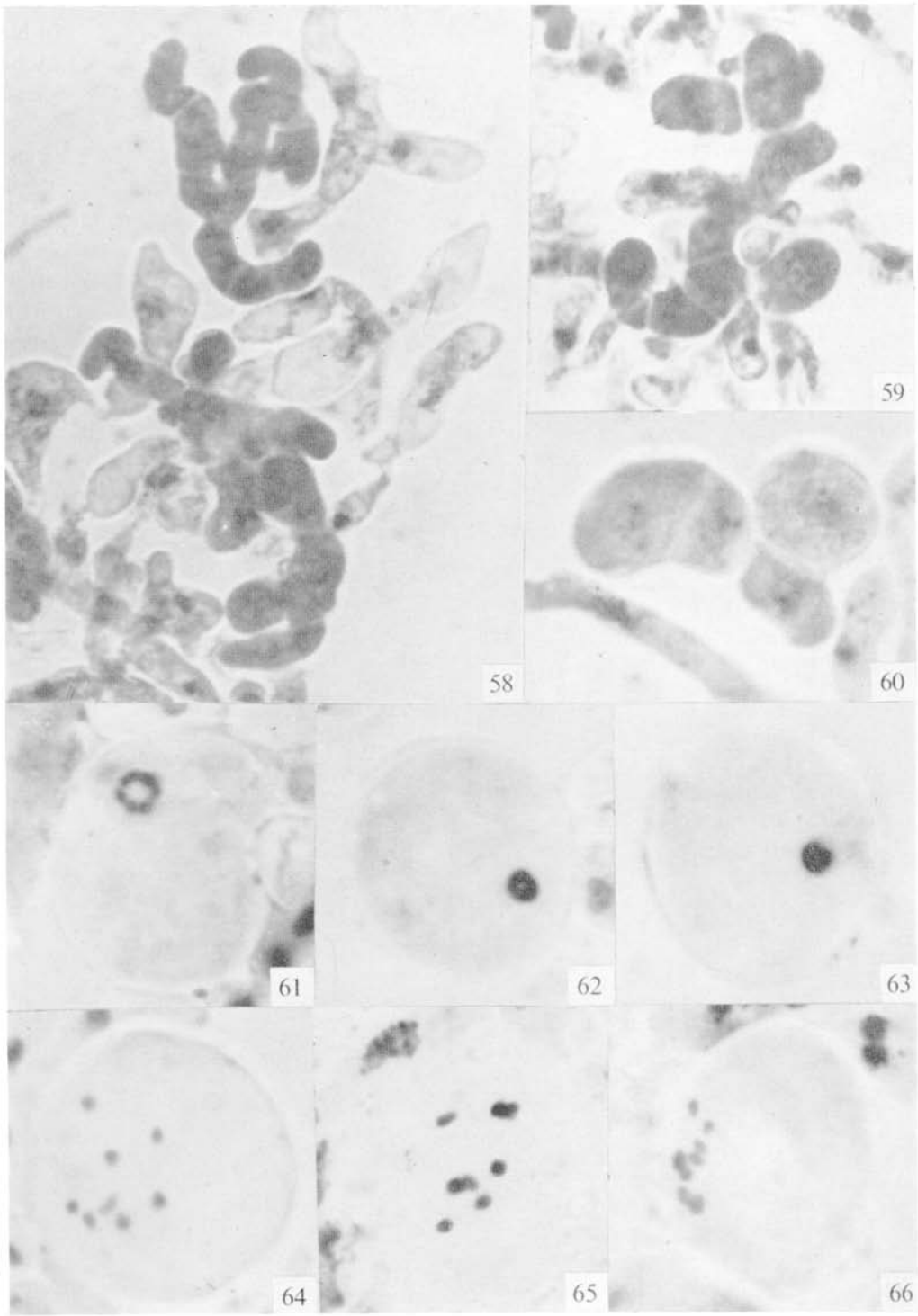

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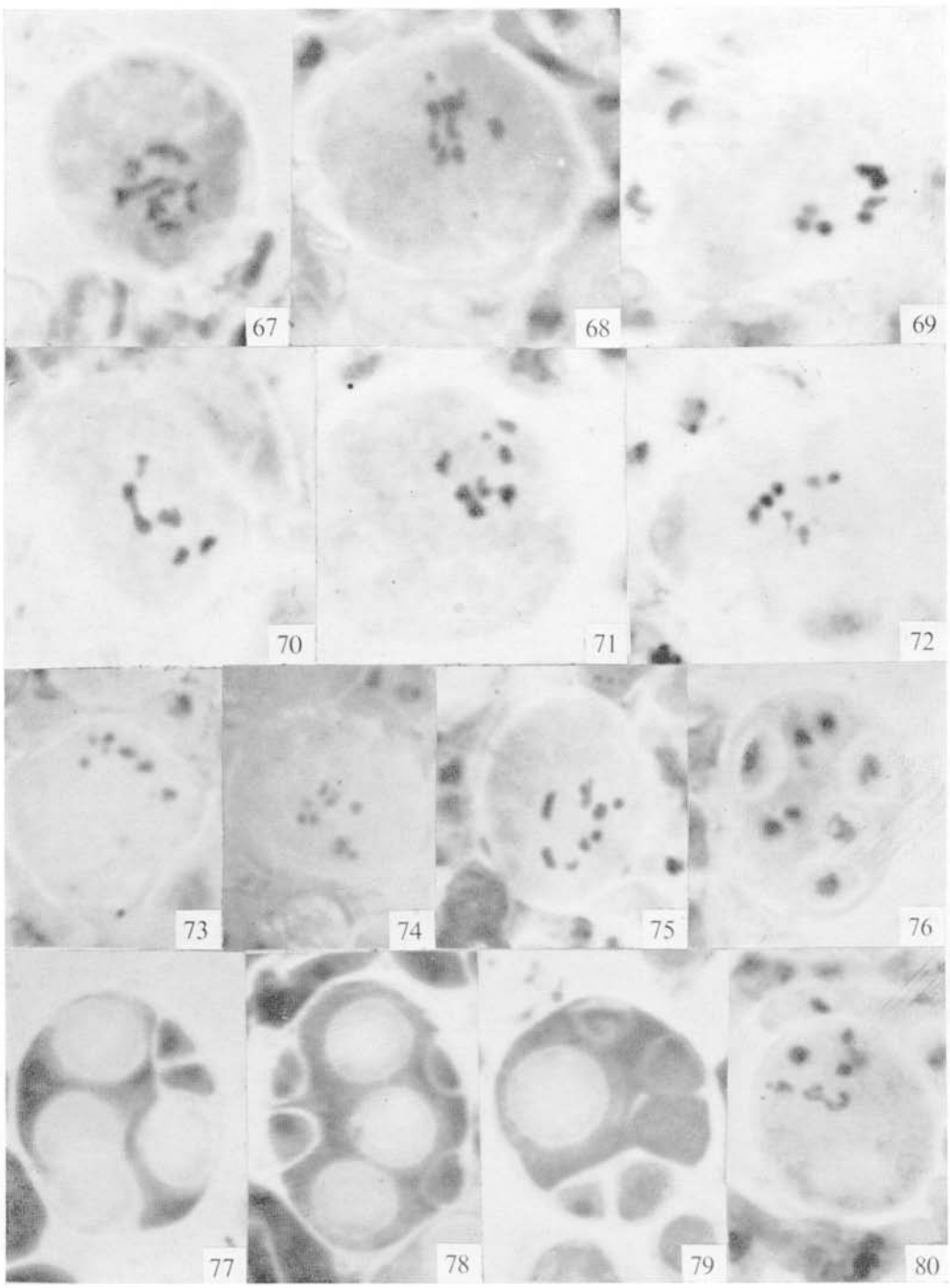

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The observation that in diploid strains the ascus has a single diploid nucleus which undergoes meiosis predicts that ascospores should be haploid. This expectation is born out by the ploidy of cultures derived from ascospores isolated by micromanipulation from perithecia of two diploids. From one diploid, 151 spores were isolated and 54 grew. From the other, that called Diploid B and studied in detail cytologically, 416 spores were isolated and 38 grew. Some cultures were derived from spores dissected from 8-spored asci, but most were from free spores. Of the 92 cultures, 91 were haploid (so classified according to the diameter of their conidia; see Pontecorvo, Tarr Gloor & Forbes, 1954) and one, from Diploid B, was diploid. The latter culture was, like the parent diploid, a green prototroph, and analysis of mitotic segregants from it showed that its genotype was the same as that of the parent strain. It may well have been a conidium isolated by mistake.

THE *DIPLOID A* FRAGMENT

In Diploid A eight bivalents and a fragment were observed at diakinesis and first metaphase. The fragment was either free (Fig. 68) or more often associated with one of the larger of the three small chromosomes (Figs. 69–71, 69*a*–71*a*). The chromosome with which the fragment pairs will be defined as Chromosome 6.

Diploid A was synthesized from the two haploid strains *ad*2 *y* and *w*1 *lys*1. The heterokaryon between these two strains was made and perithecia from it examined. These perithecia had, of course, the morphology of perithecia of a single haploid strain. The chromosome configurations seen at first metaphase were the same as in Diploid A, a single fragment being either free or associated with Chromosome 6.

In a homothallic fungus, the perithecia which develop on heterokaryons may arise from one or other of the component strains, or from both (hybrid perithecia). When the strains in a heterokaryon differ by conidial colour markers, the kinds of perithecia may be identified by streaking ascospores from them on complete medium and observing the colours of conidia which develop (Hemmons, Pontecorvo & Bufton, in Pontecorvo, 1953). The heterokaryon *ad*2 *y* + *w*1 *lys*1 was found to give a rather low proportion of hybrid perithecia; some others gave a very high proportion. The hybrid perithecia produced by *ad*2 *y* + *w*1 *lys*1 (and similar heterokaryons) growing in slants were larger and developed earlier than the selfed perithecia, and hence they could be selected for cytological examination. It was shown by Hemmons *et al.* and by Strickland (1956) that in hybrid perithecia the vast majority of *asci*, if not all of them, are hybrid (cf. Olive, 1954). Hence one can be confident that the chromosomes examined are those of hybrid asci.

In Diploid A, two fragments were sometimes present. In such cells, one fragment was as a rule associated with Chromosome 6 and the other was free (Figs. 72, 72*a*). Both in this case, and when there was only one fragment, the other chromosomes had their normal bivalent appearance. Presumably the fragment may undergo mitotic non-disjunction. The similarity in appearance of first metaphases in Diploid A and the heterokaryon, and of the chromosomes other than Chromosome 6 in cells with one and with two fragments is good evidence that in the asci of diploid strains a diploid, not a tetraploid, nucleus undergoes meiosis.

The strains from which Diploid A was made were examined separately, and the heterokaryons between each and a third strain, *ribo*1 *bi*1, were studied, to find out which strain contributed the fragment. In *ad*2 *y*, which is self-fertile, nine bivalents are normally seen at first metaphase (Figs. 73–74, 73*a*–74*a*). One of them is about half the size of the smallest of the other eight. (In Fig. 71 (from Diploid

Fig. 69*a* Fig. 70*a* Fig. 71*a* Fig. 72*a*

Figs. 69*a*–72*a*. Diploid A. Drawings of cells shown in Figs. 69–72. *f* indicates fragment.

A) the fragment is seen to be about half the size of the Chromosome 8 univalents.) In about one-fifth of cells, eight bivalents and two small univalents (presumably) were present. No abnormal asci were observed in *ad*2 *y*, apart from one with eleven spores. In the heterokaryon *ad*2 *y*+*ribo*1 *bi*1 the configurations observed at first metaphase (cf. Fig. 75) were the same as in the heterokaryon *ad*2 *y*+*w*1 *lys*1. Highly abnormal asci were found in the perithecia from these two heterokaryons.

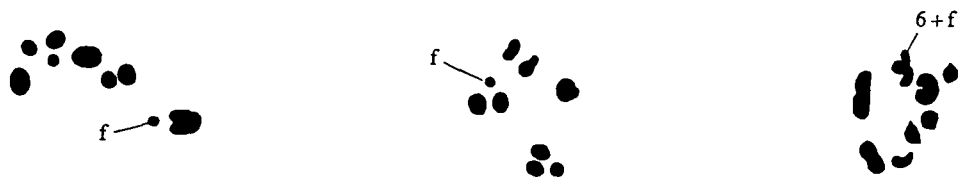

Fig. 73*a* Fig. 74*a* Fig. 75*a*

Figs. 73*a*–74*a*. *ad*2 *y*. Drawings of cells shown in Figs. 73–74. Smallest (fragment) bivalent indicated by *f*.
Fig. 75*a*. *ad*2 *y* × *ribo*1 *bi*1. Drawing of cell shown in Fig. 75. Single fragment associated with Chromosome 6 (6+*f*).

Many spores were abortive; and the number was frequently less than eight (Figs. 76–79 and Table 2). Asci with less than eight spores are the result presumably of some nuclei of an ascus failing to divide properly: Fig. 80 shows an ascus with two anaphase figures and an irregularly scattered group of chromosomes. Similar abnormal asci occur in Diploid A.

The strain *w*1 *lys*1 is completely self-sterile, and no perithecia were formed by the heterokaryon between it and *ribo*1 *bi*1. However, since the heterokaryons *ad*2 *y*+*w*1 *lys*1 and *ad*2 *y*+*ribo*1 *bi*1 were similar, it is clear that the fragment in Diploid A is contributed by *ad*2 *y*. No abnormal asci were found in crosses between *ribo*1 *bi*1 and *pro*1 *paba*1 *y* (a 'normal' strain—one of the parents of Diploid B).

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Table 2. *Abnormal asci in the cross* ad2 y × ribo1 bi1

Number of										
normal spores	8	7	6	6	5	5	5	4	4	
abnormal spores	–	–	–	2	3	2	1	4	2	
Asci observed	29	3	5	17	2	3	3	2	19	
normal spores	3	3	3	3	3	2	2	2	1	1
abnormal spores	5	4	3	2	1	5	4	2	4	3
Asci observed	2	2	2	1	1	3	1	2	1	2

The strain *ad2 y* was obtained by irradiation of *y* with X-rays. *y* has the normal complement of eight chromosomes. The persistence of the fragment in *ad2 y* through many mitotic divisions indicates that it is necessary for viability, or at least that its presence confers some advantage; but the precise nature of the change which occurred in Chromosome 6 cannot be stated on the evidence available. One possibility is that concurrently with the mutation to adenine dependence, Chromosome 6 was broken at the centromere and in one arm, giving rise to a centric fragment and a chromosome with a deletion. If this were so, the configuration seen in Diploid A would be an association of the normal Chromosome 6, the deficient chromosome and the fragment. Heterokaryon tests (Pritchard, 1955) indicate that *ad2* is allelic with *ad9*, which is located in Linkage Group I. Pritchard (1955) found abnormally low recombination fractions in crosses between *ad2 y* and 'normal' strains marked only in Linkage Group I. The information available however is not enough to show that Linkage Group I is on Chromosome 6.

DISCUSSION

The present study has shown that the haploid number of chromosomes in *Aspergillus nidulans* is eight. Earlier statements that it is four (Pontecorvo, 1953; Elliott, 1956) are incorrect. It is to be noted that eight linkage groups have been established by mitotic analysis (Käfer, 1958). The chromosomes differ somewhat in length. Analysis of the chromosomes at pachytene should be possible in diploids, though this is scarcely feasible in haploids.

Normally the diplophase in the life history of *Aspergillus* is confined to the single fusion nucleus in the young ascus. But in addition to this normal cycle, there is a 'parasexual cycle' (Pontecorvo, 1954) with a prolonged diplophase. The diplophase begins with fusion of haploid nuclei in the vegetative mycelium; it ends with mitotic breakdown of diploid nuclei through aneuploidy (Käfer, 1957) to haploidy, and, as now shown, by meiosis.

It has been shown that in the development of asci in diploid strains, the young ascus has a single diploid nucleus which undergoes meiosis, giving haploid ascospores. The most convincing evidence for this apogamous development of asci lies in the chromosome configurations themselves: they are the same as seen in the cross between the two haploid strains from which the diploid was synthesized. The structure of the chromosomes as bivalents is clearly recognizable at late dia-

kinesis in the diploids. No evidence of a tetraploid meiosis has been found, although this cannot be excluded as a rare event.

The diploids differ essentially from haploids in the absence of true croziers and presence of the coiled hyphae with dense cytoplasm. These hyphae, which may be called 'ascogenous hyphae', are made up mainly of uninucleate cells. The variability of the positions in which binucleate cells occur in them suggests that wall formation is not precisely controlled. Each of the cells is potentially an ascus: all the nuclei of the 'ascogenous hyphae' indeed resemble meiotic prophase nuclei in appearance. Some of the cells, particularly in lateral or terminal positions, enlarge (Fig. 60) and become asci. Binucleate (as well as uninucleate) cells are sometimes seen rounding off in this way, but the stages expected, were they to develop further, have not been seen.

One of the remarkable things about the diploids is their sterility. Some haploid auxotrophic strains are self-sterile, producing few small perithecia if any, but they may cross readily with other strains, producing, as with self-sterile strains, perithecia packed with asci and having very few sterile hyphae. Self-sterility in these strains seems to result from a failure of perithecium initiation. In contrast, both the diploids studied produce perithecia in great numbers: the sterility arises as a consequence of processes taking place within the perithecia. In the first place, there are many sterile hyphae in the perithecia, and the 'ascogenous hyphae' bear few asci. The number of asci varies considerably between different diploids; Diploid A has more per perithecium than Diploid B, but they are always few compared with haploid strains. Secondly, many asci degenerate at first metaphase. The numbers of asci in pachytene and diakinesis–first metaphase suggest that meiosis proceeds normally up to the latter stage. But compared with the number of first metaphases, very few asci in subsequent stages and with spores are seen. First anaphase frequently fails, and the chromosomes clump together.

The physiological basis of the sterility of the diploids is an intriguing problem. There is a striking difference in fertility between a diploid strain and the cross between its haploid parents. The diploid state of the mycelium itself must somehow be inimical to the proper functioning of the sexual processes.

SUMMARY

In haploid strains of *Aspergillus nidulans*, asci arise from croziers. The two nuclei of the young ascus (the penultimate cell of the crozier) fuse, and the zygote nucleus immediately undergoes meiosis. At diakinesis and first metaphase eight bivalents are seen: three large (one, Chromosome 2, with a satellite), two medium sized (Chromosomes 4 and 5), two small (Chromosomes 6 and 7) and one very small (Chromosome 8). The perithecia of haploid strains are packed with asci and have very few sterile hyphae.

Diploid strains (heterozygotes made by Roper's technique) are very different from haploids in that the perithecia contain many sterile hyphae with little cytoplasm and coiled hyphae with dense cytoplasm made up mainly of uninucleate cells, and there are few asci in a perithecium. Croziers are absent: some of the cells

of the coiled hyphae become asci. At diakinesis and first metaphase, the same chromosome configurations are seen as in a cross between the haploid strains from which the diploid was synthesized; the chromosomes are bivalents. The young ascus thus has a single (diploid) nucleus which undergoes meiosis, and asci develop apogamously. No evidence of nuclear fusion in the young ascus and of a tetraploid meiosis was obtained. Cultures from ascopores isolated by micromanipulation from perithecia of diploids were all haploid. Meiosis appears to proceed normally to first metaphase, but typical later stages, and asci with spores, are rarely seen. First anaphase frequently fails to occur, and the chromosomes clump together.

In the strain *ad*2 *y*, obtained from a normal strain by X-irradiation, nine bivalents are present, one a very small fragment. In crosses between *ad*2 *y* and a normal strain, and in a diploid made from them, the fragment pairs with Chromosome 6. Asci in the cross between *ad*2 *y* and a normal haploid are highly irregular, often with less than the usual eight spores.

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