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NIDULANS (EIDAM) WINT......

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THE UNIVERSITY OF ALBERTA

THE SOMATIC NUCLEAR EVENTS DURING
HYPHAL DIFFERENTIATION IN ASPERGILLUS
NIDULANS (EIDAM) WINT.,

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

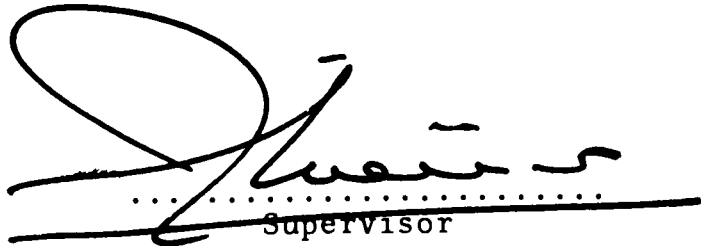
DEPARTMENT OF GENETICS

EDMONTON, ALBERTA

SEPTEMBER, 1968

UNIVERSITY OF ALBERTA
FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "The somatic nuclear events during hyphal differentiation in Aspergillus nidulans (Eidam) Wint.," submitted by Stanley Herbert Weisberg in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

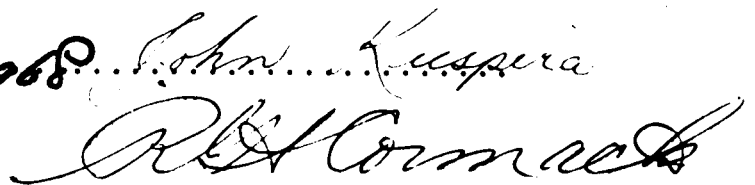

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ABSTRACT

Somatic karyokinesis in the hyphae of Aspergillus nidulans can be divided into at least two distinct chromosome cycles. The first, or Juvenile cycle, is restricted to young fast growing hyphae. During resting stage the nucleus in the conidium is contracted and irregular to spherical in shape. On incubation, the nucleus unfolds and forms a nuclear filament to which a terminal extrachromosomal body is attached. The nuclear filament is particulate in nature containing nine Feulgen-positive bodies. It is assumed that these chromatin bodies represent the eight chromosomes of A. nidulans together with a DNA-containing centriole. The nuclear filament can attain several configurations such as linear filaments, crescent- or horseshoe-shaped and ring-shaped. It is believed that crescent-, horseshoe-, and ring-shaped nuclei are nuclear filaments wound around a disc-like 'central body' (nucleolus), and that these configurations arise as a result of nuclear streaming and nucleolar development.

Prior to nuclear division, the centriole divides. No spindle apparatus can be detected during nuclear division and it is thought that cytoplasmic streaming forces separate the daughter nuclei. After division the daughter nuclei can be either filamentous, crescent- or ring-shaped.

On further incubation the juvenile nuclear structures divide endomitotically to yield large polytene filamentous nuclei. These large nuclei appear prior to the formation of the foot cell and the conidiophore. It is believed that these nuclei (Maturation cycle) are directly associated with foot cell differentiation. A nuclear fiber attaches the eight chromosomes and the centriole end to end and this fiber may become very long, spreading the chromosomes far apart from one another, and thus the nucleus may easily extend over a length of approximately 100 μ . Phase contrast microscopy after staining with Feulgen as well as bright field microscopy after staining with Acid Fuchsin reveal the nature of the nuclear fiber.

These long polytene nuclei divide longitudinally without a recognizable spindle apparatus, and transverse division of the individual chromosomes of the nucleus is often observed.

The polytene maturation nuclei are also present in the conidiophore body prior to secondary phialide formation and they arise from the original juvenile nuclei which entered the conidiophore bud from the foot cell. Phialide nuclei originate from the original conidiophore juvenile nuclei. The conidial nuclei are products of the phialide nuclei.

Possible hyphal diploid nuclei were observed as excessive polytene maturation nuclei but they did not appear to be intimately associated with the common nuclear events leading to conidial formation.

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INTRODUCTION

In an earlier publication (Weijer and Weisberg, 1966; Weisberg, 1966) it was observed that although the genetic analysis of linkage in the Ascomycete, Aspergillus nidulans by means of somatic crossing over was well established, a complete blank still existed with regard to the somatic cytology of the fungus. In this earlier study an investigation was made into the somatic nuclear events in the undifferentiated hyphae of A. nidulans. In the present study an investigation into the somatic nuclear events of hyphal differentiation leading to the production of asexual spores (conidia) is presented. As was shown previously (Weijer et al., 1965), different modes of filamentous nuclear division are operative in the related fungus Neurospora which lead to the production of asexual structures.

Although there is no direct evidence that the vegetative karyokinetic events in Aspergillus facilitate genetic exchange in a heterokaryon, the filamentous nucleus with its longitudinal mode of division as outlined, offers a more acceptable explanation for the occurrence of the parasexual cycle than the one based upon classical mitosis. Since both Aspergillus and Neurospora are functionally coenocytic (Moore and McAlear, 1962), the question also arises whether or not this filamentous mode of division and variability in nuclear structure during differentiation applies to all coenocytic organisms exhibiting a parasexual cycle.

In the present study, the DNA-specific Feulgen staining technique was employed throughout the investigation, thereby leaving no doubt as to the chemical nature and actual size of the visible structures. Since commonly accepted fixation techniques were omitted with the Feulgen technique, Navashin-fixed crystal violet stained preparations were used as well, to demonstrate that the novel nuclear configurations were not artifacts caused by the omission of fixation.

LITERATURE REVIEW

From the existing literature it becomes evident that three distinct periods of research can be distinguished with regard to the cytology of somatic nuclei in fungi: (1) early research prior to 1930; (2) the period from 1930 to 1955 and (3) research from 1955 to the present. These eras are delimited by cytological controversies prevalent during those times, as well as by the techniques and apparatus available to the researchers.

I

During the first period, cytological research on the Aspergilli was primarily concerned with the fusion of the gametes in the developing ascus (brachymeiosis) together with the gross development of the perithecia and cleistothecia. Microphotographic techniques were rarely employed and all the cytological evidence was presented by camera lucida drawings. Since the hyphal nuclei of the Aspergilli are extremely small, poor microscopic resolution prevented the researchers from seeing much nuclear detail. Also, it has been suggested (Yuill, 1950) that there might be some ambiguity and uncertainty in the allocation of the species studied in this early period, in relation to the species as they are now known and listed (Thom and Raper, 1945; Raper and Fennel, 1965). Fixation and

staining techniques universally in use during that time were later discarded as being unreliable (Olive, 1953; Pinto-Lopes, 1949; Weijer et al., 1965).

Dangeard (1907) in a fairly extensive study of the Ascomycetes investigated the cytology of 6 species of Aspergilli. Included was the early generic name of Aspergillus nidulans: Sterigmatocystis nidulans. While some species of Aspergilli were found to have multinucleate sterigmata (phialides) and conidia, S. nidulans was described as having uninucleate conidia, and uninucleate secondary and primary sterigmata. Development of the conidiophore was described as follows: A nucleus enters the primary sterigmatal bud from the conidiophore vesicle and divides to produce a nucleus for each of the secondary sterigmatal buds. When the secondary sterigma matures, this nucleus divides to produce nuclei for each of the successive conidia which on maturation bud off from the sterigma. This series of events was found to be generally common to all the Aspergilli studied. No methods were given.

Frazer and Chambers (1907) studied the morphology and cytology of Aspergillus herbariorum (= Aspergillus glaucus) using Fleming's triple stain and Heidenhain's haematoxylin. They agreed with Dangeard's observation that the sterigmata of this species were multinucleate and described the conidiophores

as also being multinucleate. The mycelium was found to be coenocytic and nuclear division was not discussed. The mature conidia contained four nuclei and the mature ascospore eight. Nuclear fusion and ascus development were discussed in detail.

Dale (1909) investigated the morphology and cytology of Aspergillus repens. The material was fixed in Fleming's solution of chrome-acetic acid and stained with aniline-gentian-violet followed by eosin in clove oil. The vegetative mycelium was described as multinucleate rather than coenocytic. The conidia were found to contain "about a dozen nuclei of different size" (p. 219). The formation of the ascus was considered in detail and the young ascospores were described as containing "several stainable bodies" (p. 223) which in the mature ascospore condensed to form one nucleus.

II

A marked decline in interest in fungal cytology was evident during the next twenty years but in the 1930's a definite advance in cytological technique was noticeable (although camera lucida drawings were still in widespread use). Many excellent reviews on fungal cytology of this period are available (Cutter, 1951; Martens, 1946; Namboodiri, 1964). However, scant interest in nuclear division and cytology of the

somatic hypha during this period is evident. Of the available reviews, Martens presents a monumental review of the research concerning the life cycles and sexuality throughout the Ascomycetes from as far back as the late 19th century.

During this middle period, some researchers were beginning to question the techniques in use. The controversy surrounding the specificity of the Feulgen nuclear reaction (Stowell, 1945) played an important role in this era. Unfortunately, even today the use of the Feulgen reaction as a nuclear specific stain is not widespread in fungal cytology (Weijer et al., 1965).

Pinto-Lopes (1949) in a comprehensive review of the nuclear structure of the fungi points out, that because of the smallness of the nucleus, investigators have used techniques which would tend to favour the detection of fungal nuclei at the expense of nuclear detail: "when the colorations by the Feulgen reaction are compared with those obtained by the haematoxylin, this latter presents the advantage of giving a strong nuclear coloration, but, on the other hand, these nuclei many times seem homogeneous, whereas those stained by the Feulgen reaction may show certain details of structure" (p. 197). He further observes that with iron haematoxylin, "the intensity of the coloration to be obtained

is determined by the observer himself, according to the differentiation." "Of the other colorations used, among those most worthy of mention is that due to the Feulgen reaction" (p. 197). Pinto-Lopes (loc. cit.) using these more precise methods, found that the structure and composition of the nuclei of fungi was not the same as in higher plants. Three types of fungal nuclei were described: homogeneous-, disperse-, and thread nuclei.

Wakayama (1931) studied the nuclear division in the sterigmata of 13 Aspergillus species not including Aspergillus nidulans. Material was fixed in Bonn's solution or Bonn's modified Fleming's solution. Sections were cut (1 - 3 μ) and stained with Heidenhain's iron-alum haematoxylin. Wakayama observed the nuclear division in uninucleate sterigmata to be essentially the same as in higher organisms. However, the nucleus in the resting stage was described as being associated with a, "large stainable body" (p. 299) which was identified as, "a caryosome, in which all or a part of the chromatin substance is localized or stored" (p. 299). At the onset of mitosis the stored chromatin separated from the caryosome and formed a definite number of chromosomes ($n = 2$). The remaining portion of the caryosome (viz. the nucleolus) usually disappeared. The centrosome was also part of the caryosome and this also separated from the caryosome at the onset of mitosis. It then divided and a spindle developed between the two bodies.

At the end of mitosis the centrosome and daughter chromosomes united at the spindle pole to constitute another caryosome. The nuclear reticulum, if present, was not intimately associated with chromosome formation -- "a state of affairs very different from that as commonly known in the higher plants and animals" (p. 299).

Whelden (1940) briefly discussed nuclear divisions in Aspergillus niger. Several fixatives were employed in conjunction with Heidenhain's haematoxylin. The conidia and sterigmata were both described as uninucleate. In a mutant "giant" form, nuclear division in the sterigmata showed chromosome numbers of $n = 2$ and $n = 4$. Nuclei at the tips of hyphae were observed to have chromosome numbers of $n = 2$ at anaphase with the annotation that: "except when these chromosomes were separating at anaphase, they were so close together that it was impossible to make out details clearly" (p. 639). The author was unable to discern the number of chromosomes in the nuclei at the hyphal tips of the "giant" form. He described the nuclear divisions in the hyphae of germinating conidia as following one another "in rapid succession" (p. 640) and stated that each nucleus showed "quite distinctly its two chromosomes, especially when the latter separated in pairs during anaphase" (p. 640). Details of nuclear divisions, however, were not discussed except for

the statement that: "The results of the present study are substantially in agreement with those of Wakayama" (p. 637).

Olive (1944) studied the vegetative hyphae in developing perithecia (cleistothecia) of Aspergillus Fischeri, using material fixed in Navashin's and stained with haematoxylin. His description of the resting nuclei of these vegetative hyphae is similar to Wakayama's (loc. cit.) description of the resting nucleus of the sterigmata. Details of nuclear division, however, are not discussed. Olive (1953) in a later review publication discussed Wakayama's karyosome and stated that: "This type of structure is frequently attributed to vegetative nuclei throughout the fungi" (p. 484) and attributed it to improper fixation and staining techniques: "Apparently, as a result of a peculiar sensitivity of these nuclei to most fixatives, the chromatin collapses around the nucleolus and both stain as one body-----this is less likely to occur with the carmine technique and with certain carefully mixed fixatives in common use" (pp. 484 and 485). He concludes that Wakayama's occasional observations of a nuclear reticulum associated with chromosomal formation was evidence that, "a few of the resting nuclei were properly fixed" (p. 485).

Baker (1945) studied the cytology of conidial formation and germination in 4 species of Aspergilli not including A. nidulans. Material was fixed in Bouin's fluid and stained in Heidenhain's iron-alum haematoxylin. The latter

three species were described as having uninucleate phialides and giving rise to uninucleate conidia. The details of the mechanics of nuclear division, however, were not given nor was the number of chromosomes per nucleus. In Aspergillus fumigatus and A. clavatus, anastomosis of the hyphae often preceded the formation of "plurinucleate" (p. 585) conidiophore initials. The developing conidiophores were described as being, "densely filled with cytoplasm accompanied by many actively dividing nuclei" (p. 585). (The nuclei as drawn in the illustrations could very easily be as small as 0.3μ , judging from their diameter in relation to the diameter of the conidiophores and hyphae drawn.) The mycelia in all four species were described as being coenocytic in early stages with septa forming at a later stage of development. Conidiophore formation did not differ markedly in the other two species (A. echinulatus and A. repens), except that in A. echinulatus the phialides and conidia were found to be multinucleate. In describing the formation of conidia, the author stated that, "nuclear divisions in the phialide are infrequently seen." "Nuclei continue to move into the phialide from the vesicle as successive conidia of a chain develop" (p. 590); and further, that "before a conidium germinates several mitotic divisions have taken place, making it multinucleate unquestionably" (p. 590).

Yuill (1950) studied the numbers of nuclei in species covering all 14 groups of the Aspergilli. Fixation was in La Cour's B.D. or in Allen's Bouin solution and staining was accomplished by La Cour's modification of Newton's Crystal Violet method. Conidia of Aspergillus nidulans and A. nidulans mut. alba were found to be uninucleate. The numbers of nuclei for the other members of the A. nidulans group were not given. Some groups of Aspergilli were found to contain both uni- and multinucleate conidial species with the diameters of these conidia varying proportionately, whereas others gave rise to either multinucleate or uninucleate conidial species.

Uninucleate conidia arose from uninucleate sterigmata and multinucleate conidia from either multinucleate or from uninucleate sterigmata after division of the single nucleus in the conidium. Dikaryotic studies of isolated conidia of heterokaryons of A. carbonarius (A. niger group) confirmed that the conidiophore vesicle supplied both nuclei to the sterigma of the species. Similar experiments on the multinucleate conidia of A. repens, suggested that the vesicle supplied only one nucleus to the multinucleate sterigma.

Sakaguchi and Ishitani (1952) made some observations on the growth and conidial development of Aspergillus oryzae, A. oryzae var. magnasporus (not listed in Raper and Fennell, 1965) and A. oryzae var. microsporus. The sterigmata and

conidia of these species were described and shown in drawings as being multinucleate. The conidial diameter varied, depending on the number of nuclei supplied by the vesicle to the sterigmata.

Pontecorvo, in "The Genetics of Aspergillus nidulans" (1953) made some observations on its cytology. Material was freshly squashed and stained in aceto-lactic-orceine. Cytological photographs were not provided although Pontecorvo (loc. cit.) provisionally concluded that there were, "four chromosome pairs (in the zygote), and of these one is very large, one very small, and two of medium length" (p. 149). The comment was made that, "as to the nuclei in the mycelium a direct cytological examination is to no avail because of the smallness of the chromosomes." "But we may take it that from the haploid nuclei of the ascospore, the nuclei of the mycelium derive by mitosis, and from these the nuclei of the conidia" (p. 148).

Elliott (1956) described four bivalents at first metaphase of meiosis in a diploid strain of A. nidulans and supported Pontecorvo's (loc. cit.) observation of $n = 4$. Käfer (1958) established eight definite genetic linkage groups in A. nidulans by mitotic recombination of 40 genetic markers. Elliott (1960) studied the cytology of haploid and diploid strains of Aspergillus nidulans. Material was fixed in acetic alcohol and stained in aceto-orcein. Detailed descriptions of the morphology of eight bivalent chromosomes seen at diakinesis in haploid strains

was presented. At the late anaphase of all three divisions in the ascus, Elliott (loc. cit.) stated that, "two rings are seen in which the individual chromosomes cannot be distinguished, and a thread, presumably representing the spindle, often connects them" (p. 465). Centrioles were said to be unstainable due to the methods employed, and no observations were made on nuclear division in the somatic hyphae.

III

Although interest in the mode of somatic nuclear division of the fungi did not become widespread until the late 1950's, Smith (1923) and Varitchak (1931) provided the later cytologists with the groundwork for their theories.

Smith (loc. cit.) visualized a 'direct division' of the somatic nucleus of Saprolegnia. A nuclear membrane was present and remained intact throughout the division. The nucleus became increasingly dumbbell-shaped as the chromoblast (stained part of the nucleus) pulled apart until the membrane constricted at the median and the daughter nuclei separated. Varitchak (1931) diagrammed a different form of non-mitotic nuclear division (p. 18) in the vegetative nucleus of Ascoidea rubescens showing chromosomes ($n = 2$) attached end-to-end and a centrosome attached to one of the chromosomes, constituting a chain of three bodies.

Division began at the centrosome and continued along the chain— as far as the end chromosomes which remained attached so that a linear chain of 5 bodies was formed, acutely angular at first, but later extending to a straight line. At this point the end chromosomes separated and moved apart. The daughter nuclei resumed a spherical interphase shape with the centrosome remaining outside and attached by a thread to the sphere.

Robinow (1956) reviewed non-mitotic nuclear division in various taxonomically "lower" forms of life including fungi. Citing only the work of Smith, and his own unpublished observations (but not those of Varitchak) he maintained that somatic nuclei of fungi divided by 'direct division.' A theory was postulated that evolution from unicellular to multicellular forms of life was correlated with a parallel evolution sequence in nuclear division. Three types of division were outlined: (1) mitosis; (2) endomitosis and (3) amitosis. Mitosis was defined as nuclear division associated with well defined chromosomes aligned on a fibrous spindle which may or may not be anchored in a centriole. The most important criterion, however, was a recognizable resting and dividing state of the chromosomes. Endomitosis was defined as division of the chromosomes without the aid of a spindle apparatus. This latter type of chromosome separation (as observed in higher plants and animals) is usually not followed by division of the nucleus itself and hence gives rise to varying degrees of polyploidy. Amitosis was defined as division of a nucleus without the emergence of definable chromosomes.

Olive (1953) in a review of fungal cytology maintained that division in the somatic hyphae was similar to that of higher plants. However, reference to Smith and Varitchak was omitted.

The publication of these theories of amitotic nuclear division in fungi initiated a controversy among fungal cytologists. Many schools of research have subsequently arisen either rejecting or supporting the theory of classical mitosis in somatic nuclei of fungi. This controversy, however, has not yet involved the Aspergilli (Weisberg, 1966), although somatic nuclear division in the related Ascomycete, Neurospora crassa has been the subject of a vast amount of discussion (Bakerspigel 1959, 1962; Robinow and Bakerspigel, 1965; Somers et al., 1960; Ward and Ciurysek, 1962; Dowding and Weijer, 1960, 1962; Weijer and Dowding, 1960; Weijer et al., 1963, 1965; Weijer and Koopmans, 1964; Weijer, 1964; Dowding, 1966; Bianchi and Turian, 1967; Oulevey and Turian, 1967).

Bakerspigel (1959) fixed Neurospora material in acid alcohol and osmium tetroxide and stained with HCl Giemsa, Feulgen, Azure SO₂ and iron haematoxylin techniques. Phase contrast observations were made on in vivo material as well. Using HCl Giemsa, interdivisional somatic nuclei are described as being "spherical and oval in shape and are composed of granular chromatin which envelope unstained areas, the sites of the Feulgen-negative

central bodies" (p. 183). In a later publication, Bakerspigel (1962) defines the central body as the nucleolus. At division the chromatin becomes increasingly granular and forms into "a complex of chromosomal filaments in various arrangements" (p. 185). This complex somehow takes the shape of an "irregular crescent mass" around the central body (nucleolus). The central body stretches into a dumbbell-like shape pushing the ends of the crescent shaped chromatin apart. When the central body reaches its maximum length the mid-region constricts, cutting off the two daughter nuclei. A nuclear membrane was not observed.

A complete review of other fungal species (mainly Phycomycetes) studied by Robinow, Bakerspigel and co-workers appears in Robinow and Bakerspigel (1965). In most fungi investigated, somatic nuclear division was found to be quite similar to the direct division described by Smith (loc. cit.) and Bakerspigel (loc. cit.). The one exception was found in Basidiobolus ranarium (Robinow, 1963). In this Zygomycete, a large barrel-shaped spindle is observed at mitosis and division is considered to be classically mitotic.

Somers et al., (loc. cit.) fixed Neurospora hyphae in acetic acid: alcohol, (1:3) and stained with an HCl acetic orcein method. Spindle fibers are not discussed although all the classical mitotic nuclear figures are described, with the exception that (at telophase) "during the stage of

despiralization of the chromosomes the daughter groups assume a much elongated, instead of the regular roundish shape" (p. 805-806). Also in one fourth of the observations an unexplained "tiny eighth" chromosome was noted. Other non-mitotic nuclear figures are discounted as "disintegrating nuclear material" (p. 903). Nucleoli were seen arising at telophase and at late telophase the nucleolus located centrally with a nuclear ring surrounding it. Somers et al. (loc. cit.) concluded that their observations differed from those of Bakerspigel (loc. cit.) only in interpretation for "the division figures recorded in Bakerspigel's photographs are similar to those presented in this report" (p. 806).

Ward and Ciurysek (loc. cit.) used alcohol : acetic acid : lactic acid, (6:1:1), as a fixative and stained with HCl Giemsa. Although somatic nuclear division was described as mitotic, a centriole was seen to be closely associated with the resting nucleus. Non-mitotic nuclear configurations were attributed to be degenerating nuclei, as noted by Somers et al. (loc. cit.). Although Ward and Ciurysek (loc. cit.) did not observe well defined spindle fibers, they stated that "many configurations were seen which suggested the presence of a spindle and the separation of the daughter chromosomes to spindle poles"

The 6 to 9 hour staining of Bakerspigel was compared to their own 15 minute treatment with the observation that "staining for lengthy periods resulted in loss of chromosome definition, only gross nuclear outline remaining." "When detail is obliterated in this way, division may appear , superficially,

to be amitotic" (p. 399).

Weijer and co-workers (loc. cit.) have evolved an unique concept of somatic nuclear division (karyokinesis) in Neurospora crassa (Weijer et al., 1965). This work was originally based on the observations of Varitchak (1931) of a linear nucleus and centriole in Ascoidia rubescens. McGinnis (1953,1956) observed in Puccinia graminis (a Basidiomycete) linear nuclear configurations which he called "spiremes".

Hyphae were heat-dried at 60°C and stained with the Feulgen technique. Nucleoli were stained with crystal violet after fixation. Three distinct karyokinetic cycles were observed in Neurospora: (1) Juvenile cycle of young undifferentiated hyphae, (2) Maturation I leading to the formation of macroconidia, (3) Maturation II, leading to the formation of micro-conidia.

In the Juvenile cycle, karyokinesis proceeds from a globular interphase nucleus to which a triangular plate-shaped centriole is attached by a weakly Feulgen-positive thread. The interphase nucleus gradually becomes ring-shaped, composed of seven particulate chromosome bodies plus the deeply stained centriole. These appear to be attached to one another by a weakly Feulgen-positive thread. At division the ring breaks, forming a double-stranded beaded nucleus which divides longitudinally.

Maturation cycle I precedes formation of the macro-conidiophore and arises from the late juvenile nucleus. The chromosomes of Maturation I are much larger but are still attached end-to-end. This large nucleus divides longitudinally and provides the smaller nuclei for the conidia of the macro-conidiophore.

Maturation cycle II precedes microconidial formation. The nucleus of this cycle also consists of chromosomes and centriole attached end-to-end. A prominent nucleolus is characteristic of this cycle but the nucleus still divides longitudinally. Division products of this nucleus supply the nuclei for the microconidia.

Centrioles of all three cycles were strongly Feulgen-positive and were attached to the nuclear ring or to one end of the nuclear filament by a weakly Feulgen-positive thread. A centriole was seen in the Neurospora ascus by both Dodge (1927) and Singleton (1953) and the presence of DNA in this body has recently been established (McDonald and Weijer, 1965).

The studies of Weijer and co-workers on Neurospora (loc. cit.) and Gelasinospora (Dowding and Weijer, 1962) represent a major change in direction in the theory of division of somatic nuclei of fungi. This theory, based upon the chromosomes existing in an end-to-end attachment and the longitudinal division of this linear nucleus, has been very unacceptable to many authors (Somers et al., loc. cit.; Ward and Ciurysek, loc. cit.). These latter authors have described linear nuclei as "migrating nuclei" and other non-mitotic figures as "degenerating" or "autolysing" nuclei without attempting to demonstrate that hyphal nuclei do degenerate at any particular stage. Their own observations of classical mitosis in Neurospora have excluded demonstration of a well defined spindle apparatus, suggested as a criterion for mitosis by Robinow (loc. cit.). Other authors finding the observations of Weijer and co-workers unacceptable to their own theories of nuclear division, have ignored reference to this research (Robinow and Bakerspiegel, 1965). However, the last few years have witnessed increasing observations of linear nuclear structures and their longitudinal division. While not conforming in every instance to the interpretations suggested by Weijer and co-workers, these observations lend credibility to them.

Wilson et al. (1966) fixing in Carnoy's fluid and staining Neurospora crassa with an HCl-Giemsa technique describe hyphal

chromosomes as being "associated linearly into a bar-shaped metaphase plate" (p. 18). Anaphase occurs when the chromosomes within the metaphase are only seen between "chromatids or groups of chromatids which have already separated" (p. 18). Division occurs within an "enlarged envelope" (p. 20). After division, new membranes are formed around the daughter nuclei and these migrate out of the old membrane, which disintegrates.

Wilson and co-workers also studied somatic nuclear division in Ceratocystis fagecearum (Aist and Wilson, 1965, 1967) and other plant pathogenic fungi (Brushaber et al., 1967). These reports of somatic nuclear division conform closely to that described for Neurospora (Wilson et al., loc. cit.).

In a subsequent publication, however, Aist and Wilson (1968) remark that their interpretation of vegetative nuclear division in fungi is confusing. In this later publication, observations presented in earlier papers are reinterpreted, although no alternative conclusions are offered. It is suggested that overstaining may explain the "taffy pull" and "amitotic figures" observed by workers (p. 877). It was also concluded that, "some of our figures illustrated as telophase may have been caused by fracture of nuclei during fixation and staining" (p. 877). The difficulties encountered in defining the nuclear envelope and thus distinguishing nuclei and chromatin is also discussed. In their work the

existence of a centriole is indicated together with the occurrence of a spindle-like apparatus situated between daughter nuclei which are enclosed in a common nuclear envelope. Reference in this regard is made to an unconfirmed communication and abstract (Robinow and Caten, 1966) of a cytological study of A. nidulans.

In the concluding remarks, the authors state that: "We believe that many disputed aspects of nuclear division can be resolved by observation of the living fungal cell with phase contrast" (p. 877). In the light of this statement it is therefore surprising that the pertinent information on in vivo and in vitro observations in N. crassa as communicated by Weijer and co-workers (loc. cit.) has been omitted in this publication (although not in earlier ones).

Bianchi and Turian (1967) fixed Neurospora material in acetic acid, chloroform and ethanol (1:3:6) hydrolysed in perchloric acid and stained in buffered Giemsa. Nuclear division during conidiation and conidial germination was described. Both mitotic and non-mitotic figures are observed and the problems of interpretation of nuclear configurations are discussed.

In active rapidly growing parts of the hyphae and in hyphae constricting to form conidia it was observed that "the dividing nuclei are often forced out and may display an

elongated appearance" (p. 195). However, it was also noted that: "In older hyphae where movement of the cytoplasm is not as rapid, and in large hyphae, the distortion of the nucleus is not as pronounced and a more typical mitosis is observed" (p. 4). Bianchi and Turian therefore concluded that "for certain developmental stages of Neurospora, a facultative mechanism of longitudinal division of filamentous nuclei as advanced by Keeping (1966) and Weijer et al., (1965) must therefore be considered" (p. 195).

Oulevey and Turian (1967), in electron-micrographs of Neurospora hyphae noticed that nuclear migration through a hyphal pore appeared to be guided by a "granule" attached to, but preceding a nucleus. This granule is described as being composed of a material of the same density as that of the rest of the nucleus. Analogies with regard to structure and location were made between it and the centriole as described by Weijer and colleagues (loc. cit.).

Previous to this publication, Turian (1958, 1959) and Turian and Cantino (1960) studied the cytology of Allomyces macrogynus and Blastocladiella emersonii. In these interpretations they leaned towards a direct division of a ring-like nucleus with the nucleolus elongating and constricting in the center.

Namboodiri and Lowry (1967) studied Neurospora somatic nuclei fixed in acetic-alcohol (1:3) or alcohol-acetic acid-

lactic acid (6:1:1) and stained by HCL-Giemsa. The observations support the concept of the linear nucleus and longitudinal splitting of the thread-like nucleus as proposed by Weijer and co-workers. The existence of a centriole attached to the nucleus is not supported, although it is noted that, "as in the elongated nuclei the rounded ones may show an attached bead-like structure." "Similar displaced, Feulgen-positive structures are seen in rounded nuclei undergoing division" (p. 745). The authors state that their observations differ from those of Weijer and co-workers in that the elongated nucleus is a "distinct nuclear form and not just a stage in division" (p.745) and that the division of these elongated nuclei and rounded (ring) nuclei are not sequences in the same cycle but "follow independent though parallel pathways" (p.747). This review of the controversy concerning division of the somatic nuclei of Neurospora crassa, encompasses most of the major theories and methods of somatic nuclear division subsequently observed in other species of fungi.

Hall (1963) investigated the cytology of the asexual stage of Monilia fructicola, a Discomycete having the same form genus as Neurospora crassa (Alexopoulos, 1962). Staining was with HCL-Giemsa. Although elongated nuclei were noted in the hyphae, Hall described the division of the vegetative nuclei as classically mitotic.

Moore (1964) in an electron microscope study of Cordyceps militaris (Ascomycete, Alexopoulos, 1962) proposed a new non-mitotic mode of somatic nuclear division called karyochorisis. The main feature of karyochorisis (or nuclear sundrance) is that during division the nuclear membrane remains intact and invaginates at division to separate the daughter nuclei. At first, the inner membrane invaginates producing two daughter "karyomes." This is followed by invagination of the outer membrane along the same axis, completely separating the karyomes to give rise to daughter nuclei.

Duncan and MacDonald (1965) examined somatic nuclear division in the Basidiomycete Marasmius using Feulgen and aceto-orcein staining methods. Their observations follow quite closely those of Weijer et al. (1965) on the Juvenile cycle in Neurospora. However, the authors maintain that the, "individual beads on the strands are not individual chromosomes, as thought by other workers, because they are greater in number than the chromosome complement or even more numerous than twice the complement" (p. 135).

Yamasaki and Niizeki (1965) studied the hyphal nuclear cytology of Piricularia oryzae (Moniliales, Ainsworth and Bisby, (1945). Stains employed were Giemsa, basic fuchsin, aceto-orcein and aceto-carmine. It was found that nuclear division in

germinating conidia appeared to be typically mitotic. Yamasaki and Niizeki (loc. cit.) state however that, "detail of the nuclear division in the mycelium is still obscure." "It is not considered to be a typical mitosis" (p. 265). In the explanation of their photographs (Plate I-D) it is stated that "chromatin masses connected in irregular rosary form split longitudinally and separate into two groups" (p. 267). Other photographs show linear nuclei (p. 271, figs. 29, 30 and 31) as described by Weijer and co-workers.

Weijer and Weisberg (1966) studied the Juvenile cycle of the somatic nucleus of Aspergillus nidulans. Hyphae were heat dried and stained with the Feulgen technique. Division was seen to be related to the earlier observations of Weijer and co-workers in Neurospora. It was found that resting conidia are globose rugose, dark green and 3 to 3.5 μ in diameter (Plate I, Figs. 1 and 2, in this thesis). The nuclei within these resting conidia varied in size (0.5 to 1.0 μ) and shape but were generally round and compact. Occasionally a Feulgen-positive extrachromosomal body (centriole) was seen attached to the nucleus by a weakly Feulgen-positive thread (Fig. 1).

Three nuclear forms (filament, crescent, and ring) were seen in germinating conidia (Figs. 3 to 15). All three forms were found to be homologous and were caused by different degrees of association of a linear nucleus around a central Feulgen-

negative body (nucleolus). A triangular centriole was sometimes seen attached to one end of the filamentous nucleus (Fig. 3) and crescent nucleus (Fig. 10) and on the ring in the ring shaped nucleus (Fig. 12).

These three nuclear forms gave rise to three different separation figures. However, the centriole always appeared to separate prior to separation of the chromosomes (Figs. 4 and 13). In the separation of the filamentous nucleus (Figs. 3, 4 and 5), longitudinal division was clearly observed. This longitudinal division was not as clearly demonstrated in the separation of crescent nuclei (Figs. 6 to 10) and separation of the ring nuclei (Figs. 12 to 15).

In older actively dividing hyphae (Plate I, Figs. 16 to 19 and Plate II, Figs. 20 and 21) all these nuclear forms were observed within a single hypha. Moreover, division figures giving rise to more than one form of daughter nucleus were often observed (Fig. 18, top center; Fig. 20, center; Fig. 21, bottom left). These figures indicate that the three forms of nuclei were homologous.

When the medium was exhausted the nuclei began to condense (Fig. 22) into the rounded nuclei (Figs. 23 and 24) reminiscent of the nucleus of the resting conidium. A centriole was also sometimes seen attached to the nucleus (Fig. 24, top).

At the end of the growing stage a large filamentous nucleus was sometimes seen (Fig. 25). This nucleus consisted of 8 definite chromosomes attached end to end in a linear order by a weakly Feulgen-positive thread and with a triangular shaped centriole at one end. This was the pre-maturation nucleus and was indicative of the transition from the small Juvenile cycle nucleus to a large polytenic nucleus of the Maturation cycle. This large nucleus was also cytological proof of the 8 chromosomes and triangular centriole of the A. nidulans nucleus.

Knox-Davies (1967) investigated the cytology of the vegetative hyphae of Macrophomia phaseoli with HCL-Giemsa and orcein staining methods. They described the division in nuclei of the vegetative hyphae of this member of the Fungi imperfecti as being mitotic. No centrioles or spindles were resolved although all mitotic stages were described.

Hejtmankova-Uhrova and Hejtmanek (1967) and Hejtmanek et al. (1967) investigated the division of the hyphal nuclei of several Microsporum species (Nannizzia= perfect stage), a dermatophytic Ascomycete. Giemsa and Feulgen staining techniques were employed. The observations in these and other studies of dermatophytes (Hejtmanek and Hejtmankova-Uhrova, 1967) agree quite closely with those of Weijer and Weisberg (1966). However, the existence of a centriole attached to one end of the linearly arranged chromosomes is not discussed.

Laane (1967) investigated nuclear division in the hyphae of Penicillium expansum using a 5N HCl-aceto-orcein staining technique as well as in vivo phase contrast observations. Nuclear division was found to be analagous to that observed in Aspergillus nidulans (Weijer and Weisberg, loc. cit.) with some minor exceptions. The nucleus contained five stainable bodies and either the center or end bodies seemed to exhibit the properties of a centriole. Thus V-shaped nuclear configurations as well as rod shaped ones were commonly seen and the longitudinal division was observed to initiate either from the center body outwards or from one end to the other. It was agreed that, as in Aspergillus, nuclear streaming plays an important role in separating the daughter nuclei.

Heale et al. (1968) investigated nuclear division in conidia and hyphae of Verticillium albo-atrum using HCl Giemsa, Feulgen and Acid Fuchsin staining methods. Nuclear configurations and divisions were found to be analogous to these described in the Juvenile cycle of N. crassa (Weijer et al., 1965). However, the nucleolus is described as disintegrating before nuclear division and daughter nucleoli arise de novo after division of the nucleus. A fiber (demonstrated with Acid Fuchsin) was believed to control the spatial distribution of the chromosomes, but whether or not the chromosomes were attached to this fiber was not determined.

Materials and Methods

Preparation of Specimen

The wild type haploid strain of Aspergillus nidulans, UAM #798* was used throughout the present study.

The medium employed for maintenance of stock cultures and incubation broth was Vogel's minimal medium supplemented with trace elements and biotin, as described in "Stanford Neurospora Methods" (1963). A 1% (w/v) concentration of 'Ionagar' No. 2. (Oxoid Ltd.) was used for agar slants and agar gels.

Partial synchrony of conidial germination was induced by keeping conidiating cultures on agar slants for 3 - 4 days at 40C, after which a conidial suspension was prepared in minimal medium broth supplemented with .01% Ion Agar. The suspension was kept under refrigeration (4°C) for 24 hours prior to use.

Cultures were grown on 22 mm. square Corning glass coverslips (No. 1 1/2) which prior to inoculation were prepared as follows. Coverslips were washed in 95% Ethanol and then heated overnight at approximately 400°C and then cooled. The coverslips were then dipped into the cold suspension of conidia in minimal medium broth and placed in groups of 4 on the surface of 100 x 15 mm.

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Petri culture plates containing 10 cc. agar gel. A sterilized filter paper was placed inside the cover of each dish and moistened to saturation with sterile distilled water to maintain moisture conditions during culture. The dishes were then inverted and incubated at 30°C. Coverslip cultures were removed at hourly intervals during the period from 8 to 44 hours incubation and when taken off, the culture coverslips were placed in trays, frozen in liquid nitrogen, and dried for one hour under vacuum in a Virtis freeze-drying apparatus. The cultures were then placed in a vacuum jar and stored under vacuum.

Staining and Mounting of Preparations

Feulgen

The Feulgen stain was prepared as follows:

Basic Fuchsin	5 g.
$\text{Na}_2\text{S}_2\text{O}_5$	9.5 g.
0.15 N HCl	500 ml. (room temperature)

The resulting solution was shaken for two hours. Two and a half grams of activated carbon were then added, and the suspension was shaken for two minutes and then filtered to remove the carbon. The filtrate was stored at 4°C. in an air tight amber bottle.

Coverslip cultures were pretreated with a 25% Glycerol-albumin spray and then hydrolysed for 30 to 90 minutes in 5 N HCl at room temperature (Decosse and Aiello, 1966). The optimum time for hydrolysis was found to be around 75 minutes. The preparations then were transferred serially to 2 1/2 N, 1 N HCl, and distilled water. They were washed for 10 minutes in several changes of distilled H₂O and placed into the Feulgen solution for approximately 75 minutes after which they were washed in several changes of tap water until the water remained clear and not pink (5 - 10 minutes) The stained preparations were mounted directly in Gurr's Water Mounting Medium neutralized to pH 6.5 with Na OH.

Crystal Violet

The coverslip preparations were fixed in Navashin's fluid for 24 to 36 hours and then washed in tap water for 6 to 12 hours.

Staining was as follows:

1% Aqueous Crystal Violet	10 minutes
Lugol's solution (1% KI + 1% I in 80% Ethanol)	4 minutes
95% Ethanol (2 changes)	2 minutes
98% Ethanol (2 changes)	2 minutes

The stained coverslips were then cleared in 3 changes of xylol dipped in Euparal Essence and Mounted in Euparal.

Acid Fuchsin (Heale et al., 1968)

Coverslip preparations were fixed in Navashin's fluid and washed as for Crystal Violet staining, after which they were rinsed in 1% acetic acid and stained for 3 minutes in acid fuchsin (1/60,000 in 1% acetic acid). After staining they were rinsed and mounted in 1% acetic acid and sealed with nailpolish. Prior to observation, the slides were refrigerated at 4°C. for 24 hours.

Microphotography

Observations and photomicrographs were made with a Leitz Orthoplan microscope (tube factor 1.25) fitted with 10X periplan hi-point compensating oculars. A Leitz Xenon lamp was used as light source for both brightfield and phase contrast micrography.

A Leitz Aplanatic condenser fitted with oil cap (N.A. 1.40) and Leitz Oil Immersion Plano Objective (100 x, N.A. 1.32) were used in brightfield micrography. A Leitz Phase Apochromatic Oil Immersion Objective (N.A. 1.32) and Leitz Variable Phase (Heine) Condenser were used in Phase Contrast Micrography.

Photomicrographs were taken with a Leitz Orthomat 35 mm automatic camera. Photomicrographs of acid and basic fuchsin preparations were taken with a 540 μ (green) interference filter. A 576 μ (yellow) interference filter was used for crystal violet preparations. No grey or diffusing filters

were used. Phase contrast photomicrographs were taken without interference filters. Shadowing was obtained by use of the swing out collecting lens fitted below the condensor.

Kodak High Contrast Copy Extreme Resolution Panchromatic 35 mm film was used for all microphotographs. The film was developed with Kodak D76 developer for 18 minutes at 20°C. Enlargements were printed on Kodak paper of suitable gradations and developed in Kodak Dektol developer.

For publication these prints were mounted on cardboard and photographed on Kodak Pan X 35 mm film. Enlargements were then made on 8 1/2" x 11" Kodabromide A matt paper and bound directly into the thesis.

Observations

A. General observations with regard to growth.

Although a progressive shift in the growth pattern of A. nidulans was noted between the 8 to 37 hour coverslip cultures, a wide spectrum of growth stages was often encountered upon a single slide, indicating only semi-synchronous growth. Therefore, an attempt to describe the vegetative development of the fungus on an exact time-cycle basis, would be artificial and unrealistic. Other criteria were thus explored. The formation of the conidiophore was found to be progressive (Plates III and IV). Primary (1^o) phialides gave rise to secondary (2^o) phialides which in turn yielded the conidia. Both developmental sequelae were found to occur simultaneously in one and the same conidial head (Plate IV, Fig. 34). As growth progressed the width and depth (seen as an increase in refraction) of the 1^o and 2^o phialide rows increased (Plate IV, Fig. 35).

Consequently, the different growth stages of the conidiophore together with width and depth measurements of 1^o and 2^o phialide rows were used as criteria for the determination of culture age.

Development of the occasional odd shaped conidiophore was ignored (Plate VII, Fig. 50).

B. Karyology of the hypha.

1. Early karyological stages (Juvenile cycle, Weijer and Weisberg, 1966).

Hyphal growth of A. nidulans as obtained from coverslip cultures (Plate V, VI, VII and VIII) was found to be similar to that of cultures grown in broth medium (Plates I and II: see Weijer and Weisberg, 1966). The nuclear configurations, however, were much more condensed in the coverslip cultures and often the particulate nature of the nuclei became obscured (Plate V, Figs. 39 - 43, vs., Plates I and II). However, in the early germination period (8 - 12 hours), ring-shaped nuclei (Figs. 39 and 42), crescent-shaped nuclei (Figs. 40, 41 and 43) and filamentous nuclei were present. These various nuclear shapes often occurred simultaneously in the same hypha (Figs. 41 - 43).

Hyphal growth progressed very rapidly (5 μ /minute at 25°C) after germination of the conidium. The nuclei during this rapid hyphal growth phase were generally seen as filaments (Plate VI, Figs. 44, 46, 47 and 48). Occasionally, hyphal segments containing ring nuclei were found (Plate VII, Fig. 49).

These filamentous nuclei were quite often observed in the process of bending into a hyphal bud (Fig. 44, top arrow) or bending around hyphal corners (Fig. 44, bottom

arrow). Division figures suggestive of longitudinal separation of nuclear filaments were often observed (Plate VI, Fig. 46, and Plate VII, Figs. 51 and 52). Sometimes these could be interpreted as pairing of filamentous nuclei (Fig. 46). Occasionally nuclei were seen which consisted of a cluster of larger (1.5 - 2x) chromosomes (Plate VIII, Figs. 54 and 55). In the same hypha, filamentous nuclei were sometimes evident (Fig. 55, center) in which the chromatin bodies (9) were arranged in a perfectly linear order. In addition, nuclear structures were seen which suggested a further interchromosomal lengthening of the nucleus (Fig. 57). Towards the end of the early stages of growth of the hypha, an increase in variability of nuclear as well as chromosomal size was noted (Plate VI, Fig. 45; Plate VIII, Figs. 53 and 56). Clustering of filamentous nuclei (pairing?) occurred frequently (Plate VI, Fig. 46).

2. Late karyological stages (Maturation cycle, Weijer and Weisberg, 1966).

At 20 hours nuclear structures became very large with their chromosomes far apart in a long chain (80 μ ; diameter of the largest chromosome: approximately 0.7 μ , Fig. 58, chromosome a). Linear groupings of 9 stained particles (8 large polytene chromosomes with a triangular centriole at one end) were observed (Plate IX, Fig. 58) at these late

stages of hyphal development. In other instances rather short (10 - 15 μ) polytene nuclei were observed (Plate XIV, Fig. 72).

Separation of the chromosomes of the maturation nuclei during division did not appear to be synchronous. The division of the centriole either preceded or followed the onset of chromosomal separation (Figs. 59 and 60). The centrioles (labelled C) did not always appear to be terminal (Figs. 64, 65, 66 and 69). More frequently, however, one or both were found at one end of the nucleus (Figs. 58, 59, 60, 63 and 70). In several instances the centrioles could not be detected (Fig. 62).

Chromosomes were frequently observed in different stages of separation (Fig. 59, chromosomes e, d, b and c); some pairs of daughter chromosomes were widely separated (Fig. 60, chromosomes a and b) or were in the process of separation (Plate XII, Fig. 67, note weakly Feulgen-positive connections between individual chromosome pairs). Thus, full complements of either 9 stained bodies (Fig. 58) or 18 stained bodies (Plate XI, Figs. 64, 65; Plate XIV, Fig. 73) were somewhat rare to detect. However, within most hyphal sections, the homology of paired daughter chromosomes was quite clear (Plate X, Fig. 62; Plate XI, Figs. 64 and 65; Plate XII, Fig. 66; and Plate XIII, Figs. 68, 69 and 70).

Occasionally stained bodies were found (Plate X, Figs. 61 and 63) in a paired homologous condition (Fig. 61, aa and bb; Fig. 63, bb) which appeared to be shorter than juvenile nuclei but longer than polytene chromosomes. These bodies were interpreted to be decoiling polytene chromosomes. They often occurred in hyphal sections which also contained juvenile non-polytene nuclei (Fig. 61, to the right of b).

The large polytene nuclei were frequent both in undifferentiated hyphae, and in hyphal segments adjacent to the foot cells of fully formed conidiophores (Plate XIV, Fig. 72; Plate XVII, Fig. 77) and their length and morphology were about the same in both instances. A polytene nucleus sometimes occurred on both sides of the foot cell (Fig. 77).

A weakly crystal violet-positive thread (Plate XV, Fig. 74, arrow) or weakly Feulgen-positive thread (Plate XVII, Fig. 77) was sometimes seen connecting the chromosomes of the polytene nucleus. Under phase contrast, with Xenon light source, Feulgen stained chromosomes fluoresced green (in black and white photographs these fluorescing chromosomes appeared as roundish black bodies (Plate XVII, Figs. 77a and 77b). The fiber under phase contrast appeared as a very dark thread (Figs. 77a and 77b). Although this fiber was not visible throughout the entire length of the nucleus (Figs. 77 and 77a), it always appeared to pass through, and not around, the

stained bodies (Plate XVII, Figs. 77a and 77b; Plate XVIII, Figs. 78 and 79; Plate XIX, Fig. 80). Since it connects with the chromosomes, we judge it to have intranuclear continuity, and hence may refer to it as a nuclear structure. The fiber was often observed to be double-stranded, with separation between the individual strands, especially where it appeared next to separating daughter chromosomes (Fig. 77b, arrow).

In axial hyphae or hyphal side branches adjacent to the foot cell, the fiber appeared to be single (Plate XX, Figs. 81 and 82) or double-stranded (Plate XX, Figs. 83 and 84; Plate XXI, Figs. 85 and 86, arrow). Occasionally a fiber was observed in hyphal segments which were not associated with foot cells (Plate XXI, Figs. 87 and 88; Plate XXII, Figs. 89 and 91; Plate XXIV, Figs. 92 and 93). The fiber was also seen in hyphae associated with odd-shaped conidiophores (Plate XXII, Fig. 90).

Bright-field microscopy of the same hyphal sections did not always reveal the nuclear fiber (compare Plate XXI, Fig. 85 with Fig. 86; Fig. 88; Plate XXII, Figs. 89, 90 and 91; Plate XXIII, Fig. 95).

The nuclear fiber stained with acid fuchsin (Plate XXI, Fig. 87; Plate XXIII, Fig. 92), although the chromosomes did not. The morphology of the fibers and their surrounding

cytoplasm varied (Plate XXII, Figs. 89, 90 and 91; Plate XXIV, Fig. 93). In rare instances fibers connecting polytene chromosomes were observed within a conidiophore (Plate XXIV, Fig. 97).

In certain areas of various slide preparations, thickly walled hyphae were noticed (Plate XV, Fig. 75; Plate XXXIII, Figs. 133, 134 and 135). The nuclear material in these hyphae appeared to be excessively polytene (Figs. 133 and 135). A thick connection between the chromosomes was sometimes visible (Fig. 133, center). In other thick walled hyphae (Fig. 134) it was difficult to determine whether the stained structures were of a juvenile, non-polytene origin (Fig. 134, center) or polytene chromosomal structures in the process of decoiling (Fig. 134, top left and bottom right.)

C. The ontogenesis of the conidiophore.

The first sign of conidiophore differentiation consisted of a structural change of the hyphal segment adjacent to the initial conidium, into a thickly walled cell (sometimes called the foot cell; Plate III, Figs. 26 and 27). Later, other segments of the hypha became similarly differentiated (Fig. 28).

Many (5 - 15) small (juvenile) ring nuclei (nomenclature: Weijer et al., 1965) were observed in the initial foot cells

(Fig. 28). Sometimes very short differentiated cells (Fig. 26) with single large (polytene) filamentous nuclei were observed. The nuclear complement, however, of these latter cells could not be determined (Fig. 27). At the developmental stage of conidiophore formation, their nuclei were always observed to be small (juvenile; Figs. 29 and 30).

The conidiophore bud arose from the initial foot cell perpendicular to the hyphal axis, and as it elongated, nuclei from the foot cell divided and migrated into this thick walled structure (Figs. 29 and 30). Next, its apex enlarged into a spherical but thin-walled conidiophore vesicle (Fig. 30). Upon further growth 1° phialide (sterigmatal) buds started to differentiate (Fig. 29). The nuclei at the apex of the conidiophore initial became filamentous (Fig. 30) and a single nucleus migrated into each 1° phialide (Figs. 30 and 31).

After migration, a wall formed at the bottom of the phialide (Fig. 32) and a 2° phialide bud appeared and elongated at the apex of the 1° phialide (Plate III, Fig. 32; Plate IV, Fig. 33).

The nucleus, after migration into the 1° phialide, resumed a non-filamentous configuration (Plate III, Fig. 32) but elongated after division, prior to migration into the 2° phialide bud (Plate IV, Fig. 33). The 1° phialide nucleus kept on dividing and the 1° phialide produced more 2° phialides on

its apical region (Fig. 34).

The conidium was formed at the end of a slender neck produced at the apex of each 2^o phialide (Fig. 34, middle arrow). At this time, the conidium initial became larger and spherical shaped. After the conidium received a nucleus, it was cut off from the 2^o phialide at the connecting or neck region (Fig. 34, top arrow).

The conidial nucleus was supplied by division of the 2^o phialide nucleus (Fig. 35) followed by the migration of one of the daughter nuclei through the neck into the conidium (Fig. 36). Although subsequent divisions of the 2^o phialide nuclei (Fig. 35) were observed, no divisions in the attached conidia were seen. Due to the very condensed state of the nuclei in the phialides, a detailed description of the nuclear events during their division cannot be given.

As conidial production progressed the neck region of the conidiophore became larger (Fig. 38) and appeared to contain large nuclear and chromosomal structures, probably polytenic in nature (Figs. 35, 37 and 38).

D. Karyology of the conidiophore.

Maturation nuclei composed of polytene chromosomes were also found in the main body of fully formed conidiophores (Plate XXIV, Figs. 94, 95, 96 and 97). On one occasion a polytene nucleus was seen in a pre-phialide stage conidiophore (Fig. 98), although juvenile nuclei were present in the apical region of the conidiophore (Fig. 98, arrow). More commonly, non-polytene (juvenile) nuclei were found at this particular stage of conidiophore development (Plate XXVIII, Fig. 112). Their morphology resembled closely that of nuclei present during early stages of hyphal growth (see p. 37).

The chromosomal morphology of polytene nuclei in the conidiophore showed as much variation as described earlier for polytene hyphal nuclei. They were compact and arranged in a short filament (Plate XXIV, Fig. 94) or in a long filament (Fig. 96). The chromosomes appeared to be partly uncoiled when dividing (Fig. 95) as in polytene hyphal nuclei (Plate XI, Fig. 65). In some older conidiophores the nucleus sometimes lost its chromosomal morphology to become a mass of thread-like nuclear material (Plate XXIV, Fig. 99). Occasionally, a nucleus was observed with a slightly Feulgen-positive thread connecting the chromosomes in a non-linear arrangement (Plate XXIV, Fig. 97) or in a semi-linear arrangement (Plate XXVII, Fig. 105). The polytene maturation chromosomes

within the nucleus were frequently seen in small groups (Plate XXVI, Figs. 103, 105, 106, 107 and 108) with some indication of linearity of arrangement (Figs. 103, 105, 107 and 108).

Observation of conidiophores at progressive stages of aging and development showed a series of changes in the morphology of the nuclear material within the conidiophore body. Three different nuclear events were found to occur after the 1^o phialide formation and prior to 2^o phialide development: (a) No nuclear material could be detected in the conidiophore (Plate III, Fig. 32; Plate IV, Fig. 33); (b) Thread-like undetermined nuclear material was present in the conidiophore (Plate XXVII, Fig. 111; Plate XXVIII, Fig. 116); (c) A nucleus displaying varying stages of polyteny occupied the conidiophore (Plate XXVI, Fig. 104).

After formation of the first 2^o phialides the nuclear material within the conidiophore body displayed varying degrees of polyteny (Plate XXIV, Fig. 96; Plate XXV, Figs. 101 and 102; Plate XXVII, Fig. 110; Plate XXIX, Figs. 114, 115 and 117; Plate XXX, Figs. 118, 119 and 121; Plate XXXI, Fig. 123).

From observations on older conidiophores at advanced conidiating stages it became evident that the polytene nucleus progressively increased in size and stainability (Plate XXX,

Fig. 123; Plate XXVIII, Fig. 117; Plate XXVII, Fig. 109; Plate XXIV, Figs. 96 and 94; Plate XXVI, Figs. 106 and 105).

Division of a single polytene conidiophore nucleus was sometimes observed (Plate XXIV, Fig. 95). Other conidiophores were seen to contain two polytene maturation nuclei in the body region (Plate XXV, Fig. 100; Plate XXVI, Figs. 103, 107 and 108; Plate XXVII, Fig. 113).

During the development of 1^o phialides non-polytene (juvenile) nuclei streamed from the main body of the conidiophore through the conidiophore vesicle (conidiophore apex) into the newly formed 1^o phialides (Plate III, Figs. 30 and 31; Plate XXVII, Fig. 112). In other instances it appeared that polytene maturation nuclei (present in the main conidiophore body at later stages of development) were associated with the origin of these juvenile nuclei (Plate XXVI, Fig. 76; Plate XXIX, Fig. 119; Plate XXX, Fig. 122).

Nuclear divisions giving rise to non-polytene (juvenile) nuclei occurred in the 1^o as well as in 2^o phialides (Plate XXIX, Fig. 120; Plate XXX, Fig. 125).

In fully mature conidiophores strand-like maturation nuclear material became evident (Plate XXV, Fig. 99; Plate XXXI, Figs. 123 and 124) which in time disappeared leaving only one (Plate XXI, Fig. 127) or two (Figs. 128 and 129) dark stainable triangular bodies in the apex of the conidiophore. These bodies resembled the centriole (Plate II, Fig. 25).

In 37 hour cultures conidiophores were found in which no stained material could be detected and from which the phialides had disappeared (Plate XXXI, Fig. 126; Plate XXXII, Figs. 131 and 132). The hyphae surrounding these conidiophores had lost their cell walls and their cytoplasm resembled protoplast-like structures (Plate XXXI, Fig. 130; Plate XXXII, Figs. 131 and 132).

DISCUSSION

I

Cutter (1951) observed that, "in cytology, perhaps more than in any other science, progress depends upon manipulative skill and that low type of cunning which is needed to apply old methods to new uses," and further that, "This truth is beautifully illustrated in the case of the fungi where, in cytological work we deal with structures and artifacts lying close to the limits of visibility" (p. 17). However, as technical skills and knowledge progress, the value and respect shown to the earlier less sophisticated studies, should not be diminished. Although it is easy to criticize the early research, we should bear in mind that even today certain details of nuclear division still evade cytological examination.

The identification of stained bodies within the somatic hyphae of A. nidulans has always been difficult. Neither Dangeard (1907), Frazer and Chambers (1907), Dale (1909) nor Baker (1945) claimed to be able to distinguish between chromosomes. The present author has found that the chromosomes and nuclei become larger (polytene) during certain stages of fungal growth and in certain instances it remains difficult to distinguish between small nuclei and polytene chromosomes. It is clear that this difficulty in differentiation has contributed much to the confusion which is so evident in the literature.

As Pinto-Lopes (1949) pointed out in his discussion of fungal nuclear structure, stains whose intensity of colouration could be manipulated by the observer, through differentiation, were unsuitable. He also observed that the Feulgen reaction demonstrated details of nuclear structure whereas the other stains generally showed only the presence or absence of nuclei. The present author has omitted use of all bleaching agents, such as alcohol in the Feulgen nuclear technique and clove oil in the crystal violet technique, as it was found that these agents de-stained the original reactions and therefore they were considered undesirable manipulations. Comparing these two techniques, it was found that crystal violet stained the same structures as did Feulgen, but in addition crystal violet always stained numerous minute spherical structures in the phialides and occasionally in the conidiophore (Plate XXIX, Fig. 119). The DNA-specificity of the Feulgen nuclear reaction for A. nidulans was confirmed in an earlier study (Weisberg, 1966). Since all standard fixation techniques have been omitted in the preparation of Feulgen stained material, the Navashin fixation coupled with crystal violet staining has been used to demonstrate that omission of the fixation step in the Feulgen technique did not influence the quality of the preparations with regard to chromosomes as well as to intra-nuclear threads (Plate XV, Fig. 74). Pre-fixation of Feulgen stained material decreases the intensity of the Feulgen nuclear reaction. The hydrolyses with 5 N HCl at room temperature gave rise to

consistently good results with the Feulgen reaction as compared to the inconsistent results experienced with 1 N HCl at 60°C.

The present study agrees with the observations of Dangeard (1907), Wakayama (1931), Baker (1945), Sakaguchi and Ishitani (1952), and Yuill (1950) that the conidial nucleus of Aspergillus species is a product of the phialide nucleus and not of a conidiophore nucleus passing through the phialides into the conidium as was observed to occur in A. echinulatus (Baker, loc. cit.). The observations of Wakayama that the divisions in the phialides is mitotic could not be substantiated in A. nidulans because of the failure to observe nuclear detail.

Structures resembling the 'caryosome' described by Wakayama (loc. cit.) and Olive (1954) were not observed in A. nidulans in either the Feulgen or Crystal violet preparations. Olive had suggested that the 'caryosome' was an artifact caused by the collapse of chromatin around the nucleolus. The present author attempted to stain nucleoli with many different techniques and failed. However, cell-free preparations of hyphal nuclei (naked protoplasts) stained with the fluorescent dye acridine orange and viewed under ultraviolet light showed some evidence of nucleoli. In hyphal preparations stained with acridine orange, however, the intensity of the fluorescing dye in the cytoplasm made observation of nucleoli impossible.

Anastomosis of hyphae prior to conidiophore formation described by Baker was not detected in the material employed. The observation of uninucleate phialides and conidia described by Yuill in A. nidulans and Dangeard in S. nidulans is substantiated in the present study.

II

The reason that there exist so many different views with regard to somatic karyokinesis in the fungi is mainly due to the difficulties encountered in studying chromosomes which are at the extreme limit of resolution of the light microscope. Pontecorvo (1953) stated that any direct observation of the hyphal nuclei in A. nidulans was impossible because of the smallness of the nuclei. Weijer and Weisberg (1966) noted that at certain stages of development, the smaller chromosomes were below the resolution of the light microscope and therefore a full complement of chromosomes was not always ascertained.

Another factor which has contributed greatly to the differences in cytological interpretation has been, and still is, the use of questionable staining techniques. As early as 1949, Pinto-Lopez observed that nuclear stains were chosen for their ability to stain nuclear material densely at the expense of nuclear detail. This practice is still in use and hinders the solution of the problem.

To understand how these discrepancies might occur, one must primarily appreciate the tremendous difficulties associated with the study of a nucleus as small as the one present in the hyphae of fungi. Not only may a particular stain block out the fine detail of the nucleus (as suggested by Pinto-Lopes, loc. cit.), but it may also vary greatly in density of staining. This variance can be observed within particular slide preparations as well as between them. Moreover, a particular stain may be suitable for staining nuclei in certain structures but unsuitable in others. Cytologists are generally wary of these difficulties and compensate for them by looking for 'a good nucleus' or 'a good slide'. All other nuclei or slides are rejected as 'poorly stained'. When many observations are made of a particular 'poorly stained nucleus', the cytologist modifies either his techniques or his conclusions. This is not critical when studying large, well-defined nuclei where evaluation can be kept objective. However, when investigating nuclei and chromosomes with sizes approaching that of the limits of visibility under the light microscope, these evaluations tend to become increasingly subjective.

In general, differences in conclusions are governed by the observations on which they are based and possibly by prejudicial interpretation as well. It should be stressed that the present author is not taking previous investigators to task and is not suggesting that his own research is free

from subjective interpretations. However, an attempt has been made to improve staining techniques and microscopic resolution in order to minimize them.

Cytological studies on other species of fungi subscribe to one of the three theories of somatic nuclear division listed for N. crassa. Although Hall (in Monilinia, 1963), Yamasaki and Niizeki (in Piricularia, 1965) and Knox-Davies (in Macrophomina, 1967) describe somatic nuclear division as 'mitotic', linear nuclear configurations were observed by all these authors which they interpreted as migrating nuclei. Moore's (1964) description of 'Karyochorisis' in Cordyceps is an electron micrographic confirmation of 'direct division' in this species, with particular emphasis on a description of the mechanics of the nuclear membrane during nuclear division. Duncan and MacDonald (1965) in Marasmius and Hejtmanek and co-workers (loc. cit.) in Microsporium observed with some modification that somatic nuclear division was similar to the longitudinal division described by Weijer et al., as did Laane (1967), in a cytological study of Penicillium.

There are three schools of thought concerning nuclear division in Neurospora crassa: (1) Somers et al. (1960) and Ward and Ciurysek (1962) both describe 'anaphase' and 'metaphase' figures and conclude that mitosis is classical. However, the criteria as set down by Robinow (1956) of a

recognizable spindle apparatus together with a resting and a dividing stage of the nucleus, were not met; (2) Bakerspigel (1959) and Robinow and Bakerspigel (1965) described division as being a direct division of the nucleus as depicted by Smith (1923) in Saprolegnia; (3) Weijer et al. (1965) described a method of division (karyokinesis) in Neurospora which is based upon the longitudinal division of a nucleus consisting of a Feulgen-positive triangular plate centriole and seven chromosomes, attached end-to-end in a linear order by a weakly Feulgen-positive thread. Different nuclear cycles have been described for different stages (hyphae, macroconidia and microconidia) in the life cycle. These nuclear cycles are basically similar in their mode of division except that the chromosomes and the centrioles become much larger (polytene) in the developmental stages which lead to the formation of macro- and microconidia.

Other cytologists such as Namboodiri and Lowry (1967), Bianchi and Turian (1967) and Wilson et al. (1966) have contributed information with regard to the somatic nuclear division of N. crassa. While not agreeing in toto with observations of Weijer and co-workers their conclusions have favored the longitudinal division of a linear and filamentous nucleus. In a very recent publication, however, Aist and Wilson (1968) have completely rejected their earlier observations without offering alternative solutions. From a short research

note it appears that Robinow and Caten (1967) confirmed the earlier observation by Weijer and Weisberg (1966) of double stranded or filamentous nuclei during hyphal division in A. nidulans.

Many discrepancies in techniques and observations are evident among the studies listed above:

- (1) Somers et al. noted that although Bakerspigel used the same techniques and obtained the same nuclear configurations, their observations and conclusions were very different;
- (2) Ward and Ciurysek explained that although Bakerspigel had used the same staining technique (Giemsa) as they had, his observations were the result of extensive overstaining of the preparations (12 hours) as opposed to their own which were not (15 minutes);
- (3) Bakerspigel, who also used Feulgen technique, obtained results and conclusions which are very different from those reported by Weijer et al. (1965), using the same technique.

III

The Juvenile cycle

In an earlier study on the division of the somatic nucleus in germinating conidia of A. nidulans (Weijer and

Weisberg, 1966), it was found that in essence there are no major differences between the Juvenile chromosome cycles of A. nidulans and N. crassa (Weijer et al., loc. cit.), with the exception that nuclear forms such as rings, crescents and filaments are different configurations of the same linear (filamentous) nucleus rather than different stages in division. These different configurations are believed to be due to varying degrees of association of the linear nucleus around a Feulgen-negative "central body," or nucleolus. It was also postulated that nuclear streaming played an active part in the formation of these alternative nuclear configurations and in the separation of divided nuclei. Recently Laane (1967) observed that cytoplasmic streaming played an important role in the separation of the divided nuclei in Penicillium expansum. The counter flow system of cytoplasmic streaming as suggested by Isaac (1964) to occur in fungi, supports this theory. It is realized, however, that for the cytoplasm to have a separating effect on the daughter nuclei, the nuclear membrane must disappear at some stage as in higher plants and animals. Although a nuclear membrane has been demonstrated around hyphal nuclei of A. niger (Tanaka and Yanagita, 1963, a and b), its integrity throughout the entire division cycle has not been proven as yet. Moore (1964) in an electron microscope study of Cordyceps militaris was able to demonstrate that the nuclear membrane of this Ascomycete remained intact throughout the division.

In the Juvenile nuclear cycle of A. nidulans the resting nucleus in the conidium develops into a filamentous nucleus consisting of eight chromosomes and a triangular centriole which are arranged in a linear file on a weakly Feulgen-positive thread with the centriole in a terminal position. The centriole divides prior to nuclear division. Karyokinesis is accomplished by longitudinal division of the linear filamentous nucleus.

The fact that the centriole is Feulgen-positive and hence contains DNA is not surprising in view of the fact that other self duplicating bodies such as mitochondria and chloroplasts also contain DNA (Schatz et al., 1964; Chun et al., 1963). Shrader (1936) and Darlington (1937) believe that the centrosome and centriole are homologous because of their similarities in staining and behaviour. Pollister (1939) and Pollister and Pollister (1943) found that in Vivipara malleatus the supernumerary centrioles were in fact detached centromeres, whereas Roth (1964) pointed at the structural similarities between the basal bodies of cilia and metazoan centrioles. The centriole as described in the hyphae of N. crassa (Weijer et al., 1965) and A. nidulans seems to perform a function somewhere between that of a metazoan centriole and a ciliary basal body. Previous studies by McDonald and Weijer (1966) confirmed the presence of a triangular Feulgen-positive centriole during nuclear division in the ascus of N. crassa.

The Maturation Cycle

In the present study, after approximately 12 hours of incubation of the slide culture, nuclei in progressive stages of polyteny and of lengthening of interchromosomal connections appear, probably as a result of endomitotic replication. What actually initiates the formation of the polytene maturation nucleus in the hypha is not known, but it seems plausible that a progressive unbalancing of the ratio between nuclear material and cytoplasm is involved in the onset of endomitotic replication of the juvenile nucleus. The onset of the formation of these large polytene nuclei occurs just prior to the differentiation of the foot cell and of the conidiophore and it is for this reason that we believe that the initiation of differentiation as well as differentiation itself of the foot cell and the conidiophore is directly related to the formation of these polytene nuclei. A similar observation was made by Weijer et al. (loc. cit.), who observed that the appearance of large polytene nuclei in Neurospora crassa seemed to be associated with the development of macroconidia (Maturation cycle I) and microconidia (Maturation cycle II).

Whereas in Neurospora hyphae the occurrence of the Juvenile nuclear cycle is clearly separated in time from the Maturation cycle, in A. nidulans both cycles overlap considerably. Although immediately after the formation of the foot cell the main hypha may contain maturation nuclei, the nuclear content of

the foot cell will invariably be of a juvenile nature. Maturation nuclei in the foot cell and consequently in the conidiophore appear much later and their time of development is therefore not "in phase" with the rest of the hyphal structure.

The intranuclear thread connecting the individual chromosomes (8) and the centriole is very long. In fully developed hyphal maturation nuclei it is not uncommon to find a total length of the nuclear filament in excess of 100 μ . In addition, there is some indication that during the elongation process of the nuclear fiber the chromosomes partly uncoil. In the present study, the intranuclear fiber has been observed under phase contrast microscopy (Feulgen stained) as well as after staining with Acid Fuchsin. Due to its breakdown during the staining procedures, the fiber has not been demonstrated with exact regularity in all preparations. Its occurrence however is indisputable. Preliminary investigations in our laboratory into the fine structure of nuclear filaments in N. crassa (Kozar, pers. commun.) revealed the presence of intra- as well as inter-chromosomal microtubuli.

The long polytene hyphal nucleus divides by independent transverse separation of the chromosomes and centriole. The separation of the centriole does not (as in the Juvenile cycle) necessarily precede the separation of the chromosomes.

Apart from polytene maturation nuclei resulting from endomitotic replication of a haploid juvenile nucleus, another type of hyphal maturation nucleus appears at relative early stages of development. Its morphology is characterized by excessive polyteny and chromosome sizes are approximately twice those of regular Maturation cycle chromosomes. In addition, the wall of the hyphal lumen containing these excessive polytene nuclei is very thick, although not as thick as the wall associated with the foot cell. The frequency of occurrence of these nuclei approximate quite closely the estimate by Pontecorvo (1958) of 1×10^{-6} for diploid nuclei in a haploid homokaryon. Furthermore, during the Juvenile cycle nuclear clusters are observed with each cluster consisting of a pair of juvenile nuclei. The frequency of occurrence of these nuclear clusters correlates, again, well with the estimate given by Pontecorvo (loc. cit.) for fusions of haploid nuclei in a homokaryon. On the basis of these observations, we are inclined to identify the clusters of juvenile nuclei as fusion configurations of haploid juvenile nuclei. The excessive polytene maturation nuclei are therefore diploid maturation nuclei which at a later time (according to the steps in the parasexual cycle) can undergo haploidisation.

At the time of differentiation of the foot cell only juvenile nuclei are included in its lumen irrespective of whether the remainder of the hyphal structure contains maturation nuclei or not. These juvenile nuclei of the initial

foot cell constitute the nuclear material for the future 1^o phialides. They stream as juvenile nuclei through the conidiophore and even may undergo division before they are incorporated into the lumen of the 1^o phialide. Even later, when the 2^o phialides are formed and a maturation nucleus is present in the conidiophore, only juvenile nuclear material is supplied to additional developing 1^o phialides.

Although some observations are indicative of a breakdown of the conidiophore maturation nucleus into juvenile nuclei, the author believes that these observations represent the simultaneous occurrence of dividing juvenile nuclei and a maturation nucleus within the same conidiophore body.

The manner in which the polytene maturation nucleus of the conidiophore is produced, is not clear. The rather peculiar situation exists that sometime during the 1^o phialide stage (but prior to the development of 2^o phialides) many conidiophores do not contain any recognizable nuclear material whilst others display thread-like material. Therefore the remainder of the juvenile nuclei after 1^o phialide formation lose their chromosomal identity and are subsequently reconstituted into a polytene maturation nucleus or, endomitotic replication of a juvenile nucleus gives rise to the maturation nucleus.

Nuclear division within the phialides was not investigated in detail because of technical limitations. However, it is not ruled out that nuclear division in these organelles is classically mitotic, since the phialide is a uninucleate cell.

In our observations the production of conidia continues until the nutrients in the medium become limiting. At this point, the polytene maturation nucleus in the conidiophore uncoils and eventually disappears, leaving only one or two centriole bodies in the conidiophore. At the termination of growth and conidial production these centrioles disappear and the walls of the hyphae and phialides disintegrate.

Chromosome number of *A. nidulans*

The early cytologist did not discuss numbers of chromosomes in the nuclei of the Aspergilli. Later, however, Wakayama (1931) in his study of many Aspergilli stated that he invariably observed two chromosomes after separation from the 'caryosome.' Whelden (1940) also reported $n = 2$ at 'anaphase' in the nuclei at the hyphal tips of *A. niger*. This latter author observed that the chromosomal details were not clear except at anaphase. Pontecorvo (1953) in his study on the genetics of *A. nidulans* found that four pairs of chromosomes were present in the zygote nucleus. Elliott (1956) at first, supported Pontecorvo's

observation of $n = 4$ but when Kafer (1958) demonstrated 8 definite linkage groups in A. nidulans, he re-investigated the case and confirmed cytologically that the zygote nucleus contained 8 chromosome pairs (Elliott, 1960). The present study (and also Weijer and Weisberg, 1966) reports that the hyphal nucleus of A. nidulans consists of 9 stainable bodies, 8 chromosomes and a centriole arranged in a linear file. A DNA-positive centriole has been observed in Neurospora crassa (McDonald and Weijer, 1965) and this suggests that a similar situation exists in the ascus of A. nidulans. If this were so, then 18 stained bodies should be observable in the zygote of A. nidulans.

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Plate I, Figs. 1 - 19. Conidia and hyphae of *A. nidulans*.
Juvenile cycle (Weijer and Weisberg, 1966).

- Fig. 1 Conidium with spherical resting nucleus and attached centriole.
- Fig. 2 Conidia with irregularly shaped resting nuclei.
- Fig. 3 Conidium containing duplicated filament with triangularly shaped centriole.
- Fig. 4 Germinated conidium containing filamentous nucleus with separated centriole.
- Fig. 5 Dividing hyphal filamentous nucleus in "Y-shaped" configuration.
- Fig. 6 Conidium with "crescent-shaped" nucleus of approximately 5 particulate bodies.
- Fig. 7 Conidium (center) containing "horseshoe-shaped" filament of approximately 9 particulate bodies.
- Fig. 8 Germinated conidium containing daughter crescent like nuclei within the conidium.
- Fig. 9 Germinating conidium with dividing crescent nucleus forming a "S-like" configuration.
- Fig. 10 Germinating conidium containing daughter crescent nuclei within, and moving out of, the conidium.
- Fig. 11 Conidium (center) with nuclear ring of approximately 6 chromatin bodies.
- Fig. 12 Conidium with "ring-shaped" nucleus of a partially particulate nature and a centriole.
- Fig. 13 Conidium containing nuclear ring structure with attached divided centrioles.

- Fig. 14 Germinated conidium (center) containing identical daughter ring nuclei.
- Fig. 15 Germinating conidium containing daughter ring nuclei moving into the hypha.
- Fig. 16 Hypha containing dividing filamentous nuclei.
- Fig. 17 Ring nucleus and crescent nucleus within a single hypha.
- Fig. 18 Hypha containing dividing filamentous nuclei (bottom left and right) and other various nuclei.
- Fig. 19 Hyphae containing non-particulate nuclear rings with associated centrioles.

Mag. X 3420

C = centriole

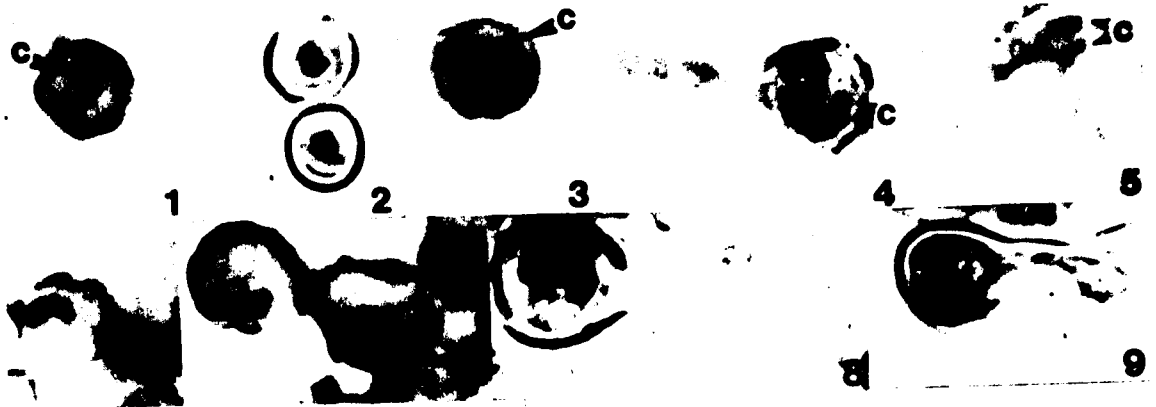




Plate II, Figs. 20 - 25. Hyphae of A. nidulans. Juvenile cycle (Weijer and Weisberg, 1966).

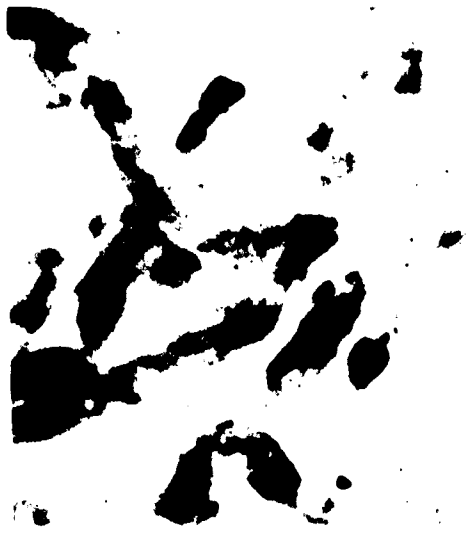
- Fig. 20 Hyphae with nuclear ring and nuclear filament structures (center).
- Fig. 21 Hyphae with non-particulate ring nuclei and an associated ring and filamentous ring nucleus (bottom left).
- Fig. 22 Hypha containing nuclei in various stages of condensation.
- Fig. 23 Hypha containing very condensed spherical nuclei.
- Fig. 24 Hypha containing condensed nuclei and a nucleus having a centriole (top).
- Fig. 25 Hypha containing a large filamentous nucleus composed of 8 particulate chromosomes and a large triangular centriole.

Figs. 20 - 24 Mag. X 3420

Fig. 25 Mag. X 4270

C = centriole





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Plate III. Figs. 26 - 32. Developing conidiophores of
A. nidulans (20 - 30 hours incubation).

- Fig. 26 A short-length but polytene maturation nucleus (left center) in a wide thick walled cell next to the conidium (top). Crystal Violet staining (20 hours incubation).
- Fig. 27 A large thick walled cell containing possible polytene maturation nuclear material. Crystal Violet staining (20 hours incubation).
- Fig. 28 A thick walled cell containing non-polytenic juvenile ring nuclei (29 hours incubation).
- Fig. 29 Early conidiophore with juvenile ring nuclei migrating from the foot cell into the developing conidiophore (22 hours incubation).
- Fig. 30 Early conidiophore with juvenile nuclei migrating from the foot cell into the developing conidiophore (30 hours incubation).
- Fig. 31 Juvenile filamentous nuclei moving into 1^o phialide buds from the conidiophore (Plate XXVII, Fig. 112 is lower focus of same conidiophore; (30 hours incubation).
- Fig. 32 Young conidiophore with fully formed 1^o phialides with a ring shaped nucleus clearly visible. No nuclear material is present in the conidiophore body (30 hours incubation).

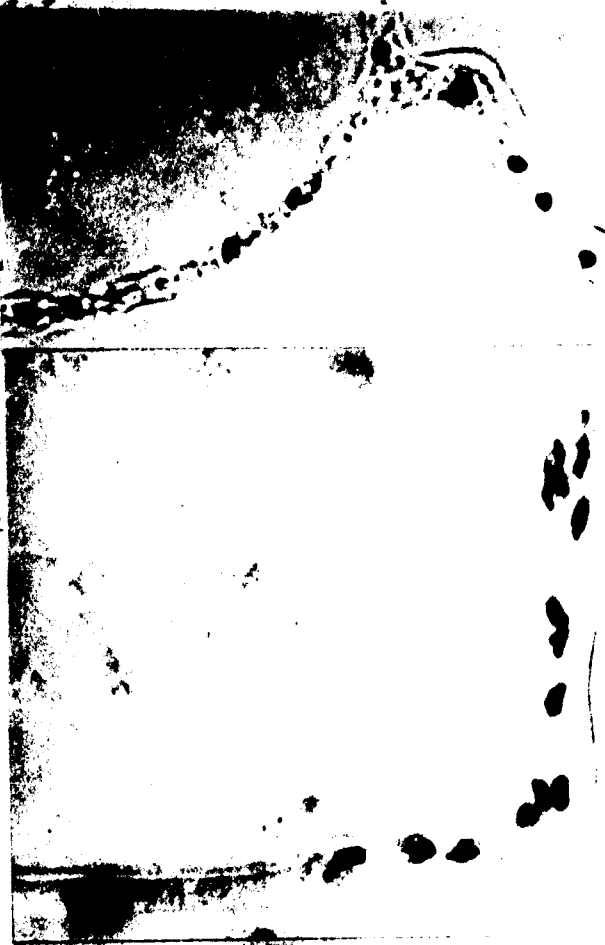
Mag. X 2300



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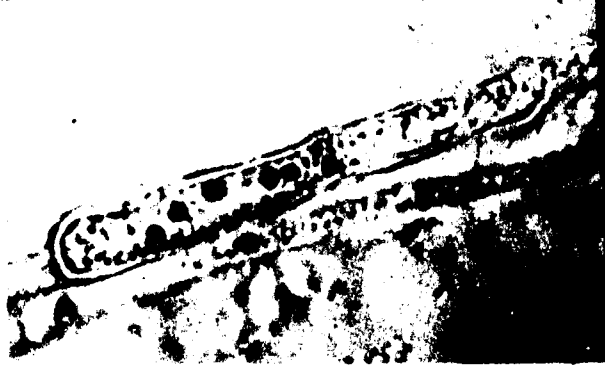
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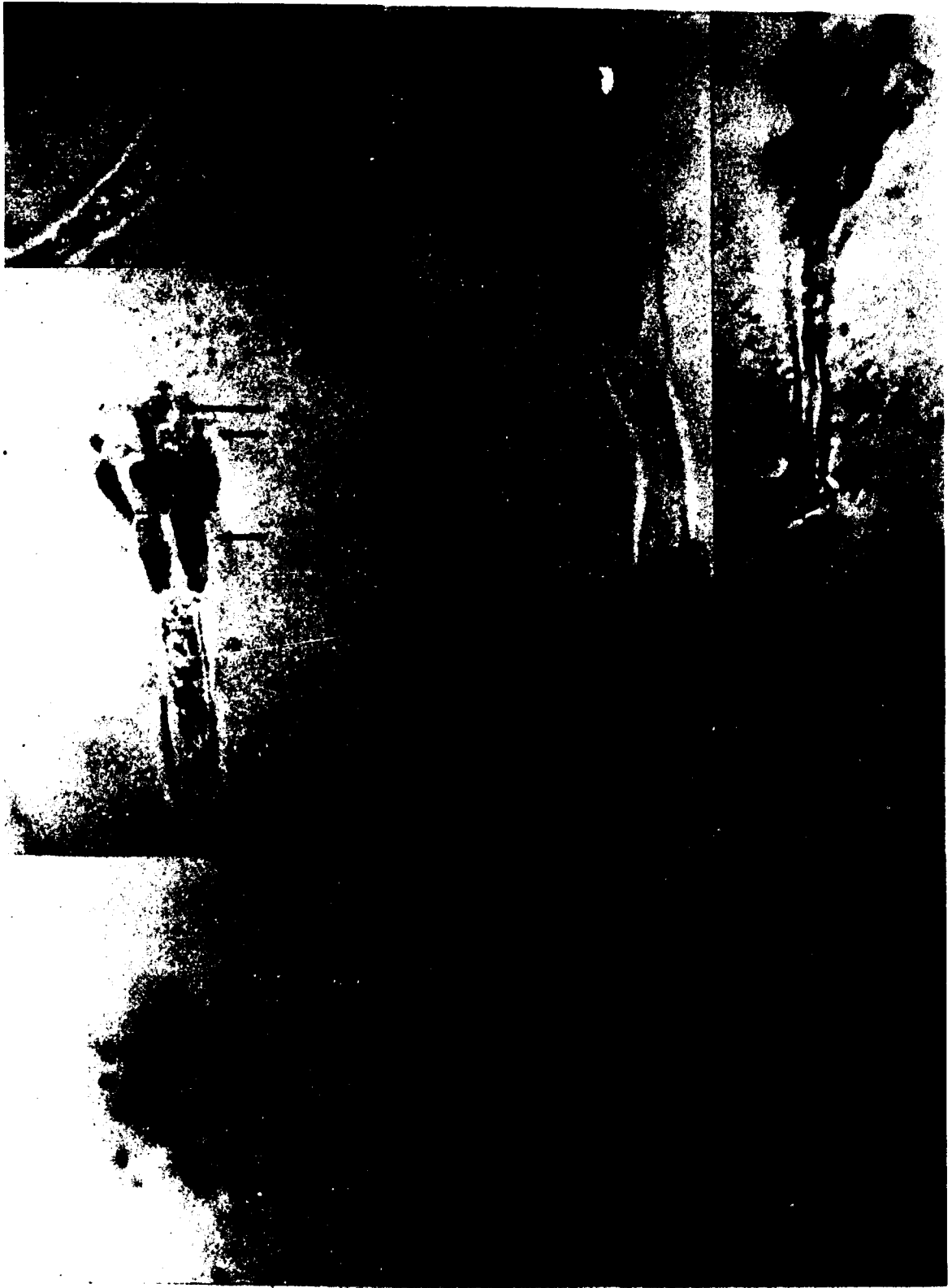
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Plate IV, Figs. 33 - 38. Further development of the conidiophore of A. nidulans (24 - 30 hours incubation).

- Fig. 33 Young conidiophore displaying 2^o phialides budding off from 1^o phialides. Nuclear material in the conidiophore is absent (30 hours incubation).
- Fig. 34 Young conidiophore showing divided nucleus in a 1^o phialide (bottom arrow) and conidial bud on 2^o phialide (middle arrow) and budded off conidial bud (top arrow) (24 hours incubation).
- Fig. 35 Conidiophore displaying divided nuclei in 2^o phialides (30 hours incubation).
- Fig. 36 Conidiophore showing a nucleus moving from a 2^o phialide into a conidial bud (arrow) but with no nuclear material in the conidiophore (30 hours incubation).
- Fig. 37 A large filament-like nucleus in a conidiophore (30 hours incubation).
- Fig. 38 A conidiophore containing large filamentous nuclear material (30 hours incubation).

Mag. X 2300





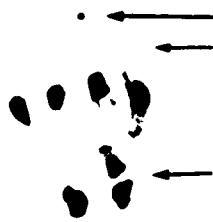
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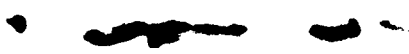


Plate V, Figs. 39 - 43. Conidia of *A. nidulans*. Juvenile nuclear cycle (8 - 12 hours incubation).

Fig. 39 Divided ring nuclei in a germinating conidium.

Fig. 40 Crescent-shaped nucleus moving into the hypha of the germinated conidium.

Fig. 41 A daughter filamentous nucleus moving into the hypha of the germinated conidium.

Fig. 42 Germinated conidium containing divided daughter nuclei in the conidium and divided daughter nuclei in the hypha.

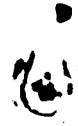
Fig. 43 Germinated conidium containing daughter nuclei of crescent and filament shapes displaying some particulate structure.

Mag. X 2300





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Plate VI, Figs. 44 - 48. Hyphae of *A. nidulans*. Juvenile nuclear cycle (22 - 30 hours incubation).

Fig. 44 Hypha containing evidence of migrating juvenile filamentous nuclei (arrows) (24 hours incubation).

Fig. 45 Hypha containing a large filamentous nucleus consisting of particulate bodies attached end to end (22 hours incubation).

Fig. 46 Hyphae containing dividing small filamentous nuclei or possible clustering (pairing?) of filamentous nuclei (24 hours incubation).

Fig. 47 Hypha containing filamentous juvenile nuclei (30 hours incubation).

Fig. 48 Hypha containing a small filamentous nucleus (30 hours incubation).

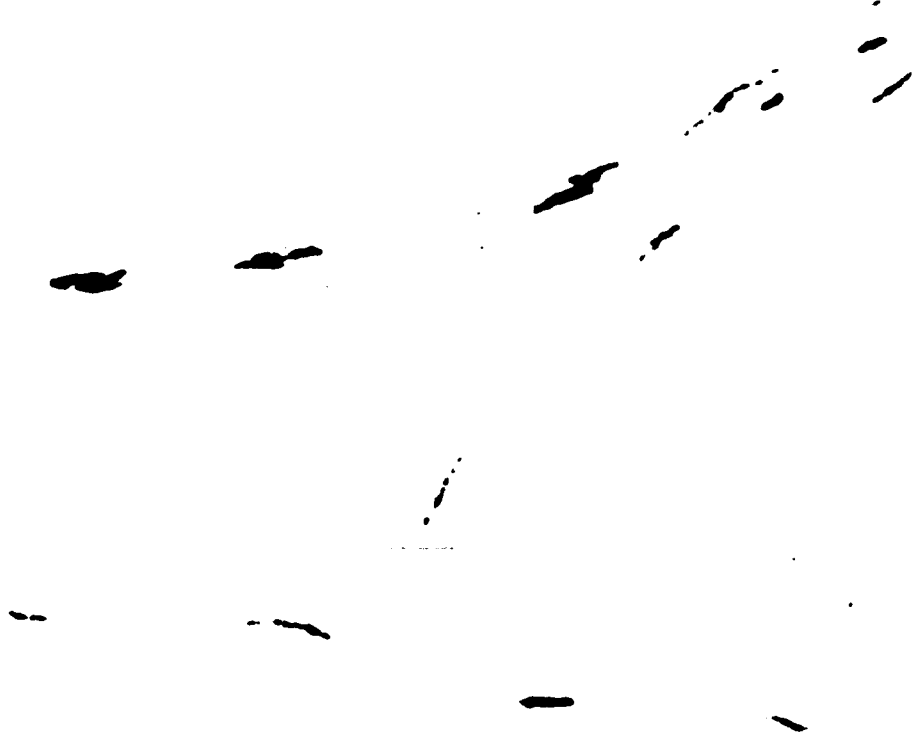
Mag. X 2300



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Plate VII, Figs. 49 - 52. Hyphae and small conidiophore
of A. nidulans (22 - 30 hours incubation).

Fig. 49 Hypha containing juvenile ring nuclei (22 hours
incubation).

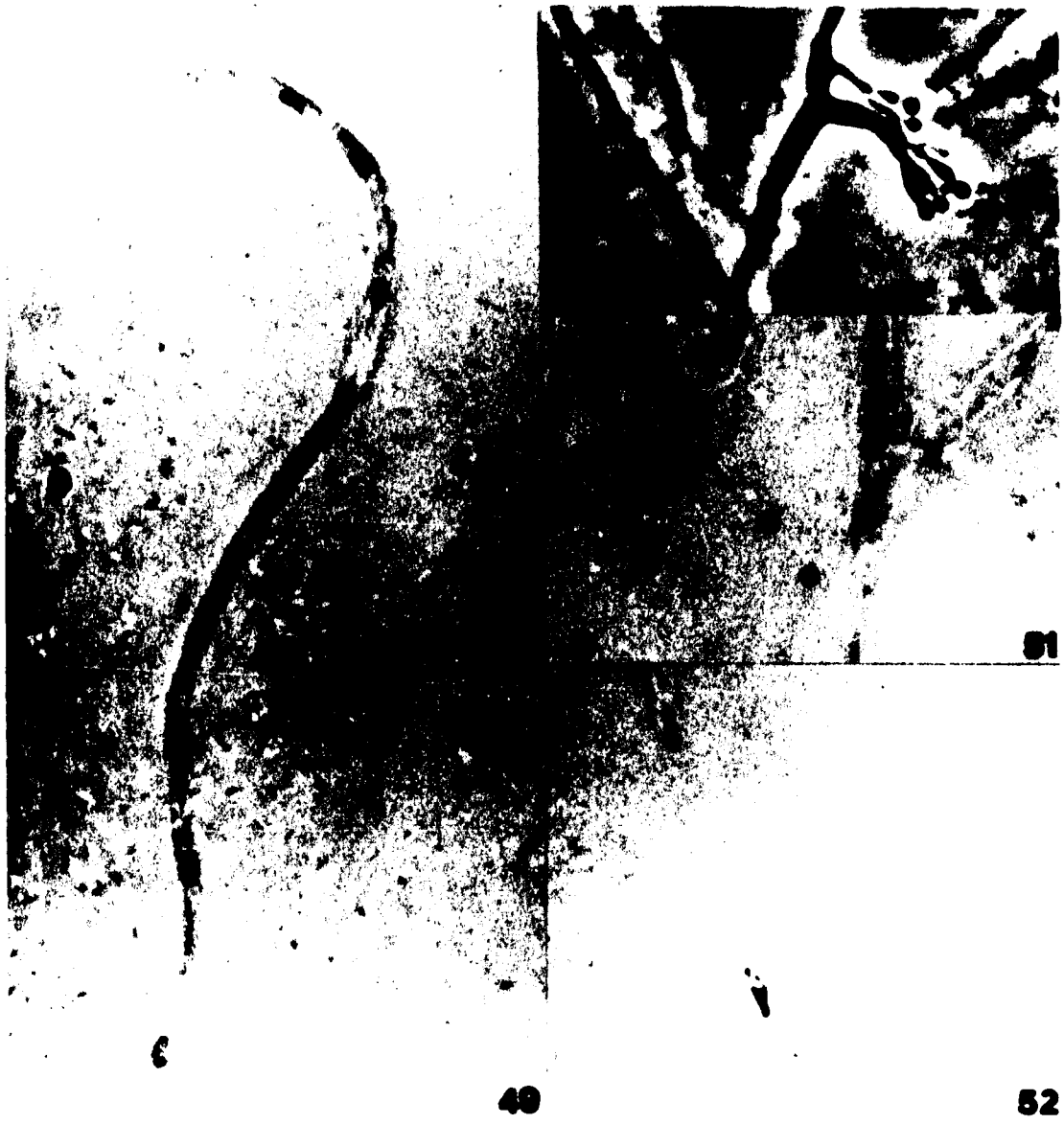
Fig. 50 Mini-conidiophores of A. nidulans (phase contrast
microscopy) (30 hours incubation).

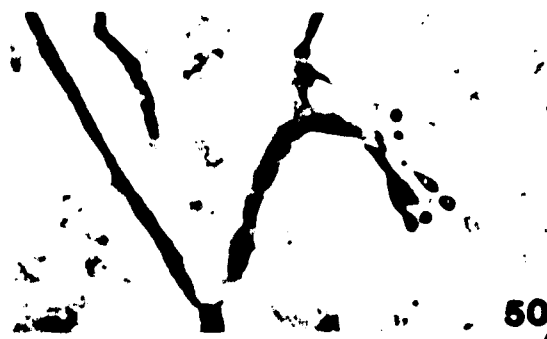
Fig. 51 Hypha containing dividing juvenile filamentous
nuclei (center) (24 hours incubation).

Fig. 52 Dividing filamentous nuclei in hypha (22 hours
incubation).

Figs. 49, 51 and 52 Mag. X 2300

Fig. 50 Mag. X 2080





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Plate VIII, Figs. 53 - 57. Hyphae of A. nidulans showing transition of the juvenile nuclear state to the polytene (maturation) nuclear state (22 - 29 hours incubation).

Fig. 53 Pre-maturation filamentous nucleus within a hypha (22 hours incubation).

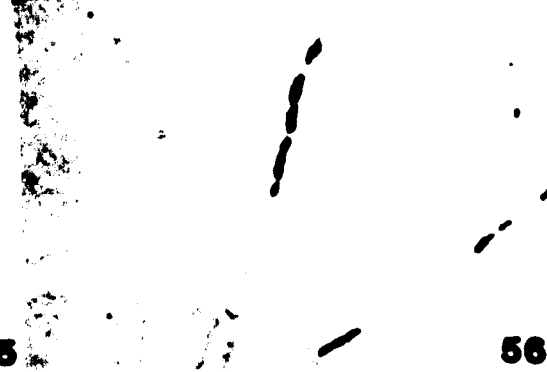
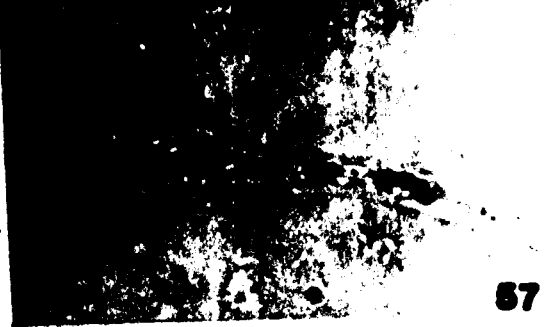
Fig. 54 Hyphae containing small juvenile filamentous nuclei (29 hours incubation).

Fig. 55 Small and a larger (center) juvenile nuclei within the same hypha (29 hours incubation).

Fig. 56 Hypha containing a large pre-maturation filamentous nucleus (see Plate II, Fig. 25) (24 hours incubation).

Fig. 57 A hypha containing a weakly stained filamentous nucleus (24 hours incubation).

Mag. X 2300



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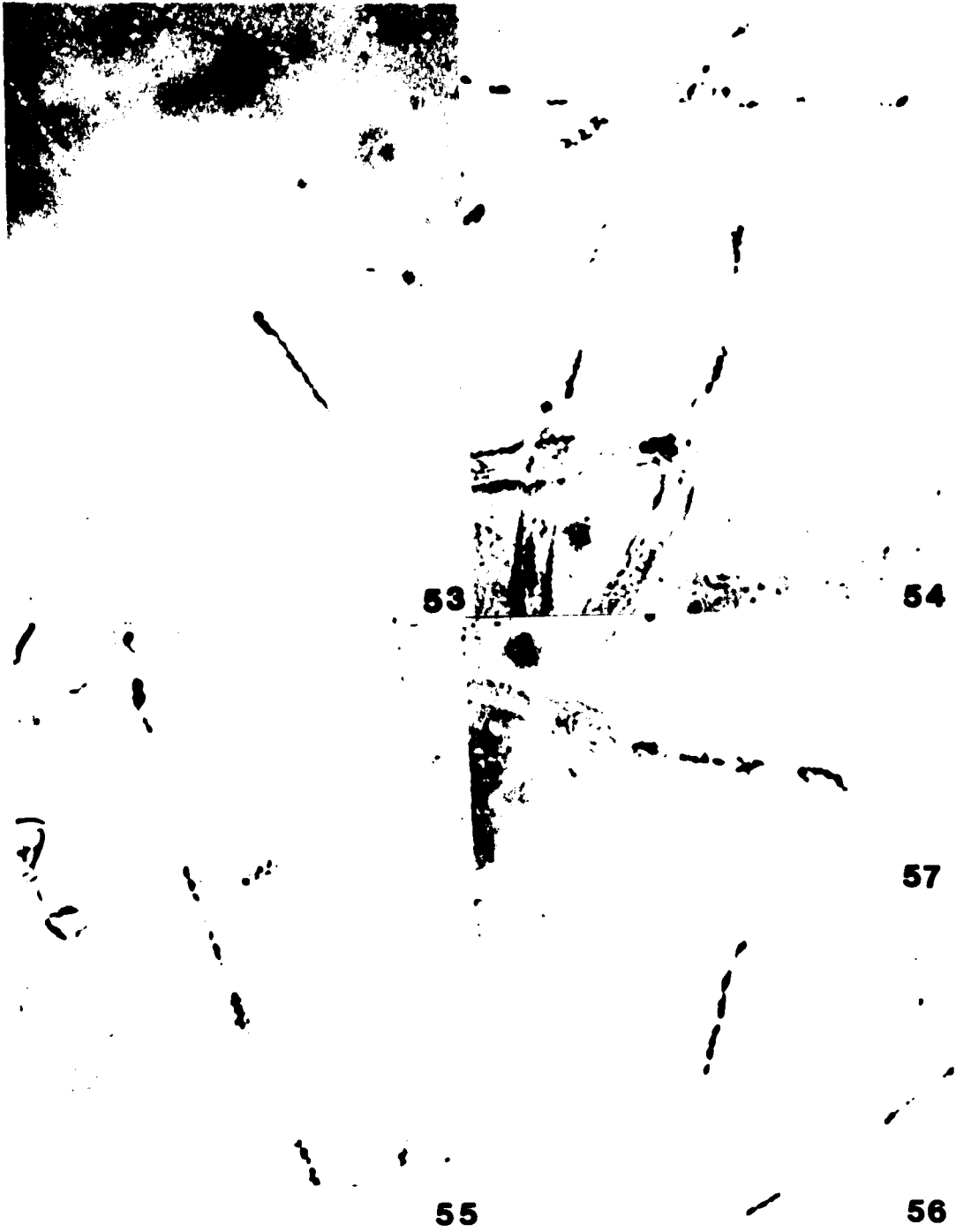


Plate IX, Figs. 58-60. Hyphae of *A. nidulans* (24-30 hours incubation).

Fig. 58 Hypha containing a very long (80 μ) nucleus of 8 polytene maturation chromosomes together with a triangular-shaped centriole (24 hours incubation).

Fig. 59 Hypha containing a long polytene maturation nucleus in which some of the chromosomes have divided (30 hours incubation).

Fig. 60 A hypha containing divided paired polytene maturation chromosomes and a centriole (24 hours incubation).

Mag. X 2300

C = centriole

Daughter chromosomes are indicated by lower-case letters.



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Plate X, Figs. 61 - 63. Hyphae of A. nidulans (30 hours incubation).

Fig. 61 Hypha containing paired polytene chromosomes and juvenile nuclei.

Fig. 62 Hypha containing paired, dividing polytene chromosomes.

Fig. 63 Hypha containing polytene chromosomes and a centriole.

Mag. X 2300

C = centriole

Daughter chromosomes are indicated by lower-case letters.



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Plate XI, Fig. 64 and 65. Hyphae of A. nidulans (30 hours incubation).

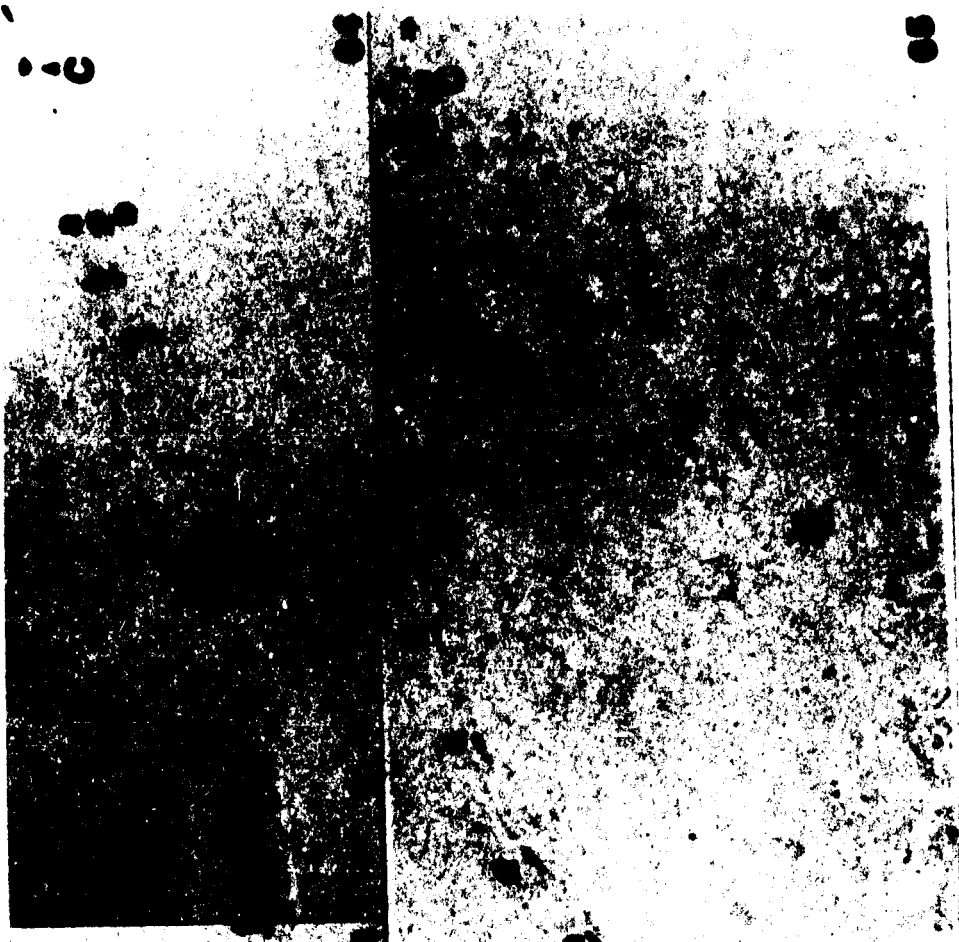
Fig. 64 Composite photograph of a hypha containing eight pairs of polytene chromosomes together with one centriole.

Fig. 65 A section of hypha containing sixteen paired polytene chromosomes together with a pair of centrioles.

Mag. X 2300

C = centriole

Daughter chromosomes are indicated by lower-case letters.



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Plate XII, Figs. 66 and 67. Hypha of A. nidulans (24 and 30 hours incubation respectively).

Fig. 66 Homology of polytene maturation chromosomes within a hyphal section.

Fig. 67 Hypha containing polytene maturation chromosomes arranged in linear file by a weakly Feulgen-positive thread.

Mag. X 2300

C = centriole

Daughter chromosomes are indicated by lower-case letters.



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Plate XIII., Figs. 68 - 70. Hyphae of A. nidulans (24 hours incubation).

Fig. 68 Extended polytene maturation chromosomes displaying homology among chromosome pairs within a section of hypha.

Fig. 69 Hypha containing sixteen polytene maturation chromosomes displaying homology among chromosome pairs and a pair of centrioles.

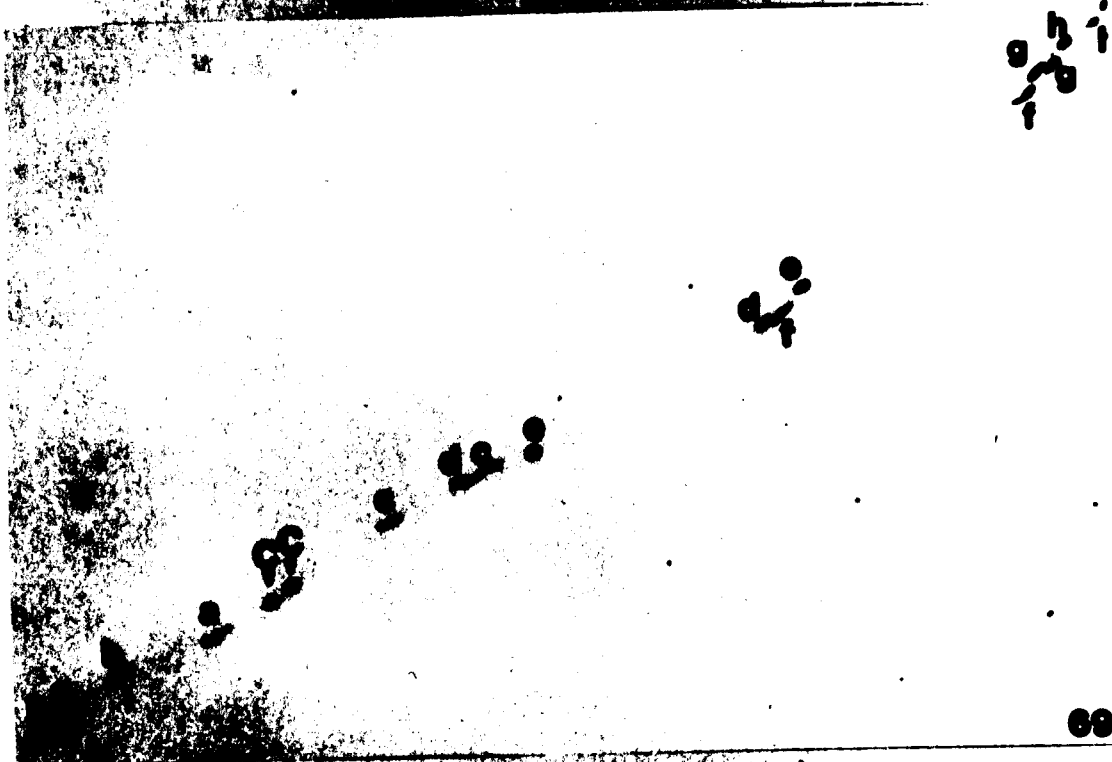
Fig. 70 Hyphal segment containing polytene maturation chromosomes displaying chromosome homology.

Mag. X 2300

C = centriole

Daughter chromosomes are indicated by lower-case letters.

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Plate XIV, Figs. 71 - 73. Conidiophore and hyphae of A. nidulans (30, 20 and 30 hours incubation, respectively).

Fig. 71 Conidiophore containing polytene maturation chromosomes.

Fig. 72 A short polytene (pre-maturation) nucleus within a conidium situated next to a foot cell (bottom).

Fig. 73 Composite photograph of a hypha containing polytene maturation chromosomes.

Mag. X 2300

C = centriole





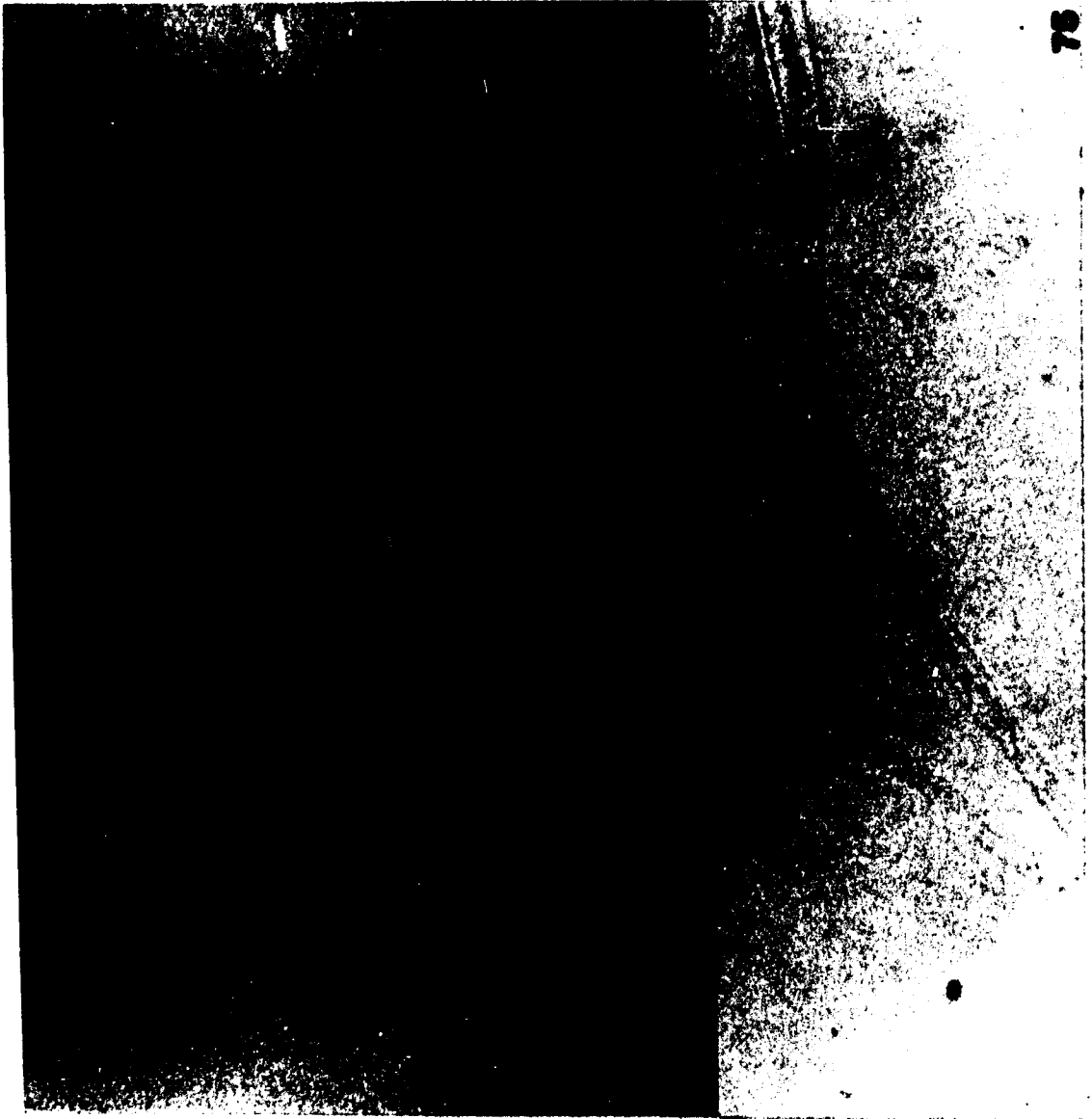
Plate XV, Figs. 74 and 75. Conidiophore and hyphae of A. nidulans. Crystal Violet staining (24 and 20 hours incubation respectively).

Fig. 74 A conidiophore and foot cell bordering a hypha containing a polytene nucleus of 8 maturation chromosomes together with a centriole attached end to end by a weakly Crystal Violet positive thread (arrows).

Fig. 75 A hyphal section (note thick walls) containing large polytene maturation chromosomes.

Mag. X 2300

C = centriole





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Plate XVI, Fig. 76. Conidiophore, foot cell and neighbouring hypha of A. nidulans (30 hours incubation).

Fig. 76 Conidiophore containing polytene maturation chromosomes and thread-like nuclear material at the apex of the nucleus. The foot cell is not clearly shown. The hypha near to the conidiophore contains polytene maturation chromosomes, some of which are homologously paired, together with a centriole.

Mag. X 1710

C = centriole





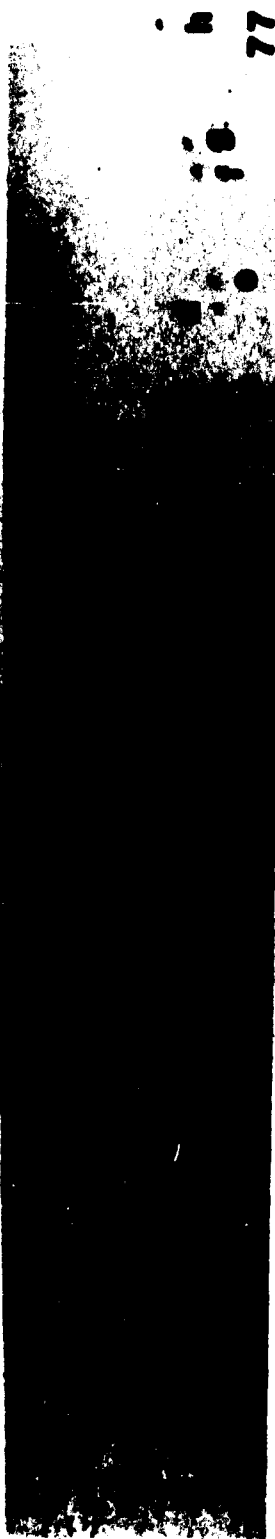
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Plate XVII, Figs. 77, 77A, 77a, 77B and 77b. Conidiophore, foot cell and neighbouring hyphal segments of A. nidulans (bright field VS phase contrast).

- Fig. 77 Composite photographs of a conidiophore and foot cell (between lower arrows). A polytene nucleus is present in the conidiophore. Both neighbouring hyphal segments contain eight polytene maturation chromosomes (some of which are divided) together with a terminal centriole. On the right [arrows; bright field observation (77A), upper arrows] a weakly Feulgen positive thread is present between the chromosomes of the nucleus. Because of focal depth limitations, the thread is not seen throughout the hypha.
- Fig. 77A A section of hypha containing a thread connecting the chromosomes (bright field observation).
- Fig. 77a A corresponding phase contrast microphotograph of Fig. 77A.
- Fig. 77B A section of hypha containing a weakly Feulgen positive thread connecting the chromosomes (bright field observation).
- Fig. 77b A corresponding phase contrast microphotograph of Fig. 77B. (arrow shows dividing thread).

Figs. 77, 77A and 77B Mag. X 1030

Figs. 77a and 77b Mag. X 2080

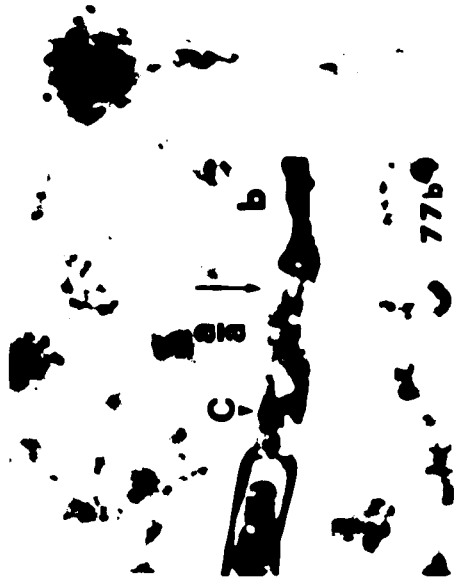




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Plate XVIII, Fig. 78 and 79. Composite photographs of a section of hypha of A.nidulans under bright field and corresponding phase contrast microphotography (30 hours incubation).

Fig. 78 A section of hypha adjacent to a foot cell (top left) containing 18 polytene maturation chromosomes (homologous chromosomes indicated by identical lettering).

Fig. 79 A corresponding phase contrast microphotograph of Fig. 78 showing a thread running through the hypha and maturation chromosomes (dark round bodies).

Mag. X 1370 and X 1230 respectively



a. b. c. d. e. f. g. h. i. j. k. l. m. n. o. p. q. r. s. t. u. v. w. x. y. z.

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Plate XIX, Fig. 80. Hypha of A. nidulans. Phase contrast
(30 hours incubation).

Fig. 80 A large magnification of the composite photograph
of Fig. 79. The sections of hypha overlap
(see arrows).

Mag. X 2080





Plate XX, Fig. 81 - 84. Hyphal segments of A. nidulans
(30 hours incubation).

Fig. 81 Hypha adjacent to a foot cell (top right)
containing polytene maturation chromosomes
and a connecting fiber (arrows).

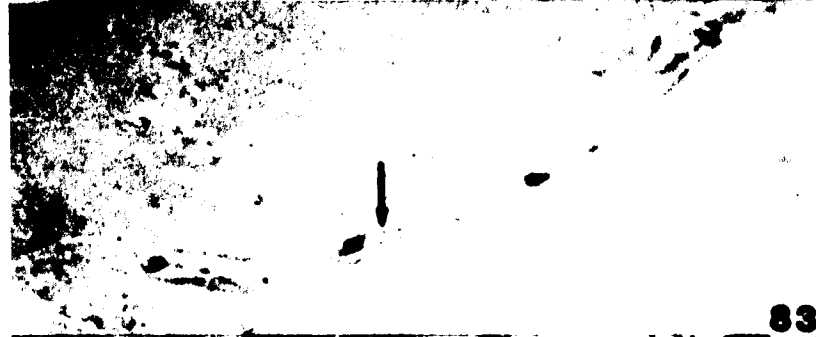
Fig. 82 Phase contrast microphotograph of Fig. 81
showing the connecting thread (arrows).

Fig. 83 Hyphal segment adjacent to a foot cell (top
right) containing polytene maturation chromosomes
and a weakly Feulgen-positive thread (arrow).

Fig. 84 Phase contrast microphotograph of Fig. 83
displaying the connecting thread (arrow).

Figs. 81 and 83 Mag. X 2300

Figs. 82 and 84 Mag. X 2080



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Plate XXI, Figs. 85 - 88. Hyphal segments of A. nidulans
(16 and 30 hours incubation).

Fig. 85 Polytene chromosomes in a hyphal section (30
hours incubation).

Fig. 86 Phase contrast microphotograph of Fig. 85
showing double strand connecting partially
divided chromosomes (arrows) (30 hours incubation).

Fig. 87 Acid fuchsin stained hyphal section showing
double stranded fiber (phase contrast condenser
and bright field objective used in this photograph)
(16 hours incubation).

Fig. 88 Phase contrast microphotograph of a single fiber
(arrow) in a hyphal section. (30 hours incubation).

Figs. 85 and 87 Mag. X 2300

Figs. 86 and 88 Mag. X 2080



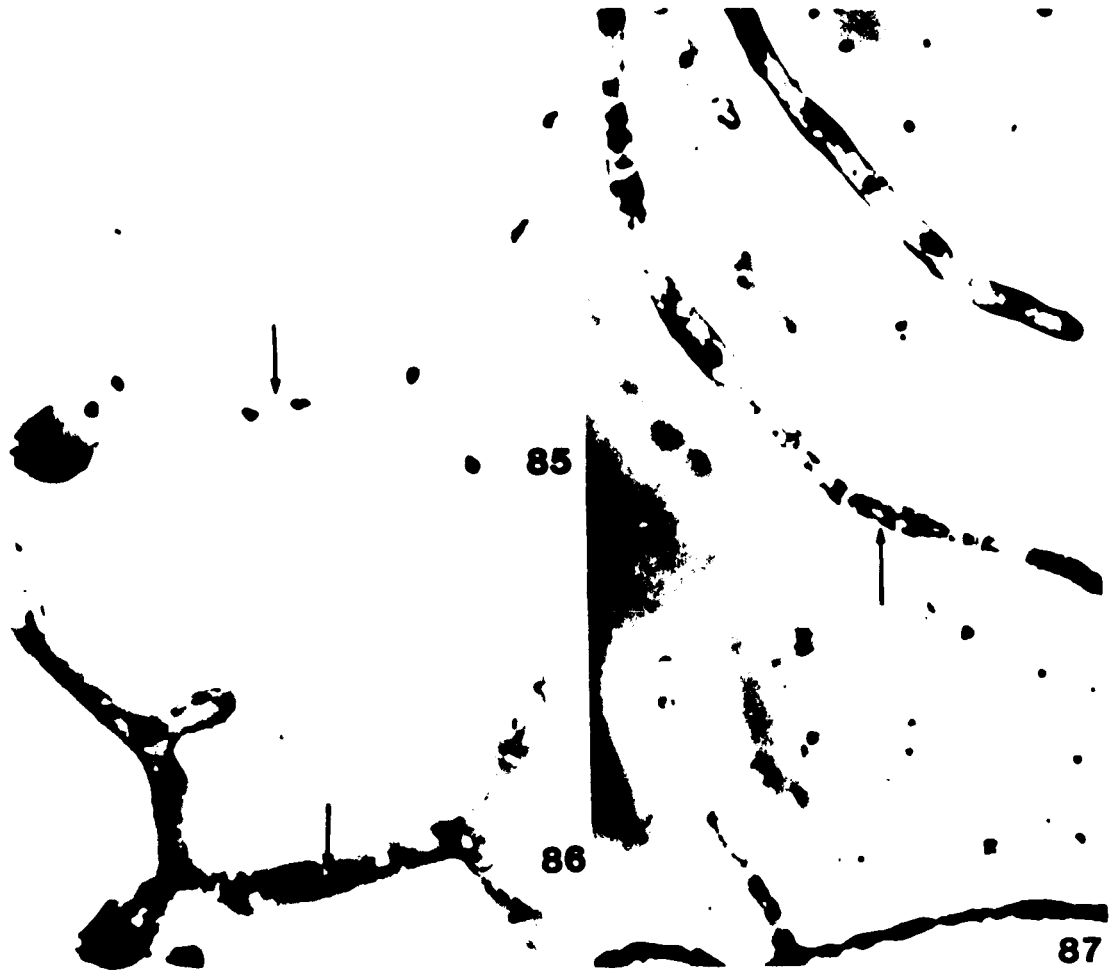


Plate XXII, Figs. 89 - 91. Hyphae of A. nidulans (30,
30 and 16 hours incubation, respectively).

Fig. 89 Feulgen stained nuclear fibers in hyphal segment
(phase contrast).

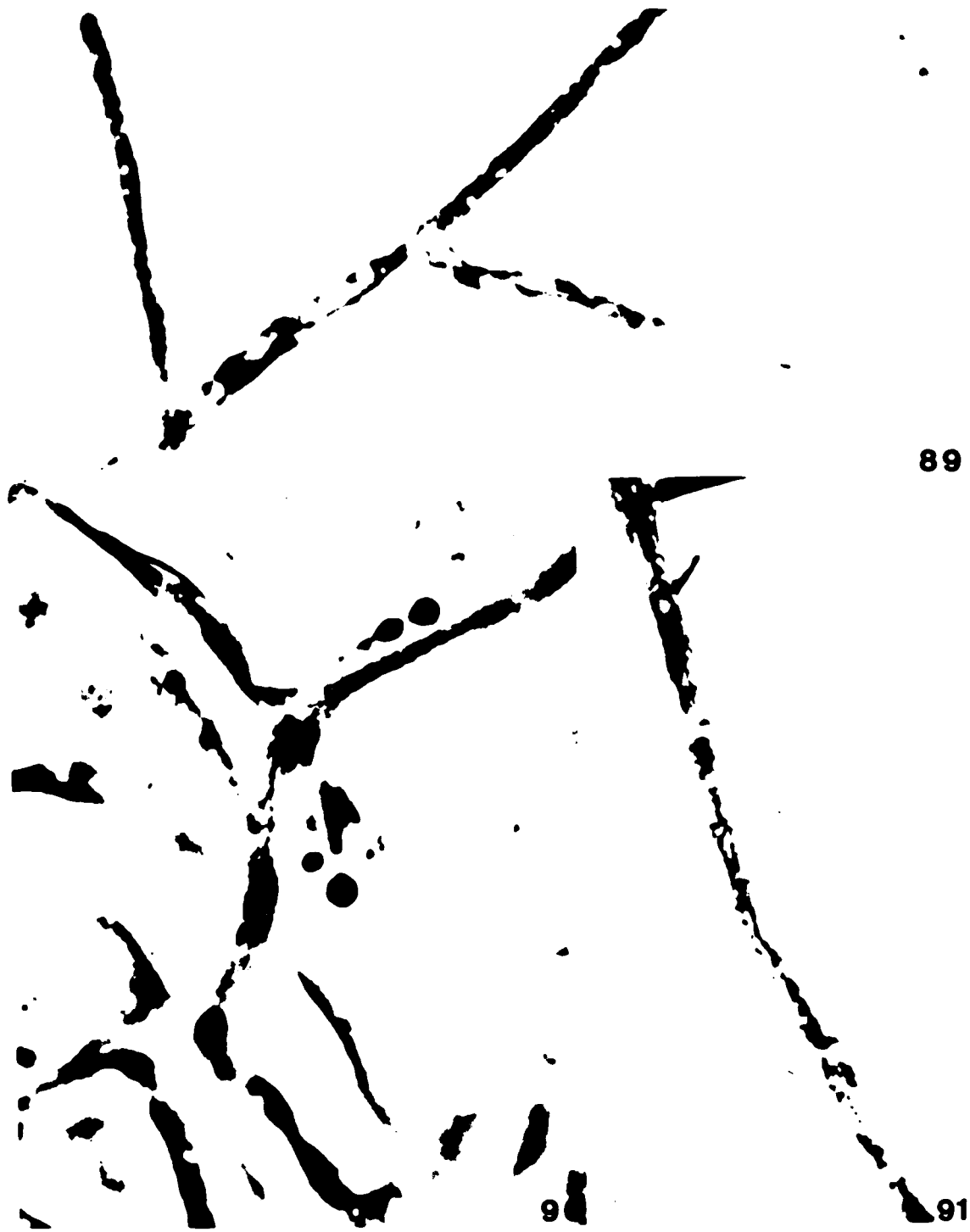
Fig. 90 Phase contrast observation of Feulgen stained
hyphae and a mini-conidiophore showing a fiber
in the hyphae.

Fig. 91 Acid fuchsin stained hypha with a stained fiber.

Figs. 89 and 90 Mag. X 2080

Fig. 91 Mag. X 2300





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Plate XXIII, Figs. 92 and 93. Hyphae of A. nidulans (22
and 30 hours incubation, respectively).

Fig. 92 Acid fuchsin stained nuclear fiber in hyphal
segment (arrow).

Fig. 93 Phase contrast microphotograph of a Feulgen
stained fiber in a hyphal segment.

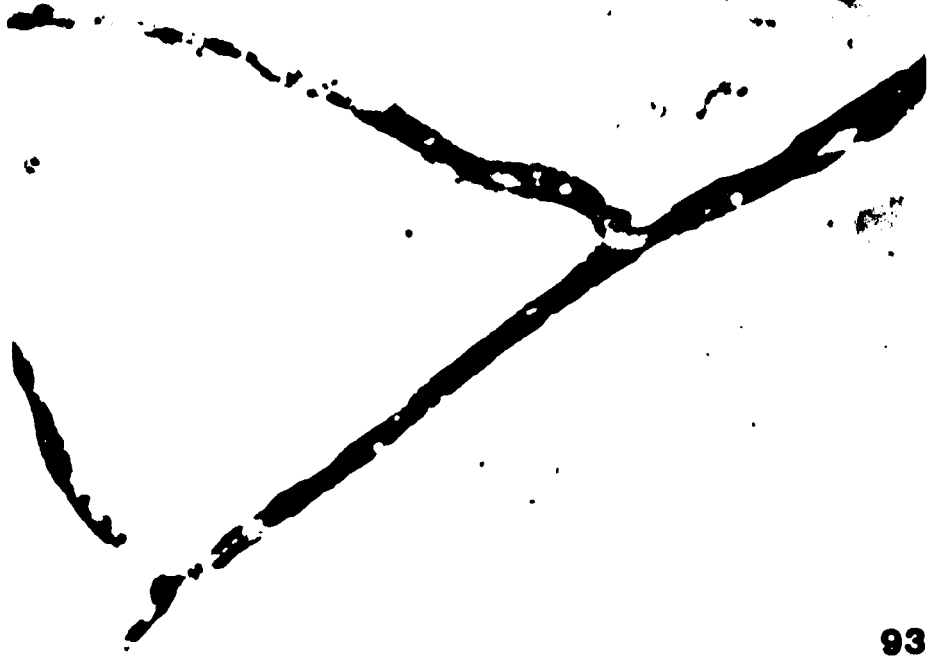
Fig. 92 Mag. X 2300

Fig. 93 Mag. X 2080





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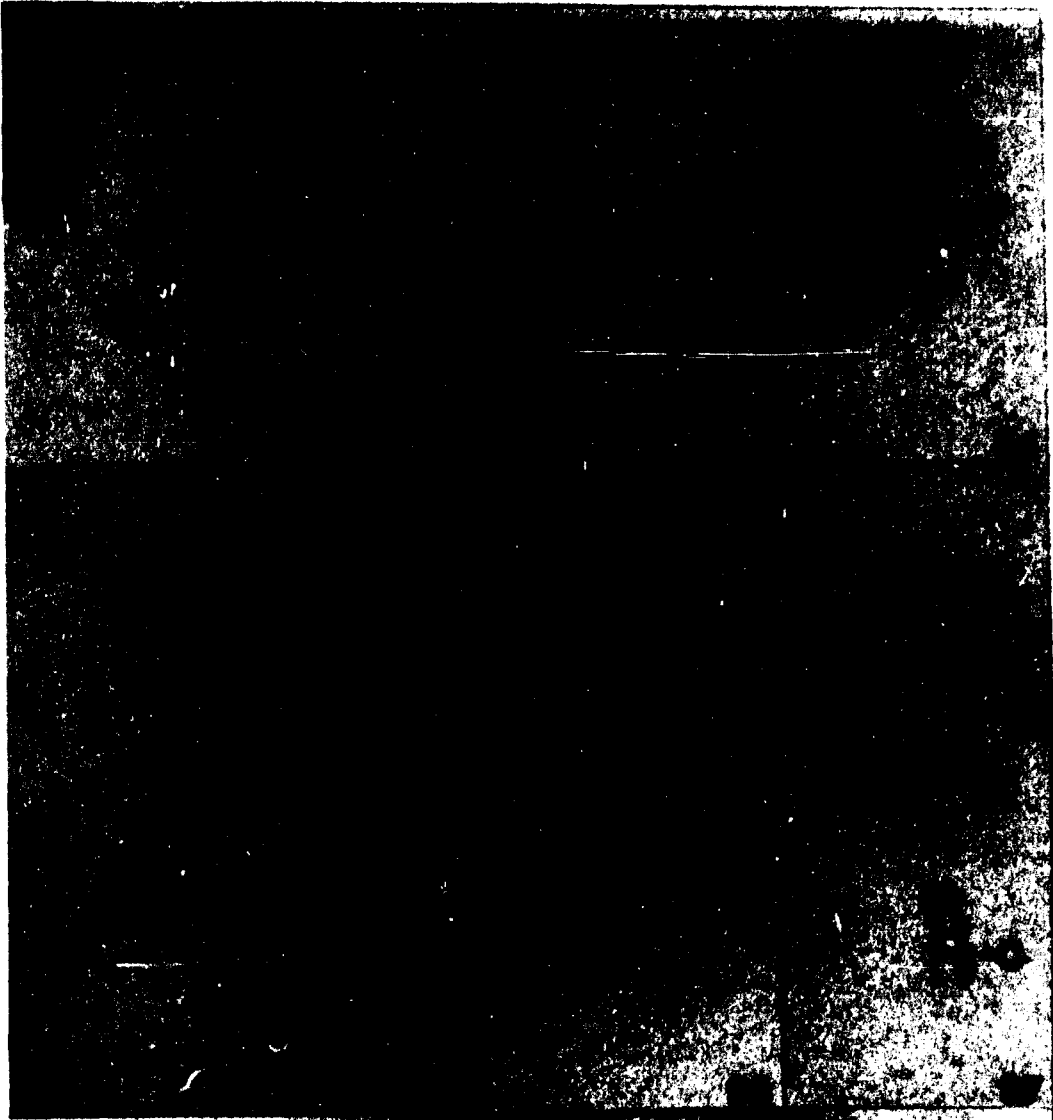
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Plate XXIV, Figs. 94 - 99. Conidiophores of A. nidulans
(30 hours incubation).

- Fig. 94 Conidiophore containing a short polytene maturation nucleus and with 2^o phialides containing divided nuclei (arrow).
- Fig. 95 Conidiophore containing fifteen uncondensed polytene maturation chromosomes (partly uncoiled).
- Fig. 96 Young conidiophore containing a linearly arranged polytene maturation nucleus.
- Fig. 97 A conidiophore nucleus containing polytene maturation chromosomes which are attached together by a weakly Feulgen-positive thread.
- Fig. 98 Very young conidiophore (lacking phialides) with a maturation nucleus containing partly uncoiled chromosomes together with two juvenile nuclei at its apex (arrow).
- Fig. 99 Mature conidiophore containing thread-like maturation nuclear material in the conidiophore head.

Mag. X 2300

C = centriole

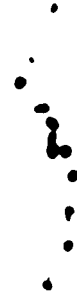
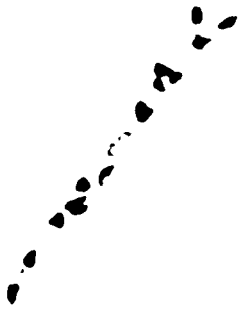




C



94



C

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96

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98

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Plate XXV, Figs. 100 - 102. Conidiophore of A. nidulans
(30 hours incubation).

Fig. 100 Large conidiophore containing two polytene
maturation nuclei.

Fig. 101 Conidiophore containing polytene maturation
chromosomes.

Fig. 102 Conidiophore containing strand-like chromosomal
material.

Mag. X 2300



101

100

102



100

101

102

Plate XXVI, Figs. 103 - 108. Conidiophores of A. nidulans
(30 hours incubation).

Fig. 103 Conidiophore containing two polytene nuclei.
The rest of the lower nucleus is shown in Fig.
108.

Fig. 104 Polytene nucleus in a young conidiophore at
the 1^o phialide stage.

Fig. 105 Single polytene nucleus in a conidiophore.

Fig. 106 Conidiophore containing polytene chromosomes
and a large triangular centriole.

Fig. 107 Conidiophore containing two nuclei with condensed
polytene chromosomes.

Fig. 108 See Fig. 103.

Mag. X 2300

C = centriole



103

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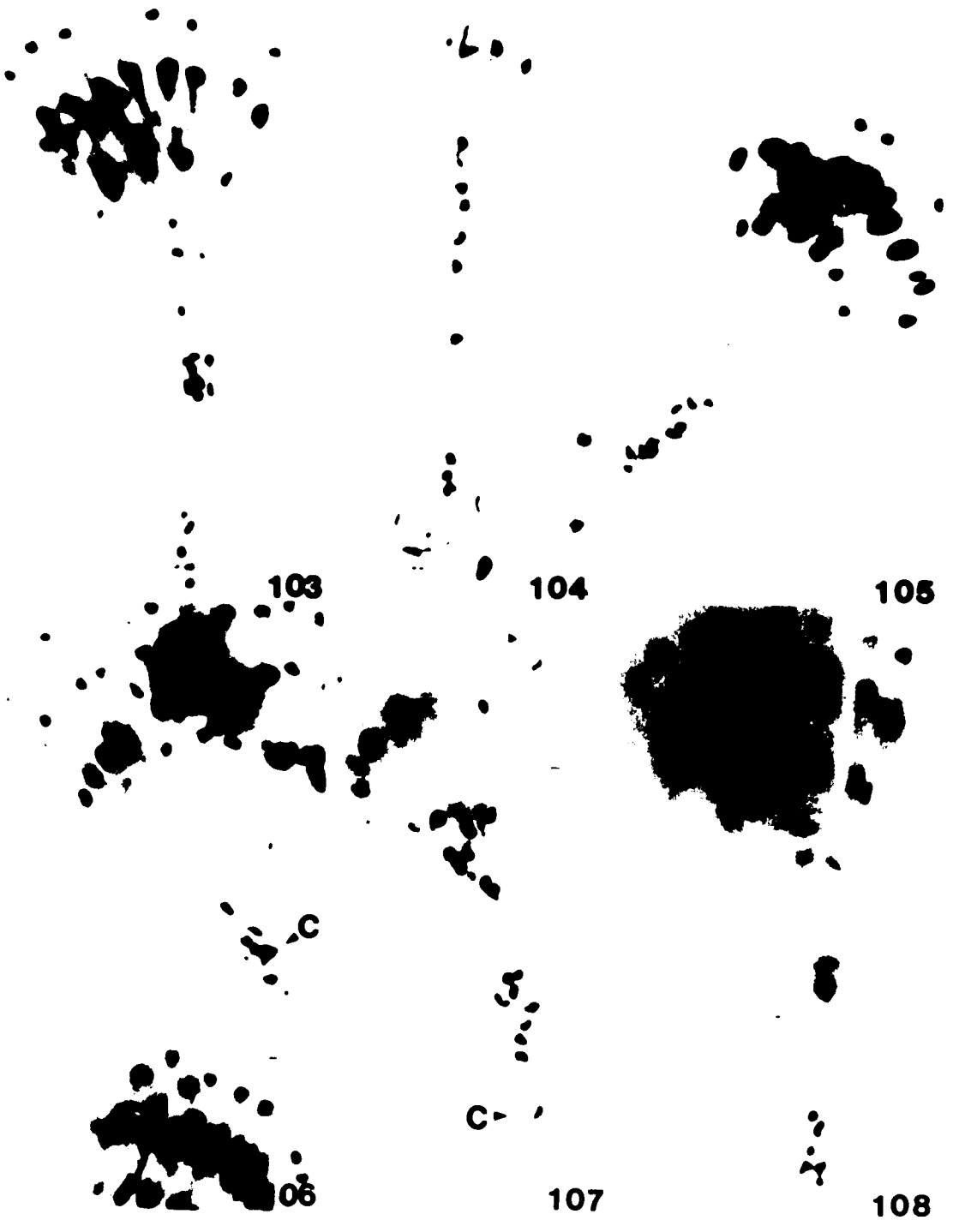
105

106

C-

107

108



103

104

105

C

C

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108

Plate XXVII, Figs. 109 - 113. Conidiophore of A. nidulans
(30 hours incubation).

Fig. 109 Centriole and polytene maturation chromosomes
in a conidiophore.

Fig. 110 Crystal Violet stained young conidiophore
containing thread-like polytene transitional
nuclear material.

Fig. 111 Young conidiophore (containing undefined
nuclear material) with a 1^o phialide carrying a
2^o phialide bud.

Fig. 112 A very young conidiophore containing filamentous
juvenile (non-polytene) nuclei migrating into
1^o phialide buds. (see also Plate III, Fig. 31).

Fig. 113 Conidiophore containing two polytene maturation
nuclei.

Mag. X 2300

C = centriole



112

A-C

113



100

110



111



112

-C

113

Plate XXVIII, Figs. 114 - 117. Conidiophores of A. nidulans (20, 20, 30 and 30 hours incubation respectively).

- Fig. 114 Crystal Violet stained early conidiophore containing partially defined polytene nuclear material. 2° phialide buds are shown on the 1° phialides and a nucleus is migrating from a 1° to 2° phialide. Polytene chromosomes are in the adjacent hypha.
- Fig. 115 Crystal Violet stained conidiophore containing divided thread-like nuclear material (arrow).
- Fig. 116 Early conidiophore (1° phialide stage) containing thread-like nuclear material.
- Fig. 117 Conidiophore containing thread-like nuclear material in the conidiophore head.

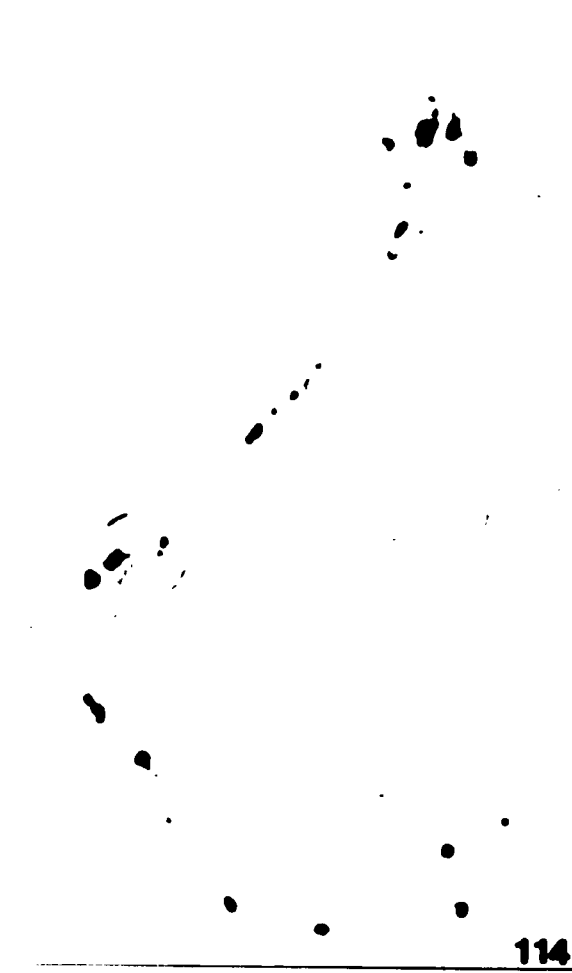
Mag. X 2300



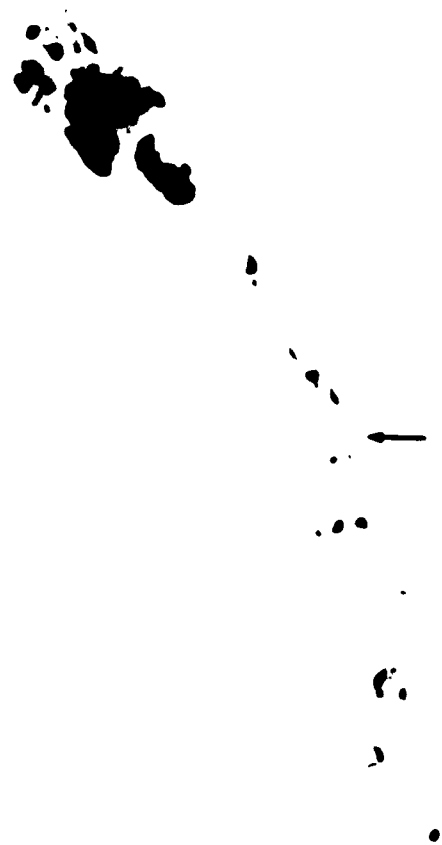
116

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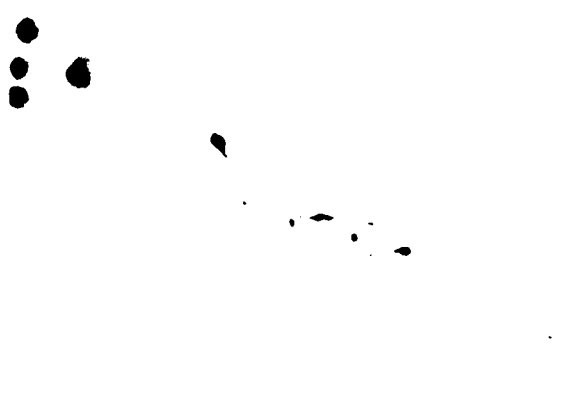
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114



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Plate XXIX, Figs. 118 - 121. Conidiophores of A. nidulans
(28, 20, 30 and 20 hours incubation respectively).

Fig. 118 Crystal Violet stained young conidiophore containing thread-like nuclear structure.

Fig. 119 Crystal Violet stained conidiophore containing polytene nucleus (bottom), strand-like nuclear material (arrow) together with oddly stained material (above arrow).

Fig. 120 Conidiophore with 2⁰ phialide containing divided nucleus (arrow; the nuclear material in the conidiophore body is out of focus).

Fig. 121 Crystal Violet stained young conidiophore containing two small polytene nuclei.

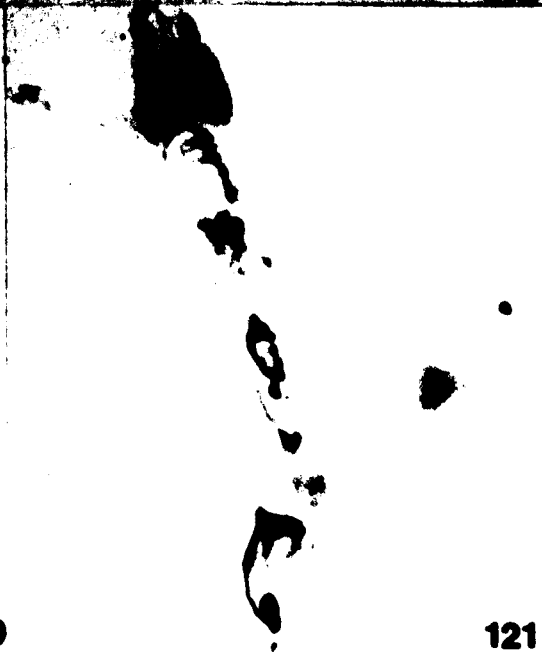
Mag. X 2300



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Plate XXX, Figs. 122 - 125. Conidiophores of A. nidulans
(30 hours incubation).

- Fig. 122 Conidiophore containing a polytene maturation nucleus and a strand of stained material which appears to be unwinding into 3 separated strands (arrow). See also Plate XVII, Fig. 76, for the complete conidiophore.
- Fig. 123 Conidiophore head containing thread-like nuclear material.
- Fig. 124 Conidiophore containing polytene maturation chromosome structures and stained thread-like material.
- Fig. 125 Conidiophore with a 2^o phialide containing a divided nucleus (the conidiophore nucleus is out of focus).

Mag. X 2300



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124



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Plate XXXI, Figs. 126 - 130. Conidiophores and hyphae of *A. nidulans*. Crystal Violet staining (36, 24, 24, 24 and 36 hours incubation respectively).

Fig. 126 Conidiophore from which the phialide rows have disappeared.

Fig. 127 Conidiophore containing a centriole in the conidial head and no other nuclear material.

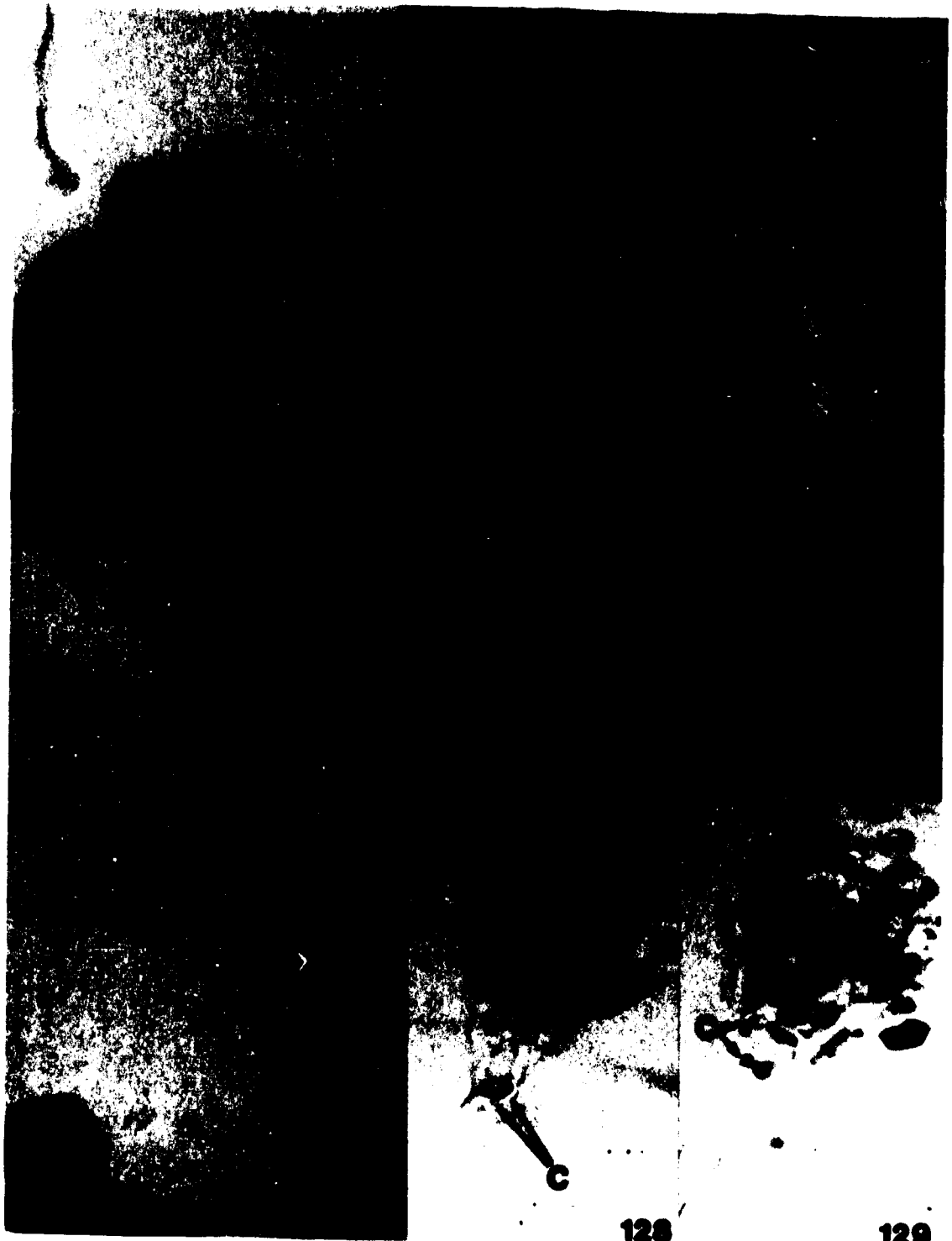
Fig. 128 Conidiophore with only a pair of centrioles in the conidiophore body.

Fig. 129 Conidiophore containing no chromosomal material but a pair of centrioles.

Fig. 130 Hypha from which the hyphal wall has mostly disappeared and the cytoplasm formed protoplast-like structures.

Mag. X 2300

C = centriole



128

129



126

128

129

Plate XXXII, Figs. 131 and 132. Conidiophores and hyphae of *A. nidulans*, Crystal Violet staining (37 hours incubation).

Fig. 131 Conidiophore from which the phialides have disintegrated.

Fig. 132 Larger field of Fig. 134, showing the conidiophore and surrounding hyphae in which the cytoplasm has formed protoplast-like structures and from which the hyphal walls have disappeared.

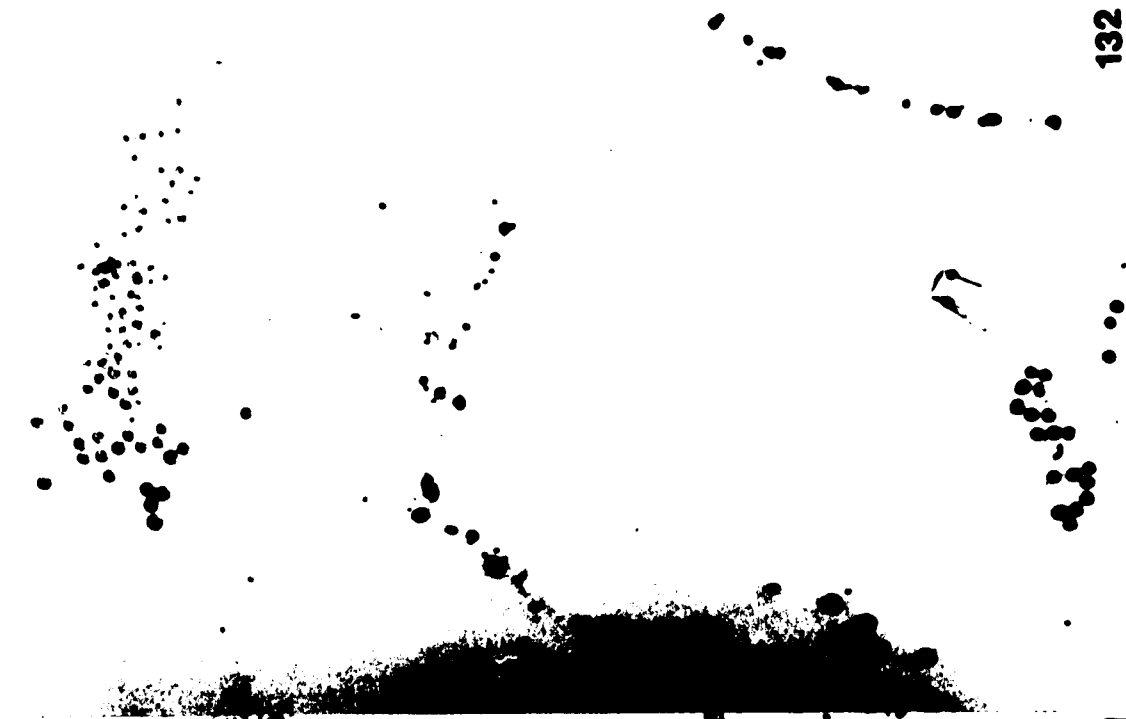
Fig. 131

Mag. X 2300

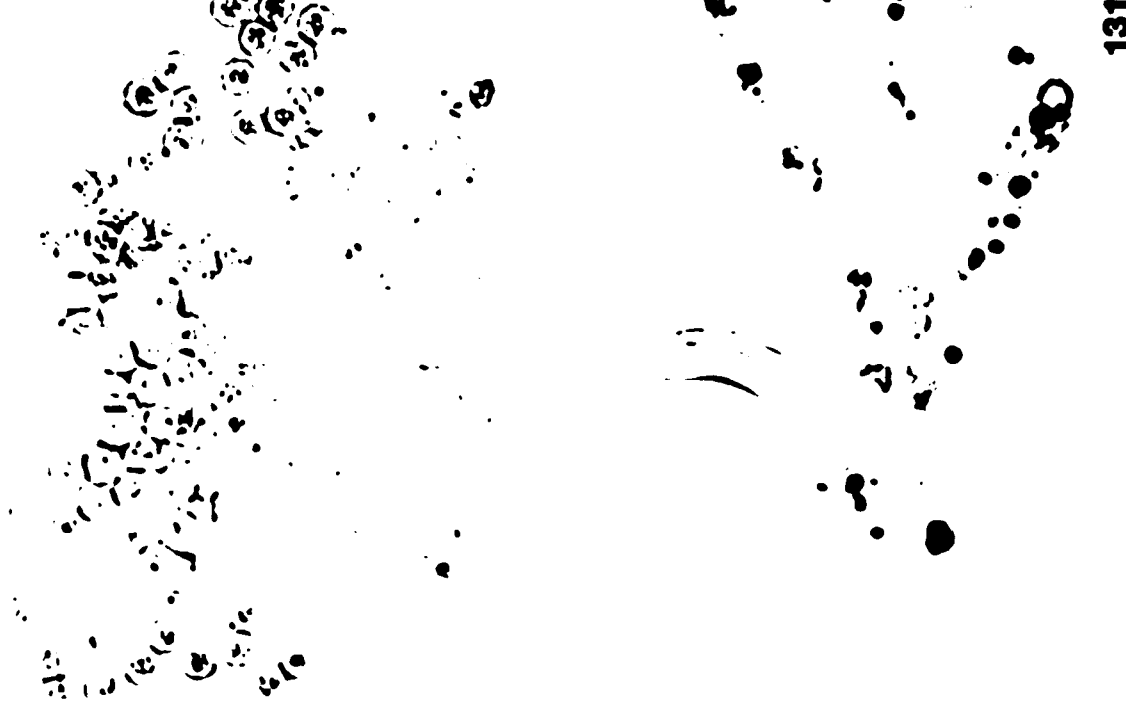
Fig. 132

Mag. X 925





132



131

Plate XXXIII, Figs. 133 - 135. Thick walled hyphae
of A. nidulans (24, 24 and 20 hours
incubation respectively).

Fig. 133 Large polytene (diploid?) maturation chromosomes
in thick walled hyphae.

Fig. 134 Thick-walled hyphae containing clusters of
juvenile structures (pairing?).

Fig. 135 Large polytene (diploid?) chromosomes in a
thick-walled hypha. Crystal Violet staining.

Mag. X 2300



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