To my parents.

POST-MEIOTIC EVENTS IN THE POLYPORACEAE

by

Fook Hon Chin B.Sc.

Thesis presented to the University of St. Andrews for the Degree of Master of Science.

Botany Department,
University of St. Andrews.
June 1974.

DECLARATION

I hereby declare that the following thesis is based on the research project done by me, that the thesis is my own composition and that it has not previously been presented for a higher degree.

The research was carried out in the Department of Botany of the University of St. Andrews under the supervision of Dr. E. G. Duncan, University of St. Andrews.

CERTIFICATE.

I certify that Fook Hon Chin B.Sc. has spent four terms of research work under my supervision, that he has fulfilled the conditions of Ordinance 51 (St.Andrews), and that he is qualified to submit the accompanying thesis in application for the Degree of Master of Science.

ACKNOWLEDGMENTS.

I would like to express my thanks to Dr. E. G. Duncan who proposed and supervised this work; and to acknowledge my indebtedness to Mr. R. B. Stephenson for instruction in photographic technique.

CONTENTS

INTRODUCTION

Duncan and Galbraith (1972) studied the post-meiotic events occurring in the Homobasidiomycetidae and found that a third nuclear division took place in all the species examined before the discharge of the mature basidiospores. However, the site of the third nuclear division varied as did the fate of the resultant nuclei. Duncan and Galbraith recognized four patterns of post-meiotic events on this basis. These were designated :-

Pattern A. The third nuclear division takes place near the apex of the basidium. One of the resultant eight nuclei migrates into each basidiospore. Any nuclei in excess of the number of basidiospores remain in the basidium and ultimately degenerate. The mature basidiospores are uninucleate.

Pattern B. The third nuclear division takes place within the sterigmata. The daughter nuclei distal to the basidium pass into the basidiospores while their sister nuclei move into the basidium and degenerate. The mature basidiospores are uninucleate.

Pattern C. The third nuclear division takes place within the basidiospores. The daughter nuclei distal to the basidium remain within the basidiospores while their sister nuclei pass into the basidium. The mature basidium bears four uninucleate basidiospores and contains four degenerating nuclei.

Pattern D. The third nuclear division takes place within the basidiospores. The daughter nuclei remain within the

basidiospores which are consequently binucleate when discharged. The aged basidium is enucleate.

Moreover their findings indicated that a given pattern was characteristic of a genus. There was also evidence to the effect that genera which were regarded as taxonomically related exhibited the same pattern. Duncan and Galbraith suggested that patterns of post-meiotic events might provide important criteria in the fields of taxonomy and phylogeny. However, they pointed out that documentation of the post-meiotic events in many more members of the Basidiomycetes was required before an evaluation of such criteria would be possible.

The first objective of the present study has been the documentation of the patterns of post-meiotic events occurring in a selection of species presently, or formerly regarded as members of the family Polyporaceae. The second objective was to attempt to correlate the patterns with existing views on the classification of the Polyporaceae and in so doing determine whether or not such cytological data has any taxonomic value.

The decision to carry out this study on the Polyporaceae was motivated by the fact that different authorities hold widely differing concepts of the family. Relevant aspects of these concepts will be discussed later. Consequently in formulating this project it was decided to regard the Polyporaceae as including all those poroid fungi and some non-poroid fungi which at some time have been included within an author's concept of the family.

The diversity encountered in the Polyporaceae is so great that it is almost impossible to compose a satisfactory definition of the

fungi under consideration. In most general terms it may be said that the Polyporaceae are fungi which produce homobasidia in a palisade hymenium which lines the inside of cavities on the surface of the basidiocarp. Nevertheless Singer (1962) has included fungi such as Pleurotus (Fr.) Quél. and Schizophyllum Fr. in his account of the family. These fungi are ostensibly gilled fungi which can scarcely be regarded as conforming to the above definition. The cavities bearing the hymenium are of variable form and depth. Shallow depressions of variable shape characterize the genus Merulius Hall. while at the other extreme well defined tubes of circular cross-section, extending to a depth of 2cm. are typical of Polyporus (Mich.) Fr. The production of tubes is a feature of most genera in the family but in cross-section these tubes may be circular, elliptical, hexagonal, rectangular or even labyrinthiform. The basidiocarps vary considerably in morphology. Basidiocarps of the genus Poria (Pers.) Fr. are typically resupinate while bracket-like (dimidiate) basidiocarps are the dominant type in the genus Polyporus (Mich.) Fr. and many others. Pileate basidiocarps also occur infrequently in a number of genera. The basidiocarps are generally annual but perennial ones are characteristic of genera such as Fomes (Fr.) Fr. and Ganoderma Karst. The family contains species which are saprophytes growing on wood and more importantly many destructive parasites of both coniferous and deciduous trees. Their distribution is world-wide.

MATERIALS AND METHODS.

A. MATERIAL.

Species of a wide range of genera classified within the Polyporaceae were collected in the vicinity of St. Andrews, Fife. Basidiocarps of these species were taken to the laboratory and the material processed immediately as described below. Identification of the species was based upon the descriptions published by Rea (1922), Wakefield and Dennis (1950), Overholts (1953), and Lange and Hora (1963).

B. CYTOLOGICAL METHODS.

Identifications of the pattern of post-meiotic events taking place in a species requires observation of the nuclear events in both the basidia and developing basidiospores. This can only be done in embedded and sectioned material since basidiospores become detached in squash preparations of the hymenium. Preparations of naturally shed basidiospores are also required in order to count the number of nuclei in mature basidiospores. The cytological methods used are now described.

(a) Preparations of the hymenium : Small blocks of material bearing the hymenium were cut from the collected basidiocarps. These blocks were then immersed for 24 hours at room temperature ($18^{\circ}C$) in the mercuric chloride-acetic acid fixative recommended by Duncan and Galbraith (1973). This fixative consists of a saturated aqueous solution of mercuric chloride to which is added 1% glacial acetic acid. The blocks were then enclosed in muslin bags and washed in running water for 12 hours. Newcomer's fixative

(Newcomer 1953) was also used on occasion to fix material from the hymenium of basidiocarps. Full details of this fixative and its usage are presented later in this section.

The fixed material was placed in a 50:50 mixture of aquax (a blend of polyethylene glycols manufactured by Gurr Ltd.) and water for 24 hours in an oven at 58 °C. The material was next transferred to a mixture of aquax and 6% glycerol for 24 hours at the same temperature. The latter mixture was replaced by a fresh batch midway through the above period. The material was subsequently embedded in aquax and 6% glycerol in moulds which were placed in a refrigerator at 4 °C to hasten the hardening of the wax. The addition of glycerol to the aquax makes the wax easier to cut with a microtome.

The embedded material was wrapped in aluminium foil and stored in tightly closed containers in the refrigerator until required. These precautions are necessary since the aquax absorbs water from the atmosphere. Sections of the embedded material were cut at 4μ, 6μ, 8μ, or 12μ depending on the size of the basidia of individual species. The sections were floated on to a drop of 4% formalin on grease-free slides bearing a thin covering of Haupt's adhesive prepared according to the formula in Johansen (1940). The preparations were left to dry slowly at room temperature.

(b) Preparations of basidiospores : Mature basidiospores were allowed to fall from the basidiocarps directly on to slides. Water was then added to the basidiospores to make a suspension of suitable concentration for mounting. Small quantities of this suspension were mixed with drops of 4% formalin on slides previously covered

with a film of Haupt's adhesive. The slides were allowed to dry before fixation.

Preparations of basidiospores were generally fixed in the mercuric chloride-acetic acid fixative described above for a period of 6 hours. These preparations were washed in running water for 12 hours.

However, in the case of members of the Polyporaceae which produce pigmented basidiospores e.g. Ganoderma considerable difficulty was experienced in obtaining preparations in which the nuclei could be observed. An alternative method of fixation appeared a possible solution to the problem. Newcomer's fixative (Newcomer 1953), a fixative containing organic solvents was selected in the hope that the latter might reduce the pigmentation or alternatively reduce the quantity of stain retained by such walls. Unfortunately, no significant improvement resulted when pigmented basidiospores were fixed in Newcomer's fixative prior to staining. However, it was found that Newcomer's fixative led to improved staining of non-pigmented basidiospores. Firstly there was a stronger affinity between the chromatin of the nuclei and the Giemsa stain. Secondly the differentiation between chromatin and cytoplasm was much greater. Indeed in some cases the cytoplasm no longer stained while the chromatin was intensely stained. Latterly Newcomer's fixative was routinely used in fixing basidiospores and was also applied in the case of species where mercuric chloride-acetic acid had been used previously. Newcomer's fixative has the following formula:-

Isopropyl alcohol	6 parts
Propionic acid	3 parts
Petroleum ether	1 part
Acetone	1 part
Dioxane	1 part

It is advisable to store Newcomer's fixative in a refrigerator to prevent the chemical composition changing. Preparations of basidiospores were fixed for a period of 1 or 2 hours and subsequently washed in 70% ethyl alcohol and hydrated by passage through 50% and 30% alcohol to water.

(c) Staining of nuclei : The sectioned material and the preparations of basidiospores were stained by the improved acid-Giemsa technique introduced by Duncan and Galbraith (1973). This technique has been found to be a reliable method of staining nuclei in a wide range of Basidiomycetes.

Preparations of sectioned material were rinsed in water to remove the water soluble aquax. These preparations and also the preparations of basidiospores were then hydrolysed in 60% orthophosphoric acid (H_3PO_4) for 4 hours at room temperature (18 °C). Hydrolysed preparations were washed consecutively in water and Sørenson's phosphate buffer at pH 6.5. Duncan and Galbraith found that the staining reaction operates most effectively at a pH of 6.5 in most species of Basidiomycetes. Hence control of pH is a most important matter. (Sørenson's phosphate buffer was prepared by dissolving 39.6gm. of KH_2PO_4 and 22.425gm. $Na_2HPO_4 \cdot 2H_2O$ in 250ml. of distilled water. This is a stock solution which is diluted x25 before use with distilled water to provide the buffer at pH 6.5.) Preparations are transferred from the buffer to a solution of Giemsa stain. This solution is prepared by adding 4ml. Giemsa R66 (Gurr

Ltd.) to 100ml. of the above buffer. Preparations are generally stained sufficiently after 30 minutes but longer staining periods often result in more intensely stained nuclei.

The stained preparations require to be dehydrated so that permanent preparations can be obtained for continued study. The preparations are first dipped in a solution of a non-ionic detergent; a 1:4000 aqueous solution of DC34 (Welton Laboratories Ltd.) in order to overcome surface tension effects. Dehydration is then carried out in the acetone:xylene series detailed below:

Acetone : xylene	Period of immersion
19 : 1	30 seconds
14 : 6	10 seconds
6 : 14	10 seconds

The process of dehydration is encouraged by the agitation of the preparations. The preparations are finally cleared in xylene for 10 minutes and mounted in Euparal.

It is occasionally found that the staining of the preparations is very intense and moreover there may be a lack of differentiation between nuclei and cytoplasm. The end result can often be improved in such cases by dipping the preparations in Plumel's sodium cacodylate-hydrochloric acid buffer at pH 5.8 before dehydration. Stain in the cytoplasm is released in the subsequent dehydration procedure and the nuclei are more easily observed.

C. PHOTOGRAPHIC TECHNIQUES.

Preparations were examined with a Reichert Zetopan microscope. The lenses used for detailed observations were x60 and x90 apochromatic oil immersion objectives and x8 compensating eyepieces.

Preparations were photographed with a Watson microscope camera. The film used was Ilford Micro-neg Panchromatic Type B, a film of high resolution and contrast. Exposures were based on readings taken with an exposure meter, an accessory of the Reichert microscope. Filters, mainly a green Wratten 74 were used to enhance contrast.

The film was developed in Ilford ID-2 for 7 minutes at 20 °C. The negatives were printed on Kodak bromide paper grade 2 or 3 depending on the contrast required. The magnification of the subject was determined by enlarging negatives of a micrometer slide to the same degree and measuring the increase in unit length. Photographs included are to magnifications of x2540, x3810, and x5080.

D. DRAWINGS.

Records of a number of items of interest in the preparations could not be obtained by photography either because of the quality of the preparation or on account of the depth of field required. In such cases drawings were made using a Zeiss drawing tube mounted on the Reichert Zetopan.

MEIOTIC AND POST-MEIOTIC EVENTS IN MEMBERS OF THE POLYPORACEAE.

The sequence of nuclear events in the basidia and in the developing basidiospores together with the complement of nuclei in the mature basidiospores have been studied in sixteen species. These species represent the genera Polyporus, Polystictus, Fomes, Pleurotus, Trametes, Merulius, and Ganoderma according to Rea (1922). It is recognized that modern authors have modified the classificatory scheme of Rea and assigned many of the species under study to new and different genera. This matter will be discussed later.

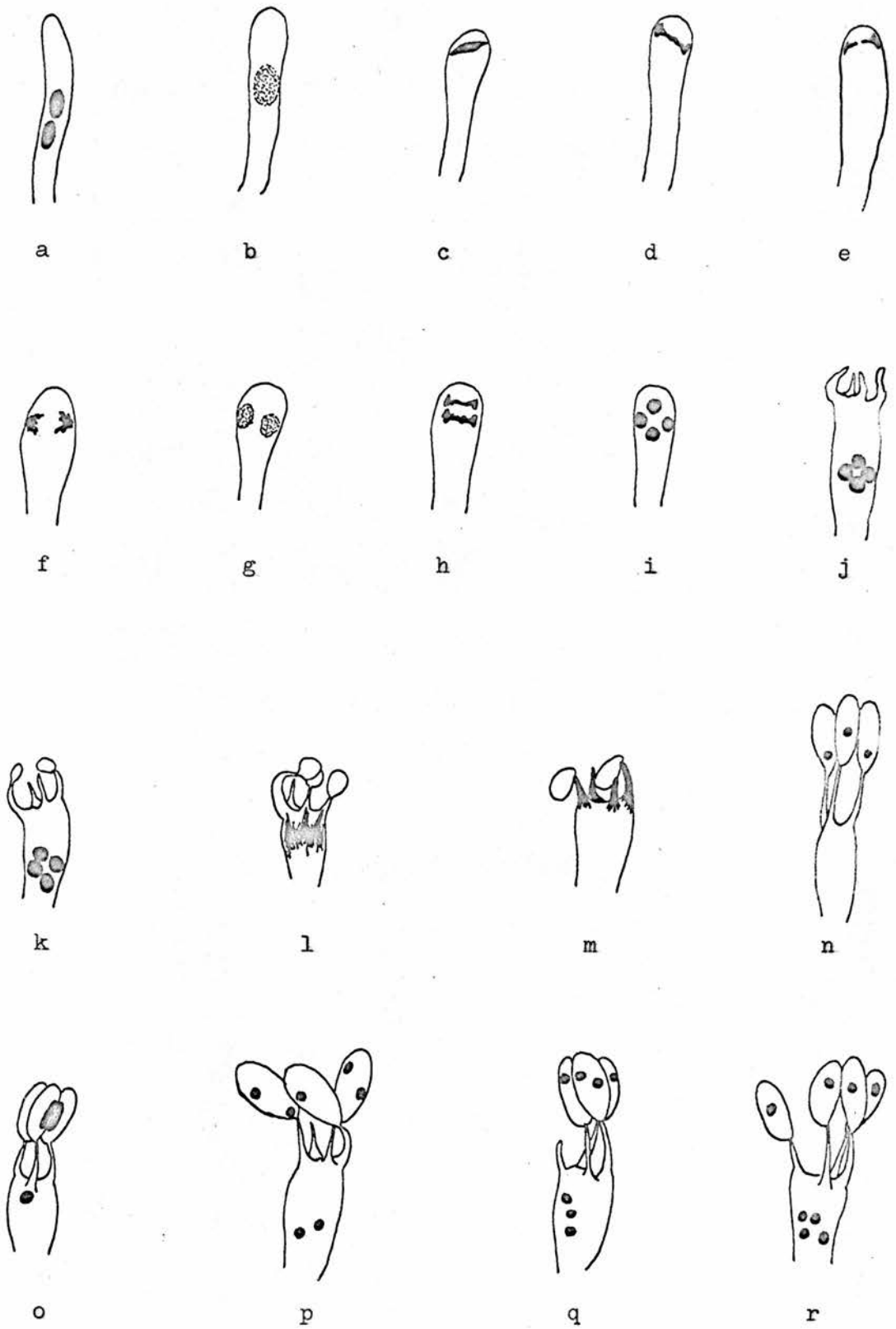
The prime aim of this study as previously stated was the determination of the patterns of post-meiotic events operating in members of the Polyporaceae. Although species may exhibit different patterns of post-meiotic events they do share certain nuclear events in common. The following sequence of nuclear events in the basidium prior to basidiospore production was common to all species in which the staining methods enabled observations to be made and is shown for Polyporus lentus in Fig. 1 a-k and for P. adustus in Fig. 2 a-g.

The young basidia were consistently binucleate with the paired nuclei arranged vertically in the long axis of the cell (Figs. 1a and 2a). The nuclei expand considerably as the basidia grow rapidly in size. The paired nuclei subsequently migrate to the centre of the cell and fuse to form a single diploid nucleus (Figs. 1b and 2b). Chromosomes become increasingly apparent in the fusion nucleus as it undergoes prophase of meiosis I. During this time the basidium continues to enlarge. The fusion nucleus moves towards the apex of

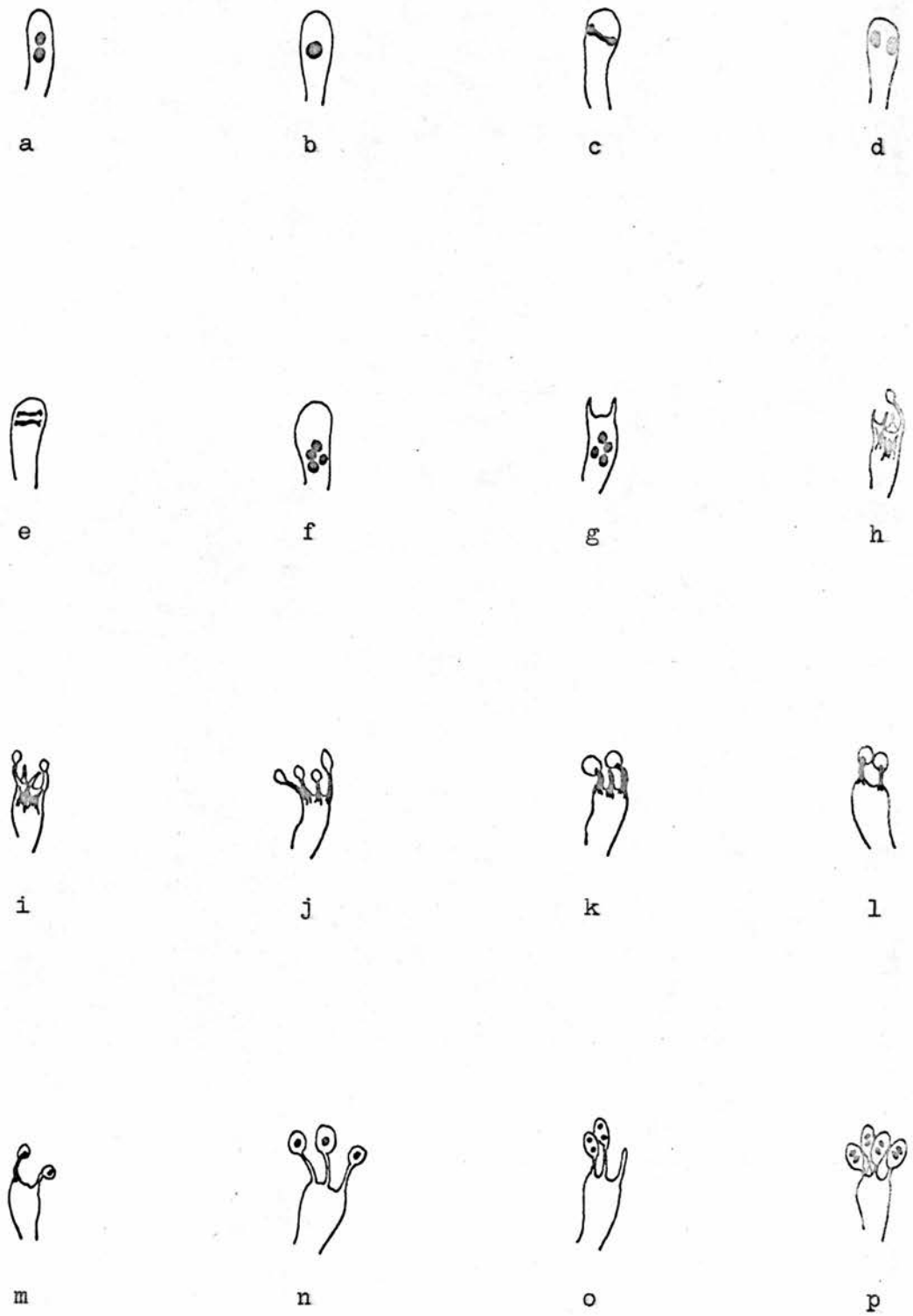
the basidium when metaphase is due. The chromosomes approach their maximum degree of contraction at this stage. The spindle develops transverse to the long axis of the basidium (Figs. 1c-f and 2c) but on occasion may be oblique. Anaphase proceeds rapidly and two groups of chromosomes move towards opposite poles. The resulting two daughter nuclei enter telophase and expand considerably before meiosis II. The stages in meiosis II all take place at the apex of the basidia but details of these stages are never as clear as those of meiosis I. The spindles are more or less parallel but noticeably smaller than the spindle of meiosis I (Figs. 1g, h and 2d, e). The two products of meiosis I simultaneously undergo anaphase of meiosis II. The four products of meiosis II lie close to the wall of the basidium (Figs. 1i and 2f). These nuclei which are termed the post-meiotic nuclei take up a central position in the basidium and expand considerably during the ensuing interphase. The subsequent nuclear events differ in different species and indicate which pattern of post-meiotic events is taking place. Ultimately the basidium commences to form four basidiospores. The sterigmata now develop and inflate apically into the basidiospores (Figs. 1i-k and 2g). The post-meiotic nuclei now pear-shaped move towards the sterigmata (Figs. 1l and 2h, i). They become extremely elongated as they enter and pass through the sterigmata (Figs. 1m and 2j-m). One post-meiotic nucleus enters each basidiospore (Figs. 1n and 2n). This nucleus takes up a more or less central position in the basidiospore. A third nuclear division now takes place in the basidiospores.

FIG. 1

Polyporus lentus. Karyogamy, meiosis, and the third nuclear division in a species exhibiting pattern C.



Polyporus adustus. Karyogamy, meiosis, and the third nuclear division in a species exhibiting pattern D.



In the case when subsequent to a third nuclear division the daughter nucleus proximal to the basidium enters the basidium pattern C is taking place (Fig. 1o-r). The daughter nucleus distal to the basidium remains in the basidiospore which therefore is uninucleate. When both the daughter nuclei of the third nuclear division remain in the basidiospore then the species exhibits pattern D (Fig. 2o-p). These were the only patterns of post-meiotic events recorded in the investigations described below.

A. EVENTS IN SPECIES WITH NON-PIGMENTED BASIDIOSPORES.

Most of the members of the Polyporaceae investigated produced basidiospores which were white when viewed in mass. The investigations on these species are described separately from those on species with pigmented basidiospores for the following reasons. The investigations of Duncan and Galbraith (1972) indicated that certain patterns of post-meiotic events were characteristic of members of the Homobasidiomycetidae which had non-pigmented basidiospores. Pattern D was the only pattern to be recorded in members with brown, purple, and black basidiospores. Moreover as the investigations progressed it became apparent that members of the Polyporaceae with pigmentation reacted differently from those without pigmentation to the cytological methods used.

Details of the investigations of members of the Polyporaceae with non-pigmented basidiospores are described first.

1. Polystictus abietinus (Dicks.) Fr. (Plate I)

Segments of the hymenium and mature basidiospores of Polystictus abietinus were fixed in mercuric chloride-acetic acid fixative. Sections of the hymenium were cut at 4μ and stained with Giemsa as previously described. The nuclei within the basidia were intensely stained red while the surrounding cytoplasm was faintly stained. It was observed that the basidia are sparsely interspersed with cystidia heavily encrusted with crystals believed to be of calcium oxalate (Lentz 1954) (Plate Ia). These cystidia are the modified ends of tramal hyphae, and contain a single nucleus which is presumably a fusion nucleus. Meiosis II takes place at the apex of the basidium (Plate Ib). The two spindles are considerably smaller than those of meiosis I; parallel with each other, and transverse to the long axis of the basidium. The post-meiotic nuclei migrate into the basidiospores where a third nuclear division takes place (Plate Ic). The spindle of the third nuclear division lies in the longitudinal axis of the basidiospore. Mature basidiospores are oblong and slightly curved with a pointed end. When the basidiospores are stained with Giemsa a single nucleus is evident in each in a more or less central position (Plate Id). It was concluded that pattern C occurs in P. abietinus.

2. Polystictus versicolor (Linn.) Fr. (Plate II)

Segments of the hymenium of Polystictus versicolor and mature basidiospores were treated in the same way as those of P. abietinus.

The hyphal pegs characteristic of P. versicolor and a few other members of the Polyporaceae were evident in sections of the hymenium (Fig. 4a). The binucleate cells of the tramal hyphae stained well but the basidia stained poorly with Giemsa. However, it was possible to observe that a single post-meiotic nucleus enters each sterigma and passes through into the young basidiospore (Plate IIa). The nucleus divides as the basidiospore enlarges and two nuclei were then present in the centre of each basidiospore (Plate IIb, c). Subsequently the daughter nucleus distal to the basidium remains within the basidiospore while the other enters the basidium and degenerates. The mature basidiospores also stain poorly with Giemsa but all were observed to have a single centrally situated nucleus. There is no doubt that pattern C operates in this species (Plate IId).

3. Fomes annosus Fr. (Plate III)

Material from the hymenium of Fomes annosus was fixed in mercuric chloride-acetic acid while mature basidiospores were fixed in Newcomer's fixative. Sections were mostly cut at 6μ. Meiosis yields four daughter nuclei which elongate as they migrate through the narrow sterigmata and enter the basidiospores (Plate IIIa). The third nuclear division takes place more or less simultaneously in all four basidiospores (Plate IIIb,c). The basidia collapsed very soon after discharging their basidiospores. Discharged basidiospores were subglobose or broadly elliptical and

apiculate. Preparations stained with Giemsa showed that mature basidiospores are binucleate. The two daughter nuclei lie some distance apart and close to the wall (Plate IIId). The binucleate state of the mature basidiospores is proof that pattern D takes place in F. annosus.

4. Pleurotus serotinus (Schrad.) Fr. (Plate IV)

All the material of Pleurotus serotinus studied was fixed in mercuric chloride-acetic acid. Sections of the hymenium were cut at 4μ and 6μ and stained in Giemsa. The small basidia were stained pink-red in colour. On completion of meiosis the four post-meiotic nuclei increased in size and occupied most of the middle part of the basidium. The increase in size of the nuclei appears to involve an unusually high degree of relaxation of the chromosomes. Thereafter the four post-meiotic nuclei move towards the apex of the basidium in a line parallel to the long axis of the latter (Plate IVa). By this time four long slender sterigmata have formed at the top of the basidium. The nuclei enter the basidiospores which become binucleate after the third nuclear division (Plate IVb,c). The mature basidiospores are very small, cylindrical and slightly curved. The Giemsa stain reveals that each has one centrally positioned nucleus. (Plate IVd). It is concluded that pattern C takes place in P. serotinus.

5. Pleurotus ostreatus (Jacq.) Fr. (Plate V)

Segments of the hymenium of Pleurotus ostreatus were fixed in mercuric chloride-acetic acid. Mature basidiospores were fixed in Newcomer's fixative. Sections of the hymenium were cut at 6μ. The basidia were stained purple by the Giemsa in contrast to those of P. serotinus. Stages in meiosis could be identified. The orientation of the spindle at anaphase of meiosis I is transverse or oblique to the long axis of the basidium (Plate Va). The post-meiotic nuclei advance towards the apex of the basidium and become very much elongated and irregular in form as they enter the sterigmata (Plate Vb). On reaching the basidiospores the nuclei undergo a third nuclear division. The daughter nucleus distal to the basidium moves towards the terminal end of the basidiospore while the other was observed to pass into the basidium (Plate Vc). Mature basidiospores appeared lilac in mass, elliptical to elliptic-oblong in shape and generally had one nucleus (rarely two). The nucleus was mostly in a central position as can be seen in Plate Vd. There is no doubt that pattern C is the main pattern in P. ostreatus.

6. Trametes mollis (Sommerf.) Fr. (Plate VI)

Segments of the hymenium of Trametes mollis were fixed in mercuric chloride-acetic acid. There was excellent differentiation between the cytoplasm and nuclei in sections cut at 6μ and stained with Giemsa. The cytoplasm was pale-blue or purple while the

chromatin stained intensely red. The hymenial layer appeared discontinuous and a proportion of the basidia were sheltered by tramal hyphae. Whether or not this was an artifact induced in the preparation of the material is uncertain. Only the later stages of the post-meiotic events were observed. There is a third nuclear division in the basidiospores. The daughter nucleus proximal to the basidium enters the basidium while the other remains in the basidiospore (Plate VIa,b,c). However, there are exceptions and binucleate mature basidiospores have been observed. It is presumed that they arise due to failure of the mechanism responsible for the retrogressive migration of the daughter nucleus proximal to the basidium. Mature basidiospores were cylindrical and curved towards the base. Preparations of the latter, fixed in Newcomer's fixative, and stained in Giemsa were all uninucleate. The nucleus was generally in a central position, rarely at one end (Plate VI d). Pattern C is unquestionably the pattern of post-meiotic events occurring in T. mollis.

7. Merulius corium (Pers.) Fr. (Plate VII)

Material of Merulius corium was fixed in mercuric chloride-acetic acid. Sections of the hymenium were cut at 4μ. This proved to be the optimum thickness for examining nuclear events in the long and cylindrical or subclavate basidia. Such basidia are termed merulioid and are typical of members of the genus Merulius and some other genera of the Polyporaceae. The basidia arise

directly from the generative hyphae.

Multinucleate hyphal cells were observed in sections stained with Giemsa (Plate VIIa). These may be interpreted as arising from either irregular wall formation or division of the two original nuclei in a mature cell. The nuclear events during the production of basidiospores could not be followed with certainty in the preparations due to age of the specimen. Only a very few basidia could be found bearing basidiospores. Mature basidiospores were oblong to elliptical in form. They were observed to be binucleate on staining with Giemsa (Plate VIIb). On the above evidence it appears that this species exhibits pattern D.

8. Polyporus adustus (Willd.) Fr. (Plates VII and VIII)

Segments of the hymenium of Polyporus adustus were fixed in mercuric chloride-acetic acid or Newcomer's fixative. The basidiocarp was seen to be monomitic. The generative hyphae possess clamp connections (Plate VIIc) and give rise directly to the basidia. The basidia were extremely small and sections were cut at 4μ for that reason. The cytoplasm of the basidia was not stained with Giemsa but the chromatin of the nuclei stained intensely red. Migration of the elongated post-meiotic nuclei into the developing basidiospores was observed (Plate VIId). Subsequently a third nuclear division occurs in the basidiospores. The axis of this division is more or less parallel to the long axis of the basidiospore (Plate VIIIa). The two daughter nuclei

in each basidiospore increase in size and lie close together in the mature basidiospores (Plate VIII b,c). Mature basidiospores are elliptical to subglobose in shape. Preparations fixed in Newcomer's fixative and stained in Giemsa confirm that the mature basidiospores are binucleate (Plate VIIId). Therefore it has been established that pattern D takes place in P. adustus (Fig. 2).

9. Polyporus lentus Berk. (Plate IX)

Mercuric chloride-acetic acid fixative was used to prepare material from the hymenium of Polyporus lentus. Sections were cut at 8μ and stained in Giemsa. The young basidia contained two nuclei arranged one above the other in the long axis of the basidium. Such nuclei were observed to fuse (Plate IXa). Many stages of meiosis and migration of the post-meiotic nuclei into the basidiospores were observed. The occurrence of a third nuclear division in the basidiospores was clearly evident (Plate IXb,c and Fig. 1). Mature basidiospores were elliptic or fusiform, pointed at the base. Preparations were fixed in Newcomer's fixative and stained with Giemsa. All basidiospores proved to be uninucleate. The nucleus was always situated at one end of the basidiospore (Plate IXd). The above observations prove that pattern C occurs in P. lentus.

10. Polyporus betulinus (Bull.) Fr. (Plate X)

Segments of the hymenium of Polyporus betulinus were fixed in mercuric chloride-acetic acid. Deposits of mature basidiospores were fixed in Newcomer's fixative. Sections cut at 6μ prove ideal for staining in Giemsa. The tramal hyphae were consistently binucleate and possessed clamp connections. There was good differentiation between the purple cytoplasm and the red chromatin of the stained basidia. Meiosis in the basidium led to the formation of four post-meiotic nuclei which entered the basidiospores. The events of a third nuclear division within the basidiospores were distinct (Plate Xa,b,c). Mature basidiospores were cylindrical and often curved in form. The cytoplasm of the basidiospores did not take up Giemsa but a single centrally placed nucleus in each basidiospore was prominently stained (Plate Xd). P. betulinus exhibits pattern C.

11. Polyporus giganteus (Pers.) Fr. (Plate XI)

Material of Polyporus giganteus was fixed as in the case of P. betulinus. Sections of the basidiocarp were cut at 6μ. The pore layer is very shallow and the basidia correspondingly small. Consequently it was extremely difficult to observe nuclear events in the basidium. Migration of elongated post-meiotic nuclei into the basidiospores was observed (Plate XIa) and also a subsequent third nuclear division in the basidiospores (Plate XIb,c). Mature basidiospores were subglobose to globose in form and with

a conspicuous apiculus. Preparations stained in Giemsa showed that the basidiospores were uninucleate. The nucleus appeared pressed against the wall of the basidiospore by a vacuole or an oil globule (Plate XId). P. giganteus exhibits pattern C.

12. Polyporus squamosus (Huds.) Fr. (Plate XII)

All material of Polyporus squamosus was fixed in mercuric chloride-acetic acid. Sections cut at either 4μ or 6μ and stained in Giemsa revealed that the tramal hyphae consisted of binucleate cells. Basidia were large and clavate. Their chromatin was stained intensely red while the cytoplasm showed an excessive affinity for the purple component of Giemsa. Post-meiotic nuclei were observed to pass through the wide robust sterigmata into the basidiospores (Plate XIIa). The third nuclear division was again parallel to the longitudinal axis of the basidiospores (Plate XIIb). Towards the end of the third nuclear division the basidiospores contained two daughter nuclei but the one adjacent to the basidium moved into the latter (Plate XIIc). The other daughter nucleus remained in the basidiospore. Preparations of mature basidiospores stained in Giemsa substantiated this interpretation, although some basidiospores were binucleate (Plate XIId). Mature basidiospores are elongate and elliptical. Pattern C occurs in P. squamosus.

13. Polyporus stipticus (Pers.) Fr. (Plate XIII)

Segments of the hymenium of Polyporus stipticus were fixed in mercuric chloride-acetic acid. Preparations of mature basidiospores were fixed in Newcomer's fixative. Sections of the hymenium were cut at 4μ and stained in Giemsa. The post-meiotic nuclei were observed to pass through the slender sterigmata into the basidiospores where the third nuclear division occurred (Plate XIIIa) as in many of the other species described. Stages in retrogressive migration of daughter nuclei from the basidiospores were observed (Plate XIIb,c). Mature basidiospores were elliptical, slightly curved and pointed at the base. All mature basidiospores were observed to have a single centrally placed nucleus. Hence it is clearly established that pattern C operates in P. stipticus (Plate XIIId).

B. EVENTS IN SPECIES WITH PIGMENTED BASIDIOSPORES.

Three additional members of the Polyporaceae which produce pigmented hyphae and basidiospores were also investigated. These were Polyporus hispidus (Bull.) Fr., Ganoderma applanatum (Pers.) Pat., and an unidentified species attributed to the genus Ganoderma.

The acid-Giemsa method of Duncan and Galbraith (1973) successful in the case of non-pigmented members of the Polyporaceae proved ineffective in demonstrating nuclear events in pigmented members of the family. Alternative methods as described below were

also tried. The results of the cytological studies on the above mentioned species are now described in detail.

14. Polyporus hispidus (Bull.) Fr.

Material from the hymenium of Polyporus hispidus and deposits of basidiospores were fixed in mercuric chloride-acetic acid. Sections of the hymenium were cut at 6μ and stained in Giemsa. Results were disappointing and further material was fixed in Newcomer's fixative.

The hyphae of the basidiocarp are brown and this pigmentation persists in the fixed material. The basidia also have this pigmentation and the presence of the organic solvents in Newcomer's fixative did little to reduce it. No nuclei could be seen in the hyphae or in the basidia. Perhaps this result is due to the pigmentation masking the stained nuclei. Alternatively the walls of the cells may not only be pigmented but modified in a way which prevents the Giemsa stain penetrating to the nuclei.

Mature basidiospores are brown when viewed in mass. They are broadly ellipsoid to subglobose in form with a small apiculus. Furthermore they appear to have a relatively thick wall. When stained with Giemsa the basidiospores develop a dark green colouration such as never occurs in the case of non-pigmented basidiospores. Two nuclei, apparently stained dark green were observed in a few basidiospores. The nuclei were very close

together and at one side of the basidiospore. In some other basidiospores only one nucleus could be seen (Fig. 3a). This observation may be misleading since such basidiospores may also be binucleate. One of the two nuclei may be less well stained and invisible. Alternatively the two nuclei may be so close together that only one appears to be present.

The results were inconclusive. The production of mature basidiospores with two nuclei suggests that P. hispidus exhibits pattern D. However, since the sequence of post-meiotic events was not apparent, and in particular since the site of the third nuclear division was not determined further research is required.

15. Ganoderma applanatum (Pers.) Pat.

Many basidiocarps of Ganoderma applanatum were collected in an attempt to obtain material suitable for cytological study. Most of the older basidiocarps did not appear to have a hymenium active in producing basidiospores. Overholts (1953) comments that he frequently failed to find basidia with basidiospores in his collections of the species. In order to locate active specimens hand cut sections of basidiocarps were stained in cotton blue in lacto-phenol and examined under the microscope. Fresh young basidiocarps in their first or second year of growth were most frequently producing basidiospores and such material was fixed in mercuric chloride-acetic acid and Newcomer's fixative.

Similar problems to those described in the case of Polyporus hispidus were met. There is pigmentation of hyphal cells and basidia and efforts to determine the sequence of post-meiotic events were unsuccessful.

Mature basidiospores are also pigmented and reddish-brown when viewed in mass. They were broadly elliptical in form with a most characteristic double wall structure. The inner wall was yellow brown in colour, smooth in immature basidiospores but becoming strongly punctate or somewhat verrucose at maturity. The outer wall was hyaline, enclosing the projections of the endospore so that the exterior of the basidiospore appeared smooth or almost so. The outer wall projected at one end to form a broad cone like structure. The collapse of this structure at maturity gave the basidiospore the truncate appearance described by many authors. Preparations fixed in mercuric chloride-acetic acid were stained in Giemsa. The wall of the basidiospores then appeared dark green and the cytoplasm purple. No nuclei were observed in the basidiospores.

It has not proved possible to reach any conclusions regarding the pattern of post-meiotic events operating in G. applanatum. The investigations carried out on an unidentified member of the genus Ganoderma are now described.

16. Ganoderma sp.

The unidentified species of Ganoderma had a large sessile

basidiocarp (18.5cm. in diameter) attached broadly to the trunk of Ligustrum vulgare L. The bracket-like basidiocarp was greyish in colour and with a concentrically zoned and rigid upper surface. The margin was acute. The basidiocarp produced basidiospores similar in all respects to those described in G. applanatum. Nevertheless the basidiocarp did not conform with the descriptions or specimens of G. applanatum.

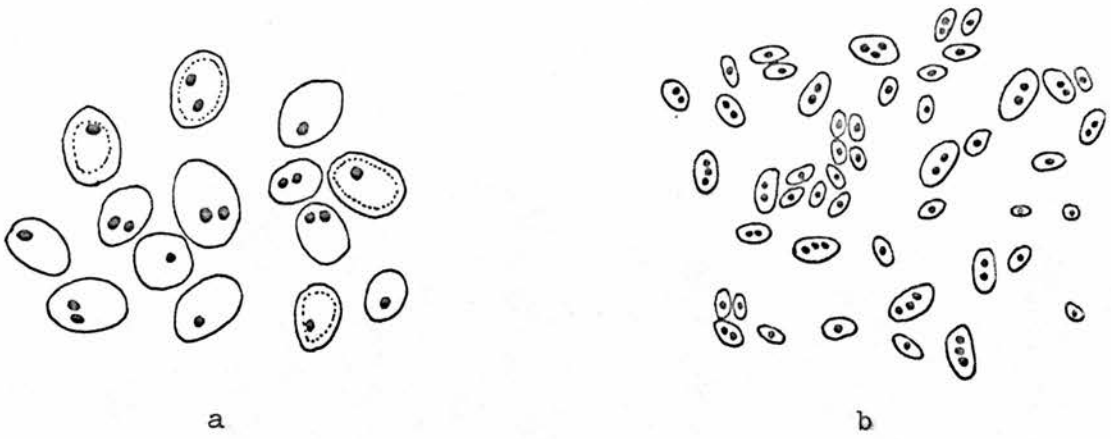
Mercuric chloride-acetic acid and Newcomer's fixative were both employed in preparing material from the unidentified member of the genus Ganoderma. Sections were cut at 12μ and stained in Giemsa. The pigmentation of cell walls again prevented a productive analysis of nuclear events.

The basidiospores reacted as did those of G. applanatum to Giemsa and no details of their nuclear complement was evident. In contrast a second type of spore was obtained from the basidiocarp in which nuclei were evident. This type of spore was unicellular, thin walled, more or less hyaline and apparently without the characteristic inner wall of the basidiospores. Most of these spores had a single centrally placed nucleus. Some had two or even three nuclei. Those with three nuclei were rather scarce and larger and more elongated than those with one or two nuclei (Fig. 3b). The nature of these spores and the manner in which some became multinucleate is not known.

The failure to observe the nuclear events in Ganoderma by means of the acid-Giemsa procedure of Duncan and Galbraith (1973)

FIG. 3

Polyporus hispidus (a). Uninucleate and binucleate basidiospores. Ganoderma sp. (b). Uninucleate, binucleate, and trinucleate spores.

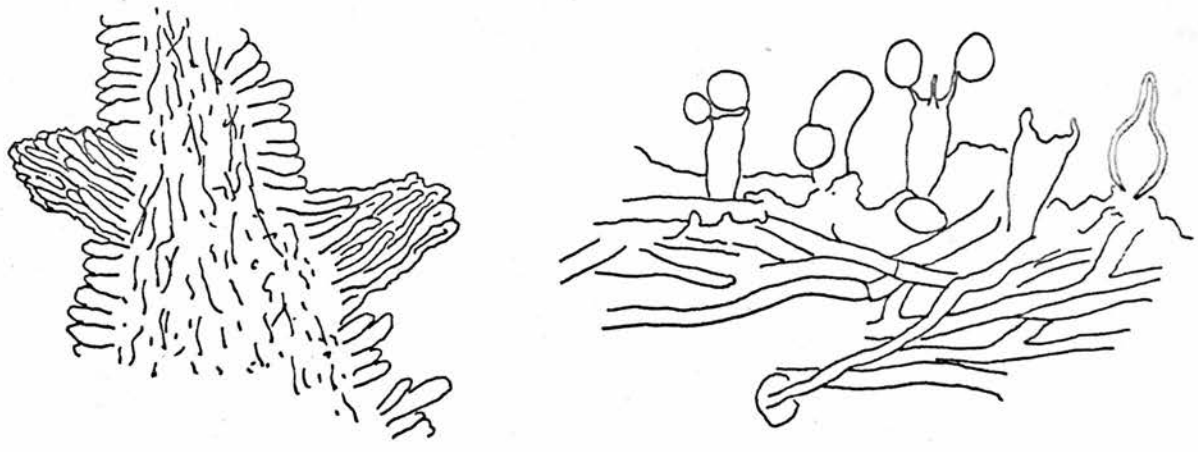


a b

0.01mm.

FIG. 4

Polystictus versicolor (a). Hyphal pegs. Polyporus hispidus (b). Hymenial layer intermingled with setae.



a b

0.01mm.

made it necessary to try other methods.

Bose (1933) carried out cytological studies on the genus Ganoderma using Leishman's stain. His method was subsequently modified by Reichert and Avizohar (1939) in their studies of the tissues of Ganoderma. They prepared Leishman's stain by dissolving 0.15gm. of the dry powder (Gurr Ltd.) in 100ml. of methyl alcohol. This mixture was allowed to stand for 24 hours at room temperature during which time it was shaken occasionally. Finally it was filtered before use. Preparations of mature basidiospores of Ganoderma were fixed in mercuric chloride-acetic acid and Newcomer's fixative. Leishman's stain was dropped on the preparations to cover the basidiospores and left for 30 seconds. Then an equal quantity of distilled water was added mixed with the stain. After a further 5-10 minutes the slides were washed in water and left to dry. The preparations were finally cleared with cedar oil and mounted in Canada balsam.

The resulting preparations were similar to those produced by the acid-Giemsa technique in appearance and failed to yield any further data on nuclear events in Ganoderma.

The results of the cytological studies on members of the Polyporaceae with non-pigmented and pigmented basidiospores are summarised in Table I.

Table I. Patterns of post-meiotic events in Polyporaceae.

SPECIES	COLOUR OF BASIDIOSPORES	NUCLEAR COMPLEMENT OF MATURE BASIDIOSPORES	PATTERN OF POST-MEIOTIC EVENTS
Polystictus abietinus	white	one	C
Polystictus versicolor	white	one	C
Fomes annosus	white	two	D
Pleurotus serotinus	white	one	C
Pleurotus ostreatus	white to lilac	one	C
Trametes mollis	white	one	C
Merulius corium	white	two	D
Polyporus adustus	white	two	D
Polyporus lentus	white	one	C
Polyporus betulinus	white	one	C
Polyporus giganteus	white	one	C
Polyporus squamosus	white	one	C
Polyporus stipticus	white	one	C
Polyporus hispidus	brown	one or two	D?
Ganoderma applanatum	reddish-brown	---	-
Ganoderma sp.	reddish-brown	---	-

DISCUSSION

A. PREVIOUS CYTOLOGICAL STUDIES ON THE POLYPORACEAE.

There have been few cytological studies on the Polyporaceae which have yielded data relevant to the project undertaken by the writer.

The main contributor to our knowledge of the cytology of the Polyporaceae has been Bose (1937). Bose studied a number of species but was primarily interested in the nuclear complement of the hyphal cells composing the basidiocarp and the processes of karyogamy and meiosis in the basidium. Nevertheless he drew attention to the fact that the basidiospores of Polyporus adustus (Willd.) Fr. were binucleate, a finding now confirmed. He also reported binucleate basidiospores in Merulius radiata Fr. currently known as Hapalopilus aurantiacus (Rostk.) Bond. and Singer and placed by Pegler (1973) as an associate genus of Bjerkandera in which P. adustus is now disposed. The binucleate condition of the basidiospores in these species indicates the occurrence of pattern D.

Schizophyllum commune Fr. included in the Polyporaceae by Singer (1962) has been the subject of many cytological and genetical studies. Essig (1922) reported that the basidiospores shed by this species were binucleate a finding confirmed by Ehrlich and McDonough (1949), and Wells (1965). The latter two authorities observed the third nuclear division to occur in the basidiospores. Therefore there is strong evidence that pattern D operates in the species. Pleurotus ostreatus (Jacq. ex Fr.) Kummer is another species unexpectedly included in the Polyporaceae by Singer (1962) which has been previously

investigated. Kawamura (1942) and Terakawa (1957) both state that the mature basidiospores are mostly uninucleate. The site of the third division was not noted but the writer has found it to occur in the basidiospores i.e. pattern C takes place.

Macdonald (1937) in his comprehensive study of Polyporus betulinus (Bull.) Fr. noted that mature basidiospores were uninucleate. The writer has also observed uninucleate basidiospores and, in addition, has found that a third nuclear division occurs in the basidiospores. Pattern C occurs in P. betulinus and also in Polyporus brumalis Pers. ex Fr. which was examined by Duncan and Galbraith (1972).

The study of Ganoderma lucidum (Leyss. ex Fr.) Karst. by Sarkar (1959) is of particular interest. There is a third nuclear division in the basidium with the result that eight nuclei are formed. Four nuclei migrate into the basidiospores while the remaining four degenerate within the basidium. The mature basidiospores are initially uninucleate. The observations of Sarkar point to the occurrence of pattern A in G. lucidum. There is no other report of events corresponding to pattern A in the Polyporaceae.

Wilson, Miller, and Griffin (1967) report that a third nuclear division takes place in the basidiospores of Fomes annosus (Fr.) Karst. The daughter nuclei remain in the basidiospores which are binucleate when shed. This finding was confirmed during the course of the present investigation and there is no doubt that pattern D takes place in this species.

The present study has established the occurrence of pattern C and pattern D in members of the Polyporaceae. Pattern C was by far

the most frequent pattern encountered, a finding in agreement with the results obtained by Duncan and Galbraith (1972) in their wide ranging investigation. There was no evidence that patterns A or B take place in the family.

B. PATTERNS OF POST-MEIOTIC EVENTS AS CRITERIA IN TAXONOMY.

Evaluation of the patterns of post-meiotic events as taxonomic criteria in the poroid fungi requires consideration of the degree of correlation between individual patterns and valid taxa. However, since many widely different views have been expressed concerning the systematic arrangement of the group it is not immediately apparent which taxa are worthy of recognition. For that reason past and present concepts of the Polyporaceae were reviewed.

1. Concepts of the Polyporaceae.

The early attempts to formulate a systematic arrangement of the poroid fungi were necessarily based on macroscopic features. Fries (1821) established the genera Polyporus, Daedalea, and Merulius which remain recognized in modern times. His main criteria were the form of the basidiocarp and in particular the arrangement of the hymenium. However, he did not recognize the poroid fungi as worthy of separation from other members of the Hymenomycetes at this time. Persoon in 1825 (Overholts 1953) was the first to segregate the poroid fungi in a group referred to as the Porodermei. Subsequent

work by Fries added considerably to the number of genera which Persoon would have regarded as Porodermei. In 1828 Fries recognized Favolus, and in 1836 (Overholts 1953) Trametes, Cyclomyces, and Hexagona. In 1855 according to Overholts (1953) he established the genus Polystictus although it did not receive recognition in his later work of 1874 when Lenzites was added to the previously mentioned genera. At this time Fries also introduced Fomes, and Poria but as subgenera within the genus Polyporus.

The work of Fries provided the basis for many subsequent taxonomists who continued to use macroscopic criteria in their attempts to improve the systematics of poroid fungi. The situation quickly became complicated by the documentation of many more species and it became apparent that the genera recognized by Fries were less well defined than previously realised. The reaction of most authorities was to create new genera. Typical of such solutions was the decision of Quélet (1888) to divide the genus Polyporus into ten genera. Karsten according to Overholts (1953) carried this approach to an extreme and recognized seventeen genera among the species assigned to Polyporus. Other existing genera were treated in the same way. Patouillard (Overholts 1953) recognized most of the genera introduced by Quélet, Karsten, and others and made further additions. Murrill, an outstanding authority on North American poroid fungi recognized a total of 58 genera between 1902 and 1915 (Overholts 1953).

The proliferation of new genera was the consequence of attempts to create a natural classification of poroid fungi; one in which the

true interrelationships between species and between larger taxa were reflected. However, these attempts did not meet with universal approval. It is evident in retrospect that their failure was largely due to the limited number of criteria on which the early workers based their interpretations.

In the above connection it is noteworthy that a number of authorities of more recent times have ignored many of the genera created by earlier authorities. Rea (1922) was content to distribute the poroid fungi in under twenty genera as did Bourdot and Galzin (1927). Overholts (1953) was even more conservative in his treatment of the North American poroid fungi and recognizes a total of eight genera. This complete reversal of previous policy was an admission that past endeavours had failed to yield the desired result.

The modern period in the systematic study of the poroid fungi began with the opportunities to study the microscopic features of the group. Consequently many additional criteria became recognized on which to judge relationships between species and delimit genera. The most significant advance lay in the discovery that poroid fungi have different hyphal systems. Corner introduced this concept and in 1932 stated that "the hyphal system of the fruit-body must be considered foremost in the morphology of polypores as it will provide a key to a natural classification." The value of this prediction was not tested for many years but now such data are used to advantage by most systematists of poroid fungi.

Corner found that the context of poroid fungi consists of three morphologically distinct hyphae. Principally there are generative

hyphae which ultimately give rise to basidia. Naturally such hyphae are found in all poroid fungi. Specialised hyphae arise from the generative hyphae. These are either skeletal or binding hyphae. Skeletal hyphae possess thick walls, have no primary septa nor clamp connections and do not branch. Binding hyphae are frequently and irregularly branched and function in strengthening the context. When only generative hyphae are present the basidiocarp is said to be monomitic; when a second type of hypha occurs the basidiocarp is termed dimitic. All three hyphal types occur in trimitic basidiocarps. Further types of hyphae have been found by subsequent investigators.

Pegler (1973) discusses the relative value of the microscopic criteria used in his and other modern treatments of the poroid fungi. In addition to the three types of hyphal system composing the context there are two main categories of cystidia which yield diagnostic data. These are tramal and hymenial cystidia. Moreover tramal cystidia are of a number of different types and these can be characteristic of a genus, although in general according to Cunningham (1965) they are of limited systematic value. The form of the basidia has not been widely used in systematic work. Cunningham (1965) recognized three forms of basidia, termed the merulioid, honeycomb, and clavate types and made use of these differences in his treatment of the group. Pegler regards the form of the basidiospores as of critical importance at specific level. Moreover a given form frequently characterizes a genus and even a family in the case of authorities who no longer recognize but a single family of poroid fungi.

The application of microscopic criteria in addition to the less informative macroscopic criteria so long the sole basis of judgements has significantly advanced our understanding of the poroid fungi. Not surprisingly there has been further proliferation of genera and more significantly the creation of new aggregates such as tribes, subfamilies, and even families additional to the Polyporaceae.

Donk (1964) includes the poroid fungi in the order Aphyllophorales and disposes the species in a number of families. Pegler (1973) follows a similar course recognizing eleven families which contain fungi regarded as being poroid or at least closely allied to them. The family Polyporaceae Corda receives recognition from both Donk and Pegler who have taken into account all known representatives of the poroid fungi. However, in Pegler's treatment the Polyporaceae is divided into five subfamilies and numerous tribes. Cunningham (1965) in his systematic arrangement of New Zealand poroid fungi groups them all within the family Polyporaceae (Fr.) Killerman and thereafter divides the latter into two subfamilies and a number of tribes. In contrast Singer (1962) has disposed a number of genera universally regarded as poroid fungi in the Agaricales which the above authors regard as the companion order to the Aphyllophorales. Singer recognizes the family Polyporaceae Fr. but includes genera such Pleurotus (Fr.) Quél. within it. No other authority includes this genus.

The systematic arrangements discussed above and in particular those proposed by Donk and Pegler are the most comprehensive possible at the present time.

2. Potential value of the data obtained.

The importance attached to the nature of the hyphal system in members of the poroid fungi by modern authorities was mentioned above. Consequently it is of interest to ascertain if any correlation exists between the patterns of post-meiotic events and a particular type of hyphal system. Pattern C occurs in species with either dimitic (e.g. Polyporus betulinus, P. squamosus) or trimitic (e.g. Polystictus versicolor) hyphal systems. Pattern D occurs in species with monomitic (e.g. Polyporus adustus) as well as dimitic (e.g. Fomes annosus) hyphal systems. It is evident that neither pattern C or D is correlated with a particular type of hyphal system.

Pegler's (1973) recent systematic arrangement appears the most suitable against which to consider the taxonomic value of the patterns of post-meiotic events identified. It is now apparent that the genera advocated by Rea (1922), in which the species collected for this project were initially placed, cannot be regarded as being other than form genera. For this reason all the species studied have been given the synonyms adopted by Pegler (or Singer, 1962, in the case of Pleurotus) and classified in accord with his interpretation. This information is presented in Table II.

It is evident that pattern C is the predominant pattern in the Polyporaceae Corda. Nine of the species studied are referred to this family by Pegler and of these seven exhibited pattern C. Three species are referable to the subfamily Tyromycetoideae of the Polyporaceae and two exhibited pattern C while the remaining one exhibited pattern D. The species exhibiting the same pattern are both members of the genus Tyromyces. Clearly there are indications

TABLE II. Current disposition of the species investigated according to Pegler (Aphyllophorales) and Singer (Agaricales).

Species investigated (according to Rea 1922)	Current synonym	ORDER Family	Subfamily	Tribe	Pattern
		APHYLLOPHORALES			
Polyporus lentus Berk.	Polyporus lentus Berk.	Polyporaceae Corda	Polyporoideae	-----	C
Polyporus squamosus (Huds.) Fr.	Polyporus squamosus (Huds.) Fr.	Polyporaceae Corda	Polyporoideae	-----	C
Polystictus versicolor (Linn.) Fr.	Coriolus versicolor (Linn. ex Fr.) Quel.	Polyporaceae Corda	Corioloideae	Corioleae	C
Polystictus abietinus (Dicks.) Fr.	Hirschioporus abietinus (Dicks. ex Fr.) Donk	Polyporaceae Corda	Corioloideae	Hirschioporaea	C
Fomes annosus Fr.	Heterobasidion annosum (Fr.) Bref.	Polyporaceae Corda	Fomitoideae	Fomiteae	D
Polyporus betulinus (Bull.) Fr.	Piptoporus betulinus (Bull. ex Fr.) Karst.	Polyporaceae Corda	Fomitoideae	Piptoporeae	C
Polyporus stipticus (Pers.) Fr.	Tyromyces stipticus (Pers. ex Fr.) Kotl. and Pouz.	Polyporaceae Corda	Tyromycetoideae	-----	C

Trametes mollis (Sommerf.) Fr.	Tyromyces mollis (Pers. ex Fr.) Kotl. and Pouz.	Polyporaceae Corda	Tyromycetoideae	-----	C
Polyporus adustus (Willd.) Fr.	Bjerkandera adusta (Willd.) ex Fr. Karst.	Polyporaceae Corda	Tyromycetoideae	-----	D
Polyporus giganteus (Pers.) Fr.	Meripilus giganteus (Pers. ex Fr.) Karst.	Scrutigeraceae	-----	-----	C
Ganoderma applanatum (Pers.) Pat.	Ganoderma applanatum (Pers. ex Wallr.) Pat.	Ganodermataceae Donk	-----	-----	-
Merulius corium (Pers.) Fr.	Merulius corium Fr.	Corticiaceae Herter	-----	-----	D
Polyporus hispidus (Bull.) Fr.	Inonotus hispidus (Bull. ex Fr.) Karst.	Hymenochaetaceae Donk	-----	-----	D
Pleurotus ostreatus (Jacq.) Fr.	Pleurotus ostreatus Jacq. ex Fr.) Kummer.	AGARICALES Polyporaceae Fr.	-----	-----	C
Pleurotus serotinus (Schrad.) Fr.	Panellus serotinus (Pers. in Hofmann ex Fr.) Kuhner	Tricholomataceae Roze.	-----	-----	C

that more than one pattern can occur in a given family and subfamily. Furthermore in the subfamily Fomitoideae one species exhibited pattern C while the second exhibited pattern D. However, these species were disposed in different tribes within the subfamily. Duncan and Galbraith (1972) suggested that a given pattern might prove characteristic of a genus. Two species studied are referred to the genus Polyporus by Pegler and both exhibited pattern C. Duncan and Galbraith themselves examined a third member of this genus (Polyporus brumalis Pers. ex Fr.) which also exhibited pattern C. There is no evidence from the present study to invalidate the prediction made by Duncan and Galbraith.

Pattern D was established to occur in Merulius corium, assigned to the Corticiaceae by Pegler. It was also suspected to take place in Polyporus (Inonotus) hispidus regarded by Pegler as a member of the Hymenochaetaceae. This latter case is of some interest since the species produces heavily pigmented basidiospores. Duncan and Galbraith found that all species producing heavily pigmented basidiospores exhibited pattern D. Consequently it is most regrettable that technical difficulties prevented determination of the pattern operating in another producer of pigmented basidiospores, namely Ganoderma applanatum.

Duncan and Galbraith found that on occasion species with non-pigmented basidiospores also exhibit pattern D. Similar findings emerge from the present study. Polyporus adustus (Bjerkandera adusta), Fomes annosus (Heterobasidion annosum) and Merulius corium all produce non-pigmented basidiospores and exhibit pattern D. The significance of

this phenomenon is impossible to judge at this time.

The above comments are made on the assumption that Pegler's classification is wholly valid. This is unlikely since experience has shown that no classification is final. All are subject to modification and improvement. Moreover it is now evident that in such a vast group an effort of considerable magnitude is required to document the patterns of post-meiotic events in a sufficient number of members to allow evaluation of the taxonomic value of such data. There are indications from the work of Duncan and Galbraith and from the evidence presented here that investigation of species producing pigmented basidiospores would be the most rewarding undertaking. Nevertheless the fact that the writer found only two of the four possible patterns of post-meiotic events in the poroid fungi raises doubts about the taxonomic value of such data in the group.

SUMMARY

1. The principal aim of the project was the determination of the patterns of post-meiotic events occurring in the Polyporaceae. It was also intended to assess the taxonomic value of the data obtained.

2. Specimens of Polystictus abietinus, P. versicolor, Fomes annosus, Pleurotus sertinus, P. ostreatus, Trametes mollis, Merulius corium, Polyporus adustus, P. lentus, P. betulinus, P. giganteus, P. squamosus, P. stipticus, P. hispidus, Ganoderma applanatum and of an unidentified species of Ganoderma were collected and subjected to cytological investigation using mainly an improved version of the acid-Giemsa technique.

3. Patterns C and D were the only patterns of post-meiotic events found in the Polyporaceae.

Conclusive identification of the patterns operating was only possible in those species which produce non-pigmented basidiospores. Thirteen such species were examined and of these ten exhibited pattern C while in three pattern D took place. The remaining species were characterized by their pigmented cells and basidiospores. The acid-Giemsa technique proved an ineffective means of elucidating the patterns of events in the latter species. Nevertheless in the case of Polyporus hispidus some evidence of pattern D was obtained. Different cytological methods were tried in an attempt to overcome the technical difficulties encountered and these are discussed.

4. Previous cytological studies on the Polyporaceae are reviewed. Few studies were found to contain sufficient information

to allow identification of the pattern of post-meiotic events operating in the species investigated. However, there was one notable report which suggested that pattern A may also occur in members of the group.

5. Past and present concepts of the Polyporaceae are discussed. Attention is drawn to the proliferation of new taxa and the radical reformation of the systematic arrangement of the group resulting from the application of new criteria. The genera in which the species investigated were placed initially have been relegated to the status of form genera. Therefore the species were disposed according to currently accepted interpretations of the group.

6. The taxonomic value of the cytological data obtained is assessed in the light of modern views of the Polyporaceae.

There appears no correlation between the pattern of post-meiotic events occurring in a species and the nature of its hyphal system. This is a finding of some interest since the nature of the hyphal system is a widely used criterion in modern arrangements of the group.

It was also apparent that patterns C and D are widely distributed in the Polyporaceae. Moreover they also occur in allied species now disposed in separate families. There was no evidence to contradict the view that a given pattern may be characteristic of a particular genus.

7. Finally the need for further documentation of the patterns of post-meiotic events in the group is stressed, and it is suggested

that investigation of those species which produce pigmented basidiospores might prove the most productive undertaking.

BIBLIOGRAPHY

Bose, S.R. 1933, Abnormal spores of some Ganoderma. Mycologia, 25:431.

Bose, S.R. 1937, Cytology-study of basidia of Polyporaceae. J. Indian Bot. Soc., 16(3):119.

Bourdot, H., and Galzin, A. 1927, Hymenomycetes de France.

Corner, E.J.H. 1932, The fruit-body of Polystictus xanthopus Fr. Ann. Bot., 46:71.

Cunningham, G.H. 1965, Polyporaceae of New Zealand. Bull. 164. New Zealand Dept. Sci. Ind. Res.

Donk, M.A. 1964, A conspectus of the families of Aphyllophorales. Persoonia, 3:199.

Duncan, E.G., and Galbraith, M.H. 1972, Post-meiotic events in the Homobasidiomycetidae. Trans. Brit. Mycol. Soc., 58:387.

Duncan, E.G., and Galbraith, M.H. 1973, Improved procedures in fungal cytology utilizing Giemsa. Stain Techn., 48, No.3:107.

Ehrlich, H.G., and McDonough, E.S. 1949, The nuclear history in the basidia and basidiospores of Schizophyllum commune Fries. Amer. J. Bot., 36:360.

Essig, F.M. 1922, The morphology, development, and economic aspects of Schizophyllum commune Fries. Univ. California Publ. Bot., 7:447.

Fries, E. 1821, Systema mycologicum, I.

Fries, E. 1828, Elenchus fungorum, I.

Johansen, D.A. 1940, Plant microtechnique, New York and London.

Kawamura, E. 1942, Studies on the sex of hymenomycetous fungi. Rev. Appl. Mycol., 21:273.

Lange, M., and Hora, F.B. 1963, Mushrooms and Toadstools, Collins, London.

Lentz, P.L. 1954, Modified hyphae of Hymenomycetes. Bot. Rev., 20:135.

Macdonald, J.A. 1937, A study of Polyporus betulinus (Bull.) Fries. Ann. Appl. Biol., 24:289.

Newcomer, E.H. 1953, A new cytological and histological fixing fluid. Science, 118:161.

Overholts, L.O. 1953, The Polyporaceae of the United States, Alaska, and Canada. Univ. Mich. Stud., Sci. Ser 19, Ann. Arbor.

Pegler, D.N. 1973, The Polypores. Supplement to Bulletin of the British Mycological Society, Vol. 7.

Quélet, L. 1888, Flore mycologique.

Rea, C. 1922, British Basidiomycetae. Univ. Cambridge.

Reichert, I., and Avizohar, Z. 1939, An anatomical study of the fruit-body of the wood-rotting fungus. Ganoderma lucidum (Leys.) Karst. in Palestine. Palest. J. Bot. Rehovot (Rehovot)., 2:251.

Sarkar, A. 1959, Nuclear phenomena in the life cycle of Ganoderma lucidum (Leyss. ex Fr.) Karst. Can. J. Bot., 37:59.

Singer, R. 1962, The Agaricales in modern taxonomy. J. Cramer, Weinheim. 2nd. ed.

Terakawa, H. 1957, The nuclear behaviour and the morphogenesis in Pleurotus ostreatus. Sci. Pap. Coll. Gen. Educ. Univ. Tokyo, 7:61.

Wakefield, E.M., and Dennis, R.W.G. 1950, Common British Fungi. P.R. Gawthorn, Ltd.

Wells, K. 1965, Ultrastructural features of developing and mature basidia and basidiospores of Schizophyllum commune. Mycologia, 57:236.

Wilson, C.L., Miller, J.C., and Griffin, B.R. 1967, Nuclear behaviour in the basidium of Fomes annosus. Amer. J. Bot., 54:1186.

Plate I

Plate I. Polystictus abietinus.

a, b, c x5080 ; d x2540.

a. Thick walled, uninucleate cystidium crusted with crystals of calcium oxalate.
b. Anaphase of meiosis II.
c. Third nuclear division in a basidiospore.
d. Uninucleate basidiospores.

Plate. I

a

b

c

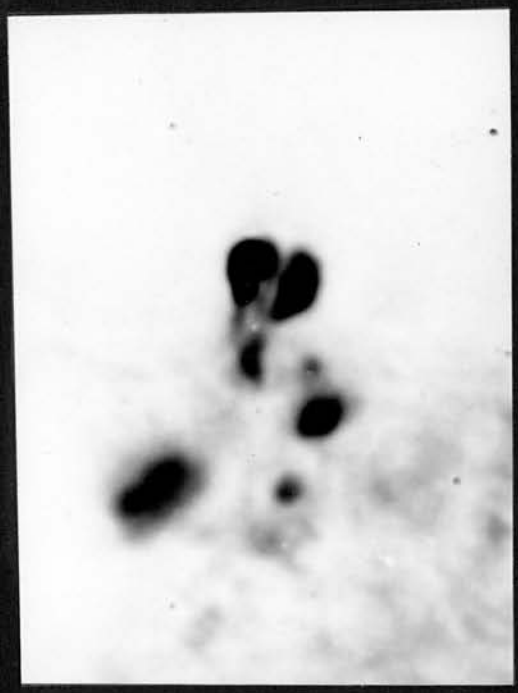
d

Plate II

Plate II. Polystictus versicolor.

a, b, c x5080 ; d x2540.

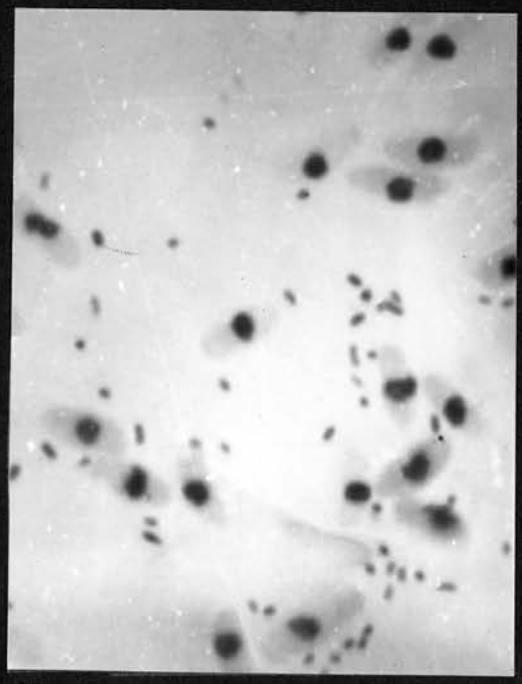
a. Migration of post-meiotic nuclei into basidiospores.
b. Third nuclear division in each of two basidiospores.
c. Two daughter nuclei in each of two basidiospores.
d. Mature basidiospores each with one nucleus.



a

b

c



d

Plate III

Plate III. Fomes annosus.

a, b x5080 ; c x3810 ; d x2540.

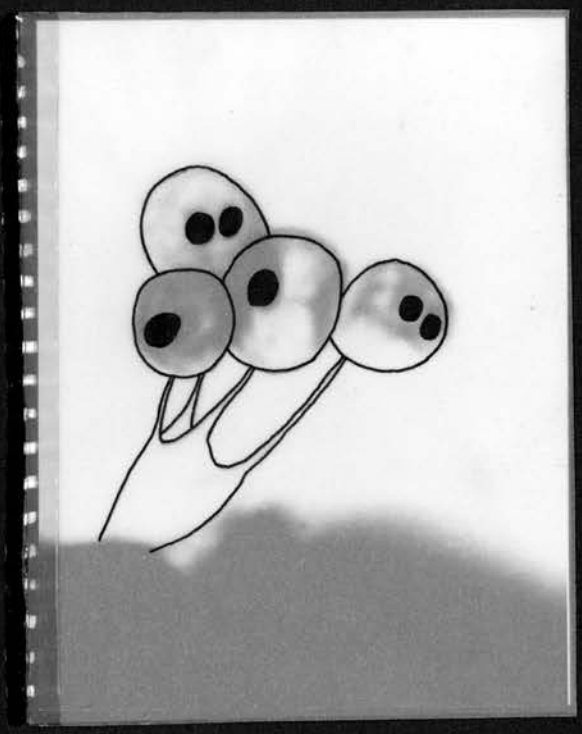
a. Post-meiotic nuclei passing through the narrow necks of the sterigmata into the young basidiospores.

b. Two basidiospores are uninucleate while the other two are binucleate as a result of the third nuclear division.

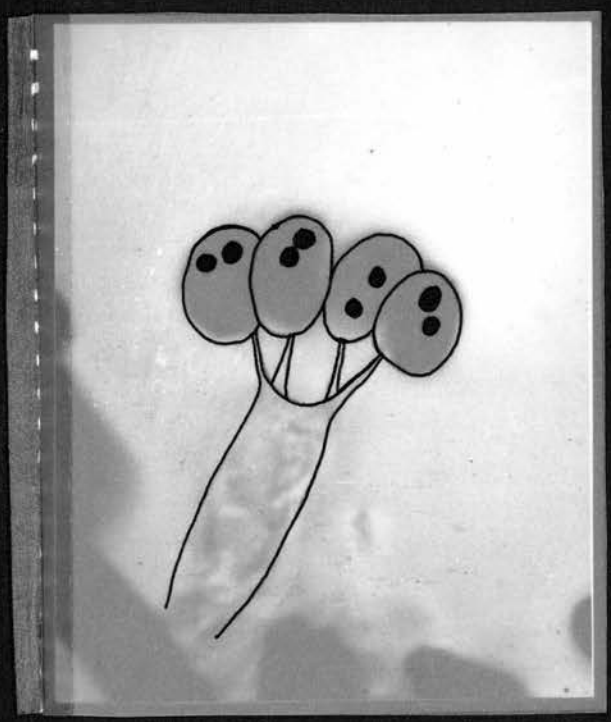
c. Four binucleate basidiospores.

d. Mature basidiospores with two nuclei.

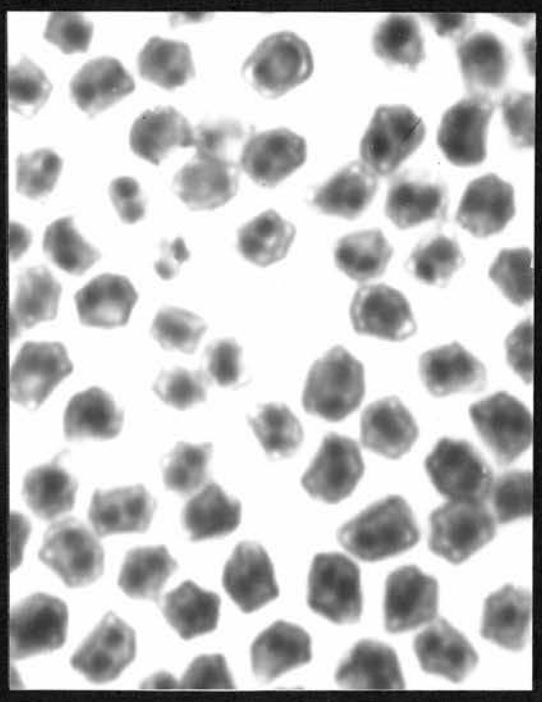
a



b



c



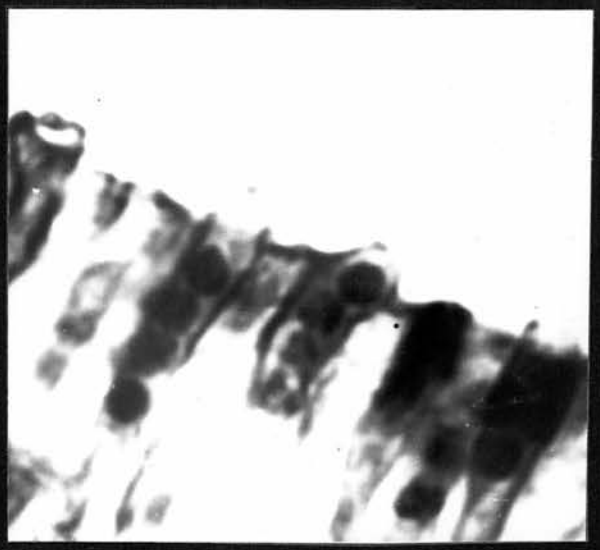
d

Plate IV

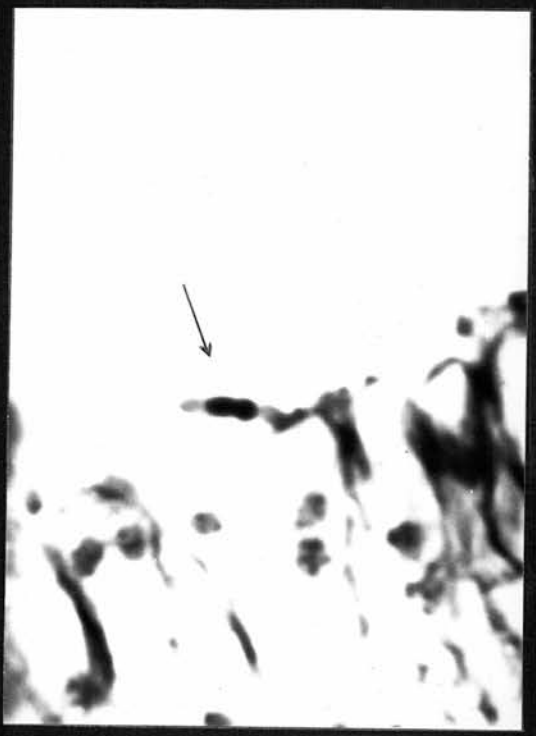
Plate IV. Pleurotus serotinus.

a, b, c x3810 ; d x2540.

a. Post-meiotic nuclei prior to migration to basidiospores.
b. Third nuclear division in basidiospore (arrowed).
c. Two daughter nuclei of third nuclear division within basidiospore.
d. Mature, uninucleate basidiospores.

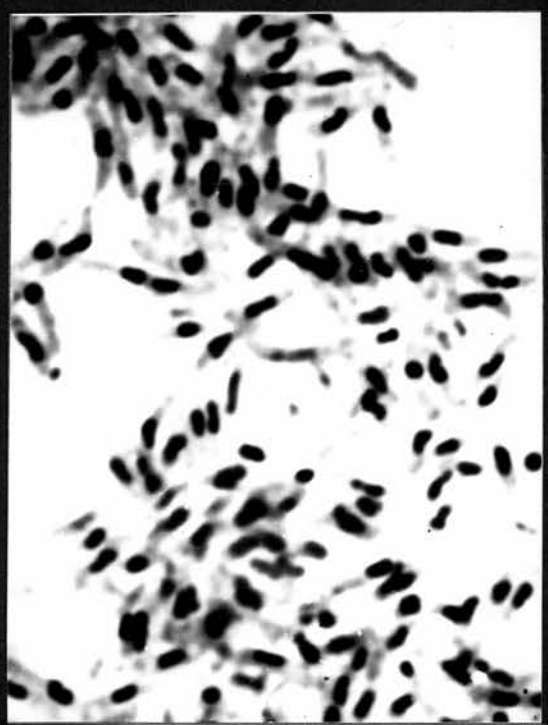


a



b

c



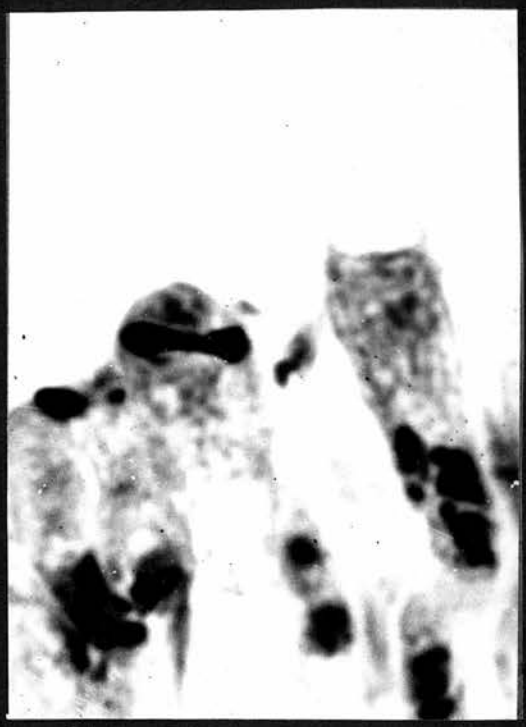
d

Plate V

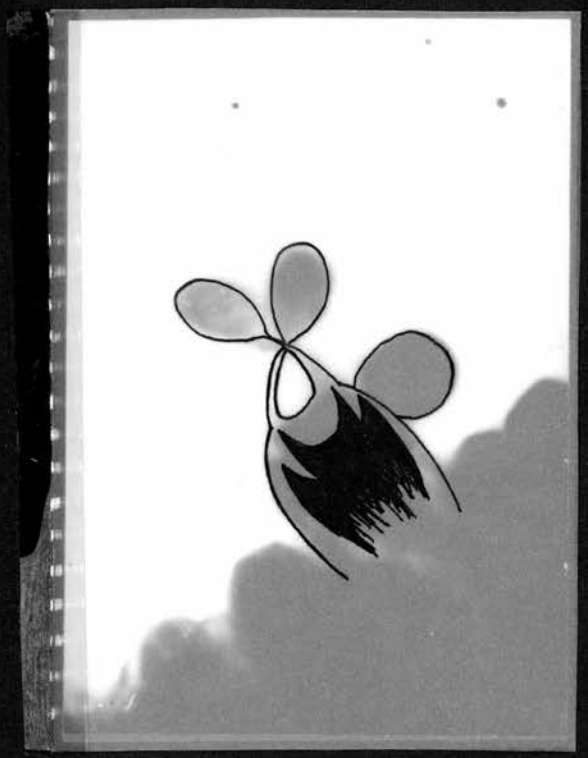
Plate V. Pleurotus ostreatus.

a, b, c x3810 ; d x2540.

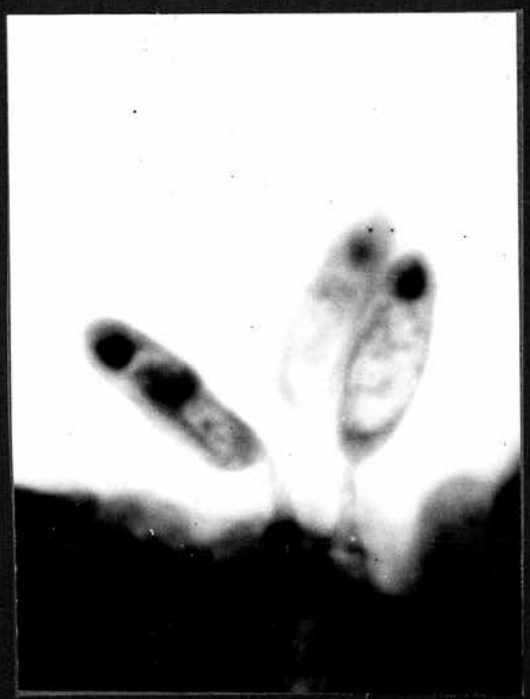
a. Anaphase of meiosis I.
b. Post-meiotic nuclei about to enter the sterigmata.
c. Stages in the third nuclear division. In the case of two basidiospores the daughter nuclei proximal to the basidium have entered it. The other basidiospore shows an earlier stage.
d. Mature basidiospores with one nucleus. The occasional basidiospore is binucleate.



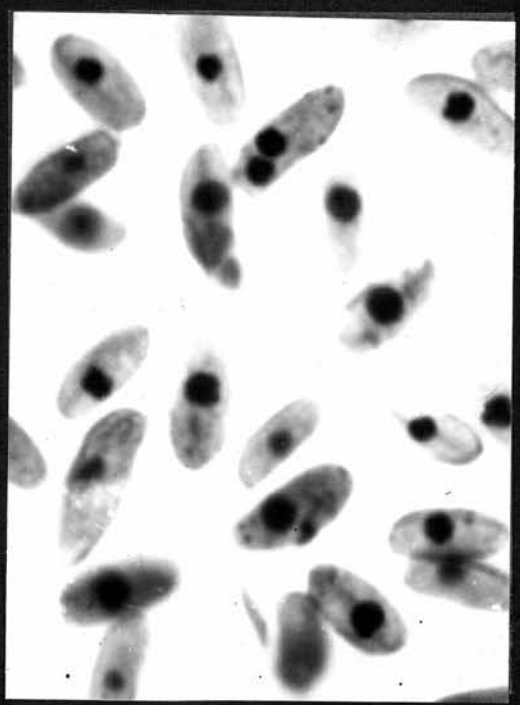
a



b



c



d

Plate VI

Plate VI. Trametes mollis.

a, b x3810 ; c x5080 ; d x2540.

a. Stages in the third nuclear division. In the basidiospore on the left the daughter nucleus proximal to the basidium is about to enter the basidium. The basidiospore on the right contains both daughter nuclei. A daughter nucleus from a missing basidiospore has already entered the basidium.

b. Stage in the third nuclear division. Note the basidiospore with two daughter nuclei. In exceptional cases mature basidiospores are binucleate.

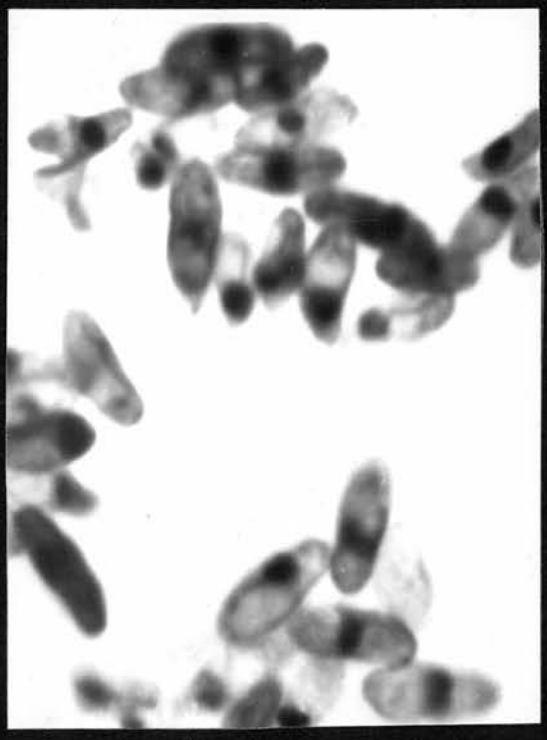
c. Typical uninucleate basidiospores at the conclusion of the third nuclear division.

d. Mature basidiospores most of which are uninucleate.

a

b

c



d

Plate VII

Plate VII. Merulius corium.

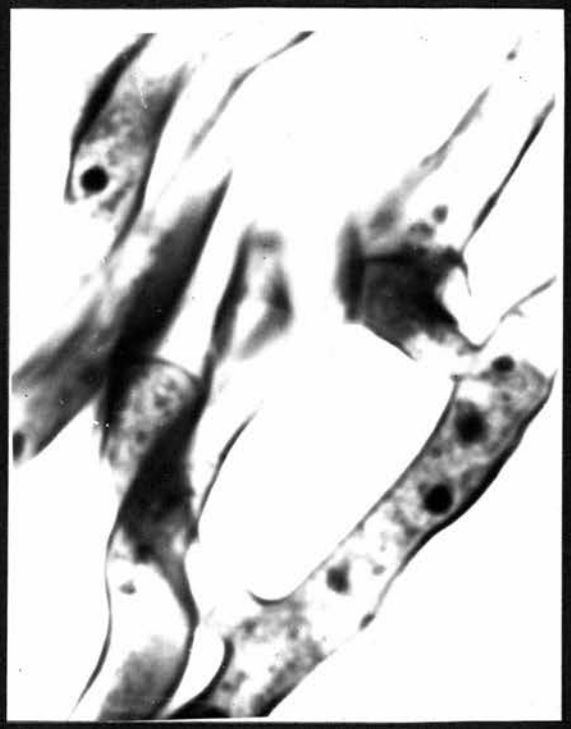
a, b x2540.

a. Multinucleate hyphal cell of trama.
b. Mature binucleate basidiospores.

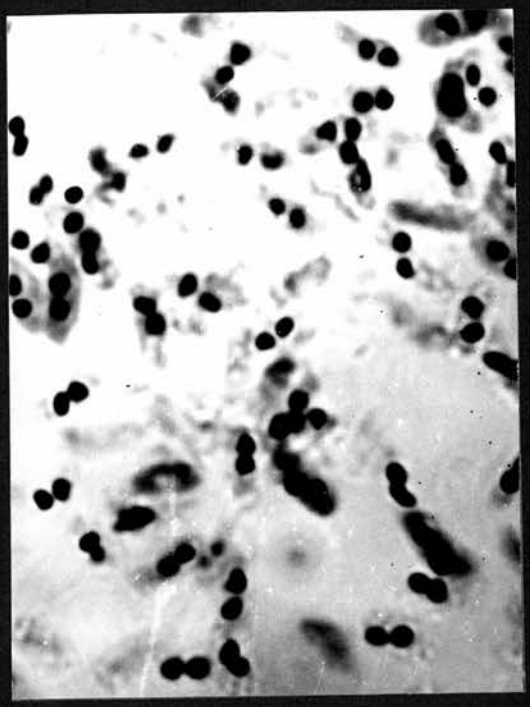
Polyporus adustus.

c x3810 ; d x5080.

c. Generative hyphae with cross walls and clamp connection. A nucleus can be seen in a clamp connection (arrowed).
d. Migration of post-meiotic nuclei through the sterigmata and into the young basidiospores.



a



b

c

d

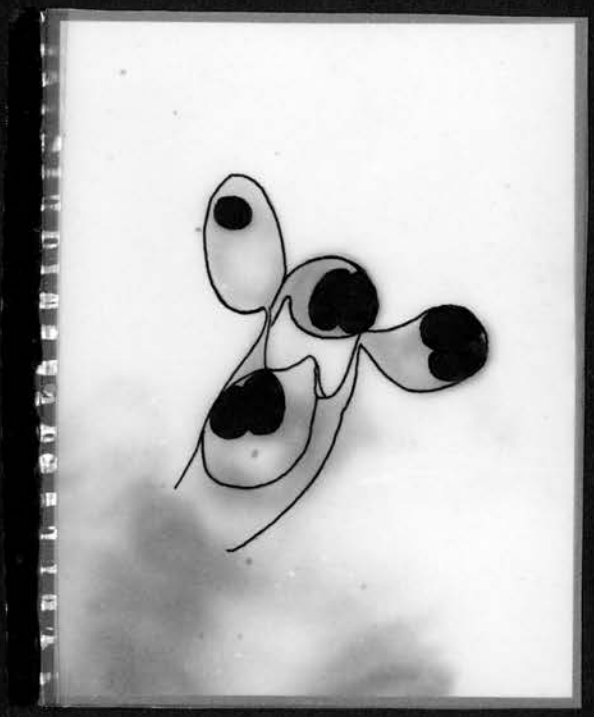
Plate VIII. Polyporus adustus.

a, b, c x5080 ; d x2540.

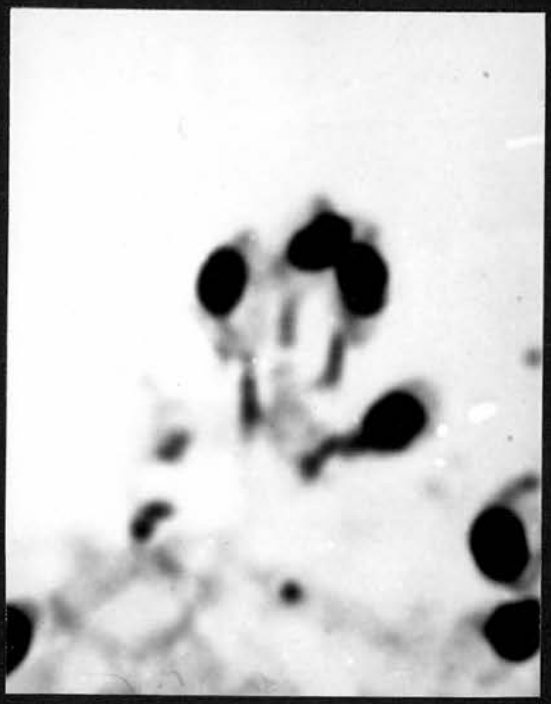
a. Third nuclear division (anaphase) in a basidiospore.
b. Three basidiospores each with two daughter nuclei resulting from the third nuclear division. The fourth basidiospore (left) contains an undivided nucleus.
c. Basidium bearing four basidiospores each of which is binucleate.
d. Shed basidiospores with two nuclei.

Plate VIII

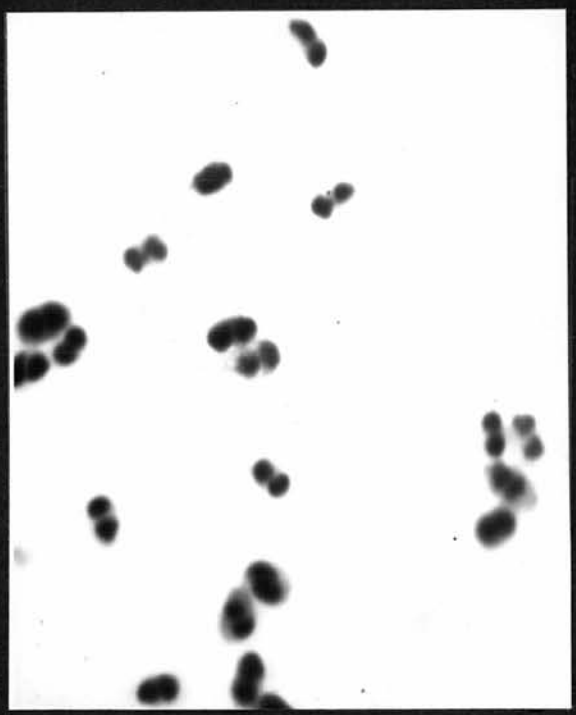
a



b



c



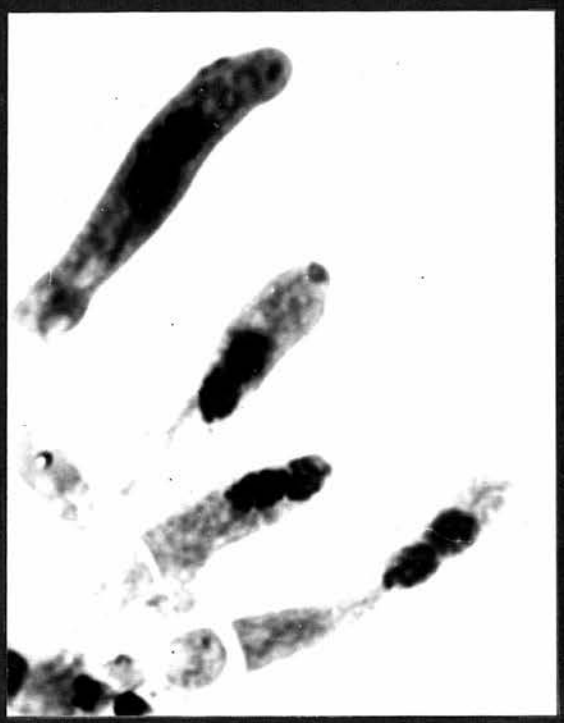
d

Plate IX

Plate IX. Polyporus lentus.

a, d x2540 ; c, b x3810

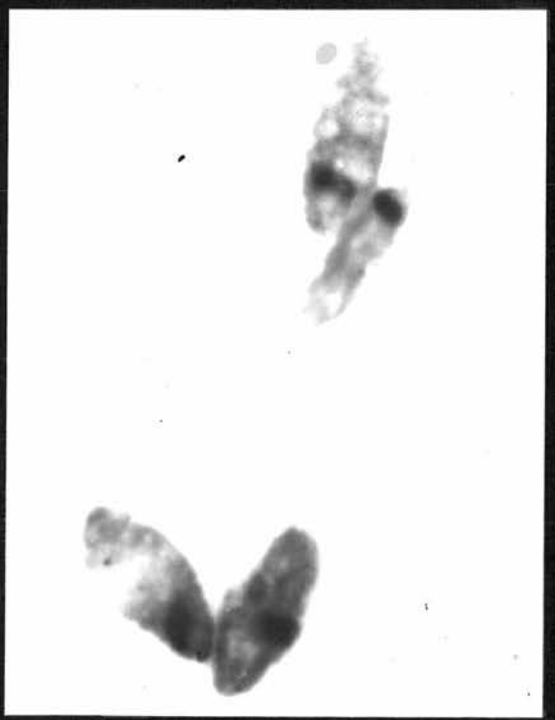
a. Young basidia with two nuclei prior to fusion.
b. Third nuclear division (anaphase) in basidiospore (arrowed). Two daughter nuclei of the third nuclear division have already entered the basidium from the sectioned basidiospore on the right or detached basidiospores.
c. Basidium with three basidiospores each containing both the daughter nuclei resulting from the third nuclear division. The lower nucleus in the basidiospore on the right is on the point of entering the basidium.
d. Mature uninucleate basidiospores. Nucleus is generally terminal in position.



a

b

c



d

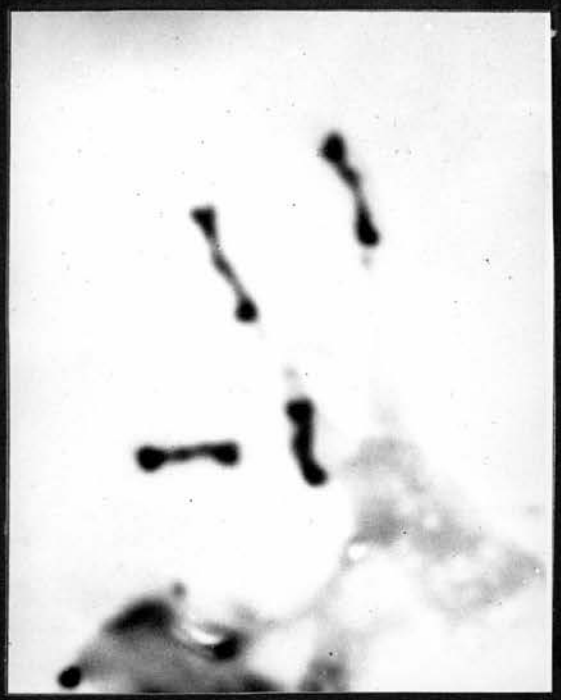
Plate X

Plate X. Polyporus betulinus.

a, b, c x5080 ; d x2540.

a,b,c. Stages in the third nuclear division in basidiospores. (a) Anaphase evident in all four basidiospores. (b) Two daughter nuclei evident in central basidiospores. (c) Basidia bearing basidiospores in which the two daughter products of the third division can be seen. The daughter nuclei proximal to the basidium are about to enter the latter.

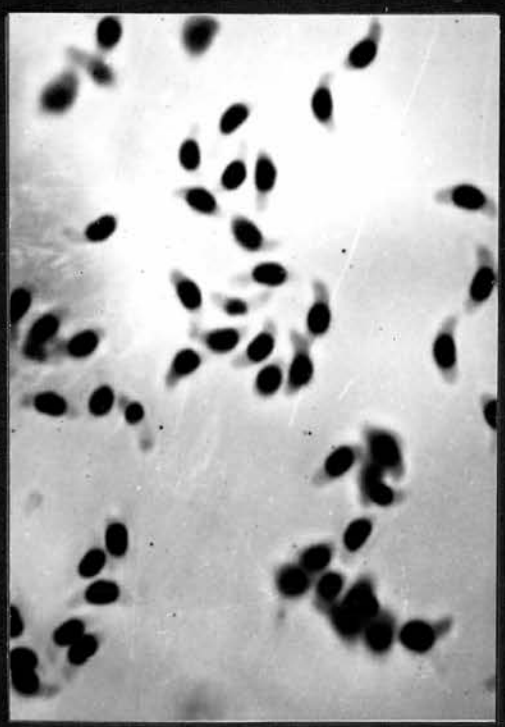
d. Mature uninucleate basidiospores.



a

b

c



d

Plate XI

Plate XI. Polyporus giganteus.

a, b, c x5080 ; d x2540.

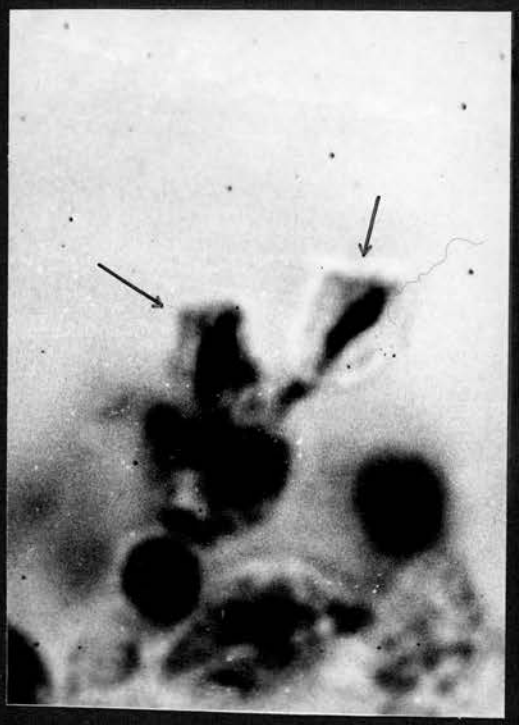
a. Migration of post-meiotic nuclei into basidiospores. The nucleus in centre is passing from a sterigma into a basidiospore.

b. Third nuclear division in basidiospores. The stage in the two basidiospores (arrowed) is early anaphase.

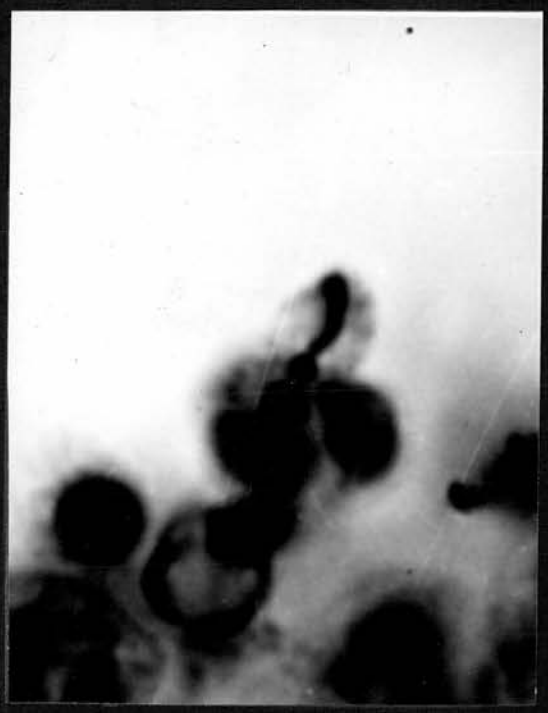
c. Third nuclear division in basidiospores. Anaphase.

d. Mature uninucleate basidiospores. Note the large central vacuole which presses the nucleus against the wall of the basidiospore.

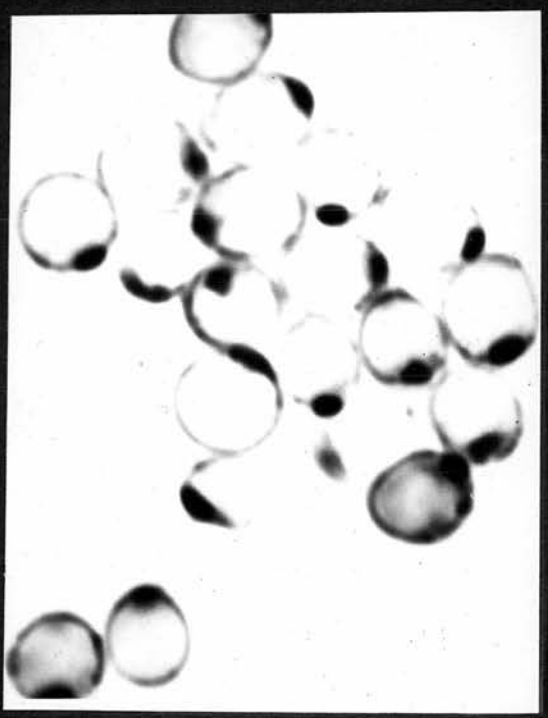
a



b



c



d

Plate XII

Plate XII. Polyporus squamosus.

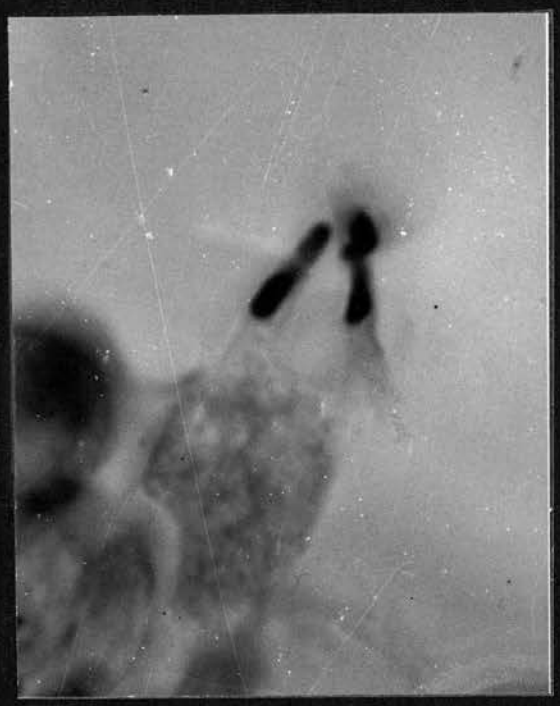
a, c x3810 ; b x5080 ; d x2540.

a. Entry of post-meiotic nuclei into the immature basidiospores.

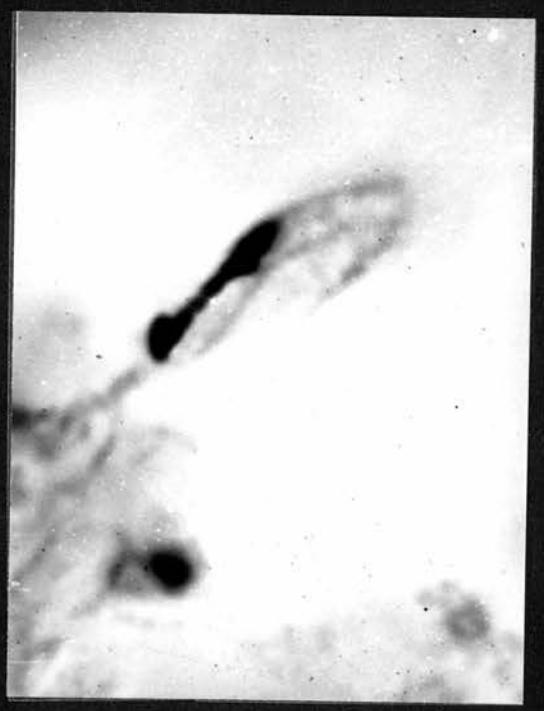
b. Third nuclear division in a basidiospore. Anaphase.

c. Concluding stages of third nuclear division. Two basidiospores each with two daughter nuclei are evident, and in addition a uninucleate basidiospore. One daughter nucleus from the uninucleate basidiospore has entered the basidium. The fourth basidiospore which has been detached must have contained two daughter nuclei.

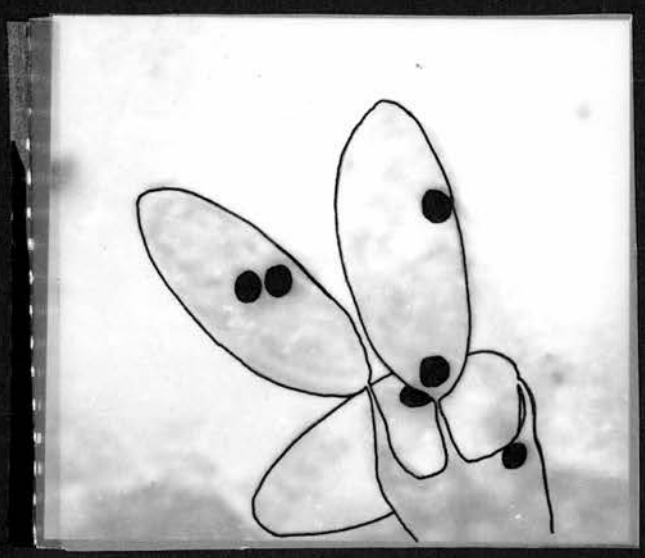
d. Mature uninucleate basidiospores.



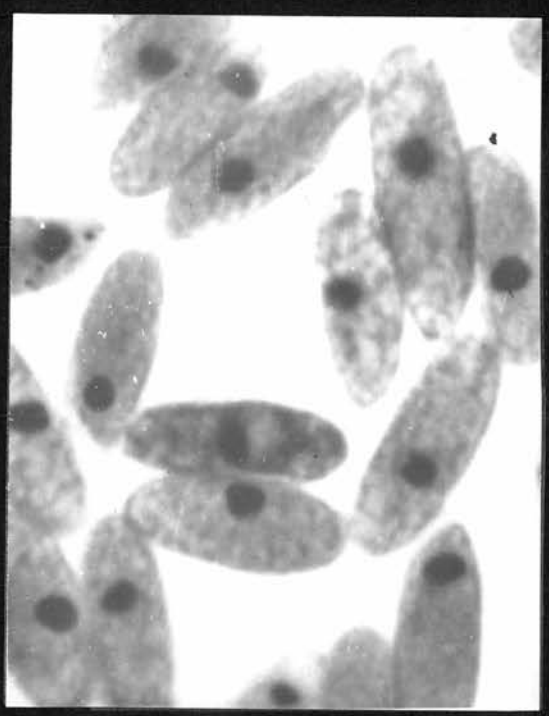
a



b



c



d

Plate XIII

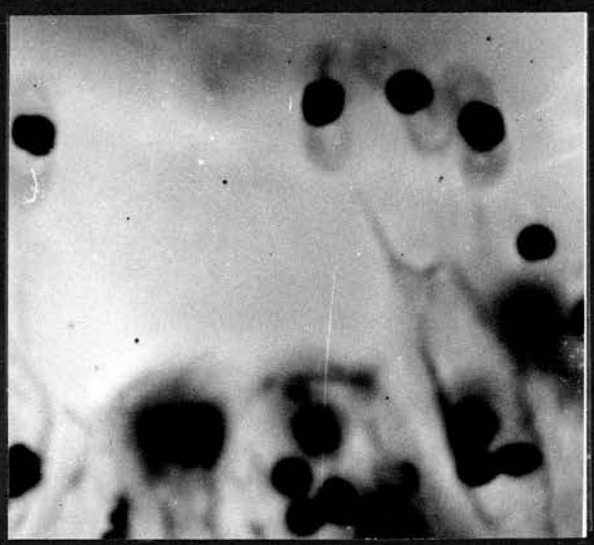
Plate XIII. Polyporus stipticus.

a, b, c x5080 ; d x2540.

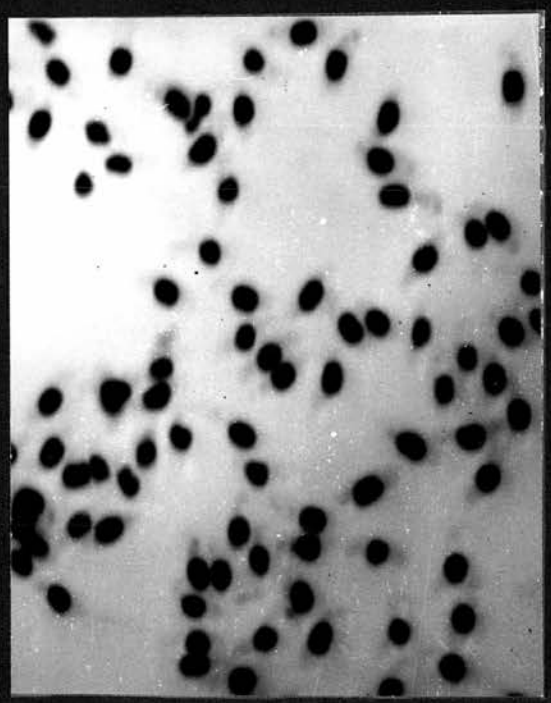
a. Third nuclear division in a basidiospore. Anaphase.
b. Later stage in third nuclear division. The two daughter nuclei are evident in each of three basidiospores.
c. Basidium (right) containing four daughter nuclei of the third nuclear division and bearing three uninucleate basidiospores.
d. Mature uninucleate basidiospores.

a

b



c



d