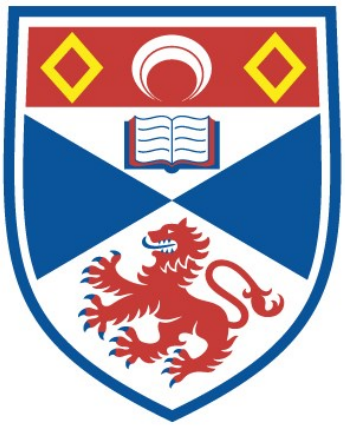
POST-MEIOTIC EVENTS IN THE BASIDIOMYCETES

Mary Huie Galbraith (or Krinos)

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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POST-MEIOTIC EVENTS IN THE BASIDIOMYCETES.

by

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D E C L A R A T I O N.

I hereby declare that the following Thesis is based on a record of work done by me, that the Thesis is my own composition, and that it has not been presented previously for a Higher Degree.

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

C E R T I F I C A T E.

I certify that Mary Huie Galbraith or Krinos has spent nine terms of research work under my direction and that she has fulfilled the conditions of Resolution of the University Court of the University of St. Andrews, 1967, No. 1, and that she is qualified to submit the accompanying Thesis in application for the degree of Doctor of Philosophy.

C A R E E R.

In October 1964, I matriculated as a mature student at the University of St. Andrews to read for the Degree of Bachelor of Science at St. Salvator's College, graduating in June, 1968 with Second Class (upper division) Honours in Botany. In October 1968, I matriculated at the above University for the Degree of Doctor of Philosophy under Resolution of the University Court of the University of St. Andrews, 1967, No. 1. From this date onwards, I have been supervised by Dr. E.G. Duncan, Lecturer in the Department of Botany, University of St. Andrews.

A C K N O W L E D G E M E N T S.

I wish to express my thanks to Dr. E.G. Duncan, my supervisor, who suggested the problem, for his advice and interest during the course of this work.

I would also like to take the opportunity to express my thanks to the Science Research Council for awarding me a Research Studentship, thereby enabling me to carry out the work presented here.

C O N T E N T S

INTRODUCTION

Throughout the past 90 years, many cytological investigations of the Basidiomycetes have been concerned with the nuclear events which take place during the final stages of sexual reproduction involving the production of basidiospores. However, the many reports on this subject are often fragmentary and not infrequently conflicting. The following review of the literature concerned focuses attention on the inadequacy of general knowledge regarding the most characteristic phase in the life cycle of the Basidiomycetes.

The first cytological investigation of the above nature was carried out by Strasburger (1884). His examination of Russula rubra was severely hampered by technical difficulties. The nuclei were extremely small and alcohol fixed material stained inadequately with haematoxylin, a cytological procedure which had been applied successfully to the nuclei of higher plants. Nevertheless, he noted that the young basidium contained a single nucleus which was considerably larger than any nucleus present in the hyphal cells. In his opinion, this nucleus divided twice and the daughter products in turn divided, so that eight nuclei were produced in the basidium. These nuclei were then presumed to pass in pairs through the sterigmata since a nucleus was observed at opposite ends of each basidiospore. Strasburger

emphasised/

emphasised that two nuclei were always clearly visible in the basidiospores. He assumed that the basidiospores remained binucleate until they were discharged, and that the now anucleate basidium had no subsequent function in basidiospore development.

Rosenvinge (1886) under Strasburger's direction, studied basidial development in 22 Basidiomycetes, three of which Tricholoma virgatum, Amanita vaginata and A. porphyria were examined in greater detail than the others. He confirmed Strasburger's earlier observation of a single large nucleus in the young basidium which, in the above three species divided twice to give four daughter nuclei while the four sterigmata and basidiospores developed. He claimed that subsequent nuclear events in the basidium and basidiospores differed in different species and that two distinct categories of behaviour were evident. These categories were exemplified by the events in Tricholoma and Amanita. In T. virgatum, the four daughter nuclei each elongated towards the sterigmata, then filled the sterigmata and finally appeared to pass into the basidiospores where they became spherical. He claimed that each basidiospore contained only one nucleus, although on one occasion there may have been two. Rosenvinge was led to conclude that the four daughter nuclei of the two Amanita species all underwent a further division since the basidiospores/

basidiospores were binucleate. He was prevented by staining difficulties from observing this division and was unable to decide where and when it took place. Nine of the remaining 19 species were presumed to fall into the Amanita category since they all possessed binucleate spores, while the other 10 species belonged to the Tricholoma category by virtue of the uninucleate condition of their basidiospores.

Rosenvinge pointed out that while the number of nuclei per basidiospore was constant in a given species, it was not constant in a given genus. Both species of Boletus examined appeared to have binucleate basidiospores. The basidiospore of Collybia velutipes had two nuclei however, while that of C. maculata had only one. He therefore concluded that the nuclear complement of the basidiospore was of no taxonomic value to the mycologist.

Wager (1892) made a careful study of the basidium of Agaricus (Stropharia) stercorarius and observed two nuclei in the mature basidiospore. He postulated that this condition had arisen as a result of the division of the migrant haploid nucleus within the basidiospore.

Von Istvanffi (1895) investigated nuclear phenomena in the development of the fungi in general, and in doing so was the first investigator to pay attention to the Heterobasidiomycetidae. He illustrated his observations on the epibasidium and/

and basidiospores of Tremella lutescens in Plate 37, Figure 35. In his text, he omitted to discuss this figure which illustrates each migrating, post-meiotic nucleus occupying the tip of the growing epibasidium as the latter elongates. When the epibasidium had reached its final length, the illustration suggests that the tip ultimately inflated to form the uninucleate basidiospore.

Dangeard (1895) carried out a cytological examination of the basidium of a number of Basidiomycetes in an attempt to establish beyond doubt that sexual reproduction did occur in this group of fungi, and that the basidium was the cell in which it took place. He clearly showed that karyogamy was a feature of the basidium and that the single large nucleus observed in this cell by earlier investigators was the fusion nucleus. He also claimed that the two divisions which this nucleus underwent were meiotic, but he did not investigate the nuclear events taking place after meiosis. His general conclusions were that 'le noyau sexuel se divise en quatre ou huit dans la baside: au sommet de celle - ci se dévellopent deux, quatre ou huit sterigmates qui se renflent à leur sommet pour les sporidies: les noyaux passent dans les sporidies en traversant les sterigmates (Fig. 22, V, D)'. His statement therefore suggests that following meiosis development of the basidium could vary as could the subsequent nuclear/

nuclear events. However, the figure to which he refers, an anucleate basidium bearing four uninucleate basidiospores, illustrates only one of the possible sequences of structural and cytological events outlined in his statement.

Dangeard's findings made a considerable impact on subsequent fungal cytologists, largely because he had established conclusively that the Basidiomycetes, although showing considerable morphological variation all appeared to undergo sexual reproduction in the basidium. The diagram (Fig. 22, V, A-D) in which he indicated the essential features of sexual reproduction was accepted without question as a general illustration of not only the normal nuclear events in the basidium, but also subsequent post-meiotic events which were not actually investigated in detail by Dangeard.

It does appear that the acceptance by later mycologists of Dangeard's diagram, and in particular Fig. 22, V, D, together with the total disregard of the text which it partly illustrates has, throughout subsequent decades led to the emergence of what may be termed the 'classical pattern' of nuclear events during the development of the basidium and basidiospores.

The essential features of this 'classical pattern' are karyogamy, followed by meiosis I and II taking place in the basidium, and subsequent migration of the four post-

meiotic/

meiotic nuclei to the four basidiospores which are then discharged in a uninucleate condition.

Although Dangeard figured the four post-meiotic nuclei within the four basidiospores, he did not suggest in his text that further division of these nuclei would or would not take place, nor did he state the nuclear complement of the basidiospore when discharged.

Ruhland (1901) investigated the basidial cytology of Hypholoma appendiculatum. Although he stated that he seldom observed nuclear migration, he nevertheless illustrated this process, and suggested that while the nucleus squeezed into the basidiospore the nucleolus changed its shape, thereby indicating, in his opinion, nucleolar division. He did not state whether or not his observations were a complete record of all nuclear events taking place prior to basidiospore discharge, but subsequent authorities appear to have assumed that they were, and have regarded Ruhland's observations as an illustration of the 'classical pattern' of nuclear events outlined above.

Nichols (1904) studied the nuclear events in the basidium and basidiospore of H. perplexum. She was able to show that the migrating nucleus divided on reaching the basidiospore which was therefore binucleate when mature. This report obviously conflicted with Ruhland's earlier/

earlier observations.

Meanwhile, Petri (1902) examined the basidial cytology of Hydnangium carneum and suggested that when the migrating nucleus reached the sterigma, it broke up into a number of granules some of which passed into the basidiospore, while the others remained in the basidium. The migrating granules then aggregated within the basidiospore to form the nucleus which later divided at least once so that the mature basidiospore contained a minimum of two nuclei together with other entities which he called 'pseudonuclei'. His conclusions conflicted with von Istvanffi's earlier findings on the same species. The latter writer stated that the young basidiospore received a descendant nucleus of the primary fusion nucleus, but his illustration implied that this descendant nucleus underwent no further division on reaching the basidiospore.

In the same year Harper (1902) illustrated, in what he termed a 'semi-diagrammatic drawing' the nuclear cytology of the hymenium of Hyphochnus subtilis. He featured a basidium with 'nearly ripe basidiospores, each with a single nucleus', thereby implying once more that no subsequent nuclear divisions took place following meiosis.

Meanwhile Maire (1902) published the results of his extensive cytological researches on a large number of Basidiomycetes/

Basidiomycetes representing many genera and families. He made use of a considerable range of fixatives and stains in the preparation of his material, and consequently achieved more success in his researches than previous investigators. His work is not altogether satisfactory because only disjointed aspects of the life-cycles were studied and no single species was examined thoroughly. Nevertheless, he pointed out that in no less than 13 of the 21 Agaricales examined, a post-meiotic mitosis took place either in the basidium, or more usually in the developing basidiospore. Similarly, the five Gasteromycetes examined all exhibited a post-meiotic mitosis. He thus positively established that a third nuclear division frequently occurred during the development of basidiospores. Maire's contribution to the state of knowledge therefore was to add to Dangeard's earlier observations on nuclear phenomena in the basidium, further important information relating to later nuclear events in the basidiospore. Maire however did not emphasise this fact and consequently he failed to cast doubts on the mistaken impression regarding post-meiotic nuclear events which by now had arisen through insufficient scrutiny of Dangeard's publication.

Lewis (1906) attempted an examination of nuclear events in the basidium and basidiospores of Amanita bisporigera.

He/

He saw the first meiotic division only and assumed that the second division must have taken place, since basidia bearing two uninucleate basidiospores and containing two residual nuclei were found in his preparations. His findings were regarded as an expression of the 'classical pattern' in a bisporic species.

Buller (1909), who was the first authority to present a general description of the life-cycle of a basidiomycete, based his remarks concerning the nuclear events in the developing basidium and basidiospores on Dangeard's and Ruhland's incomplete observations, and failed to make any reference to Maire's more extensive investigations. He described the migration of the post-meiotic nuclei to the basidiospores (p.9), but did not qualify this statement until some time later (p.11) when he pointed out that 'the nucleus which wanders into a spore soon divides into two after its entry so that each spore becomes binucleate'. He then made reference to the previous investigations by Nichols (1904) on Hypholoma perplexum. It appears that as a result of his failure to make this qualifying remark immediately after his statement on post-meiotic migration, later readers completely overlooked this important point, and assumed that no further nuclear division took place prior to basidiospore discharge.

Fries/

Fries (1911) studied the cytology of the gasteromycete Nidularia pisiformis and claimed that a third nuclear division took place in the developing basidiospores, which were consequently binucleate when discharged.

Levine (1913) concentrated his cytological investigations on the genus Boletus, and was able to confirm earlier reports (Rosenvinge, 1886; Maire, 1902) that the migrating nucleus divided in the maturing basidiospore of several boleti.

Juel (1898) had earlier exposed a fundamental dichotomy in the Basidiomycetes arising from the spindle alignment during meiosis. In one group, the Stichobasidiae, which comprised most of the families within the Heterobasidiomycetidae, the meiotic spindles lay in the long axis of the basidium, while in the second group, the Chiastobasidiae, largely comprised of the Homobasidiomycetidae, the spindles lay in the apex of the basidium at right angles to the long axis of the latter.

Later (1916) he showed that the Cantharellaceae on cytological grounds, belonged to the former group and were therefore more primitive and more closely related to a possible ascomycete ancestor than other families of the Agaricales. Juel also noted that a normal feature of the Cantharellaceae was a third nuclear division which took place in the basidium, so that eight nuclei were produced. Several species/

species within this family are bisporic, and Juel pointed out that in all instances only one of the eight nuclei passed into each basidiospore after the third nuclear division, and that any nuclei in excess of the number of basidiospores remained in the basidium and degenerated. He took care to point out that the Cantharellaceae, by exhibiting a third nuclear division were not unique in this respect, and he laid great stress on Maire's (1902) earlier observation that a third nuclear division was relatively common during basidiospore production. Juel's words appear to have gone entirely unheeded, and by the end of the third decade after Dangeard's publication, mycologists had come to accept the 'classical pattern' as the established pattern in the Basidiomycetes.

Schizophyllum commune was first examined cytologically by Essig (1922), who reported that the cells of the subhymenium, and the immature basidia were binucleate. He was unable to observe nuclear fusion in the basidium, but stated that the basidia were tetrasporic and that the basidiospores were binucleate at maturity. This suggested that a third nuclear division was a feature of yet another species.

Bauch (1926) in his study of Camarophyllus virgineus used a fixing and staining technique based upon the older methods employed by Romanowsky (1891) and later modified by Giemsa/

Giemsa (1904) to form the basis of the present Giemsa technique. Bauch observed meiosis in the tetrasporic race of C. virgineus, and illustrated the presence of two nuclei in what he termed the mature basidiospore. He pointed out that this was not an unusual feature, having been previously reported by Maire (1902). He also observed, but could not explain, the presence of degenerate but tetranucleate basidia in an otherwise healthy hymenium. He described these basidia as 'kollabiert', and noted that the cytoplasm was weak in its staining reaction and that the four nuclei lay in the middle of the basidium.

Kniep (1928) in his text-book on the sexuality of the lower plants, summarised his previous cytological researches in the fungi. Hypochnus terrestis was one of the species examined by him. He found that the nucleus which migrated to the basidiospore divided there, so that the mature basidiospore was binucleate. He summarised the sequence of nuclear events observed in Figure 210. This figure was later used in part by Smith (1938, 1955, Fig. 273 A-H) to illustrate the 'classical pattern' of nuclear events, but this author neither included Kniep's statement regarding a third nuclear division, nor the latter's illustration of a mature binucleate basidiospore. Kniep also pointed out that the developing basidiospores of Coprinus narcoticus, C. stercorarius/

stercorarius and C. sterquilinus received a single nucleus which divided so that the ripe basidiospores were binucleate.

Kniep's findings threw considerable doubt on the earlier claims made by Harper (1902) concerning the post-meiotic events of Hypochnus subtilis. Harper's claim regarding the post-meiotic nuclear events fitted the 'classical pattern' perfectly, and this may well have been the reason why Gwynne-Vaughan and Barnes (1927/1937) in their widely used textbook, made use of Harper's figure of a section of the hymenium to illustrate what they claimed were the nuclear events which take place in the basidium and basidiospores up to the point at which the latter are discharged.

The bisporic forms of Psalliota campestris, Galera tenera, Naucoria semiorbicularis and Coprinus ephemerus were studied cytologically by Sass (1929), who found that both the sub-hymenial cells and the young basidia were binucleate. Karyogamy and meiosis took place in the basidium, after which pairs of post-meiotic nuclei migrated to each basidiospore where they divided, producing mature tetranucleate basidio-spores. Sass confirmed Maire's (1902) previous observation on P. campestris f. bisporus, and his own observations on this species were later confirmed by Colson (1935), Sarazin (1938) and Evans (1959).

Another bisporic species, Nolanea cetrata, was investigated by/

by Buhr (1932), but his account of the post-meiotic events in this species appear to conflict with those of Sass and others above. Buhr claimed that only two of the post-meiotic nuclei migrated to the basidiospores while the other two remained behind in the basidium. He failed to establish whether or not nuclear division took place in the basidiospore. His observations recall the earlier ones of Lewis (1906) concerning Amanita bisporigera.

Smith (1934) accepted unquestioningly Buhr's contentions regarding the post-meiotic events in N. cetrata although they completely contradicted his own observations on seven Mycena species (M. metata, M. clavicularis, M. graveolens, M. immaculata, M. margaritospora, M. mirata and M. viscosa). Smith found that the four post-meiotic nuclei moved in tandem to the basidiospores. The leading nucleus invariably passed into the basidiospore where it divided, but the behaviour of the other partner was more erratic. Sometimes it also passed into the basidiospore and divided. In other instances, it divided in the sterigma, whereupon the daughter nucleus distal to the basidium entered the basidiospore while the proximal one remained in the basidium. He also observed that a third mitotic division, which took place as the migrating nuclei passed through the sterigmata, was a feature of all the tetrasporic Mycena species investigated

by/

by him, and he postulated that this behaviour was possibly typical for the genus as a whole.

Earlier Wakayama (1930), when summarising his cytological researches on a number of Basidiomycetes, stated that occasionally a mitosis took place after the entrance of the post-meiotic nucleus into the basidiospore, giving rise to a binucleate basidiospore. He noted this behaviour particularly in Cortinarius cinnamomeus, Hypholoma fasciculare and a species of Pholiota.

Shortly afterwards Vokes (1931) published the results of her study of Coprinus atramentarius. Although she made no detailed comment regarding post-meiotic events, her illustration (Fig. 46) suggests that division of the migrating nucleus occurred in the developing basidiospore. Sass (1933) pointed out that each basidiospore of C. sterquilinus received a single nucleus which divided at least once, but he could not decide how many further divisions, if any, occurred. In view of these two results and the earlier ones of Kniep (1928) concerning three more Coprinus species, it is more than surprising to find that Burnett (1968), in his text-book (p. 16) makes no reference to a division of the migrant nucleus in the basidiospore of an unspecified member of this genus.

One of the earliest cytological investigations of the Uredinales/

Uredinales was carried out by Allen (1933) who observed that in Puccinia malvacearum, the post-meiotic nuclei divided within the basidiospores. Her observations were later confirmed by Saville (1939), who reported that the same behaviour was also typical of Melampsora bigelowii and Uromyces lespedezae-procumbentis.

Whelden (1934, 1935) carried out a cytological investigation of the basidium of Tremella mesenterica, T. frondosa, T. grilletii, Exidia nucleata, E. saccharina, E. glandulosa and E. recisa. His preparations of the Tremella species proved that the sterigma and basidiospore are well differentiated before the migrating nucleus reaches the former. This finding clearly arouses doubts regarding von Istwanffi's (1895) earlier illustration, but Wheldon made no critical comment on the former's assertions.

The results of Whelden's cytological study of Tremella species led him to believe that the basidiospore contained only one nucleus, the post-meiotic nucleus, which divided when germination of the discharged basidiospore was about to take place. He also came to the same conclusion about the species of Exidia examined, but in this genus he did notice that the migrating nucleus was extremely attenuated as it passed through the sterigma. He also pointed out that 'in rare cases, the nucleus may round up in the epibasidium, seeming/

seeming to remain in this condition for some time (Pl. 10, Fig. 28)'.

Lander (1935) examined the cytology of Pisolithus tinctorius and indicated that a division of the post-meiotic nucleus was initiated in the basidium, but division actually took place in the basidiospore which was consequently binucleate at maturity.

Bose (1937) carried out a cytological study of the Polyporaceae, and reported the occurrence of a third nuclear division in the basidiospore of two species, Polyporus adustus and P. versicolor. This finding in the latter species in particular, conflicted with Dangeard's (1895) earlier report, but the fact was completely overlooked by Bose in his discussion.

Kemper (1937) studied a number of members of the Thelephoraceae, and claimed that in Coniophora cerebella, the mature basidiospores were binucleate as a result of division of the migrant post-meiotic nucleus. The following year Biggs (1938) published her findings on members of the same family and pointed out that in Peniophora ludoviciana, the post-meiotic nuclei divided in the basidium, and that four of the resultant nuclei migrated to the basidiospores while the remaining four degenerated in the basidium.

Baker (1941), in a somewhat confused account, described the/

the developing basidiospore of Physalacria inflata as 'apparently uninucleate', and claimed to have seen two nuclei in a basidiospore on only one occasion. She found that she could not confirm Peck's (1885) earlier claim that this species was bisporic, but she surprisingly tried to explain the presence of two degenerating nuclei in an aged basidium on the basis of bispory.

Skolko (1944) carried out a cytological investigation of Aleurodiscus canadensis, a bisporic member of the Thelephoraceae, and observed double migration of the post-meiotic nuclei to the basidiospores. He observed division of these nuclei in the basidiospores and suggested that the ripe basidiospores would be tetranucleate when discharged. His finding recalls the similar type of behaviour observed by Sass (1929) and others on Psalliota campestris f. bisporus and other related bisporic species, and also that observed by Smith (1934) in his Mycena metata group. They conflict with Buhr's (1932) interpretation of his observations of Nolanea cetrata and Lewis's (1906) claims regarding Amanita bisporigera.

Meanwhile, Ritchie (1941) studied the effect of different fixatives in preparations of the developing basidium of Russula emetica. Throughout his observations, he saw no division of the post-meiotic nucleus for he states that 'after/

'after the spores have received the (post-meiotic) nuclei, there is no further activity, the finished spore being uninucleate'. His statement regarding the nuclear complement of the basidiospore would suggest that R.emetica and R. rubra (studied by Strasburger, 1884) are radically different in their post-meiotic behaviour. Later Ritchie (1948) observed a third nuclear division in the basidiospores of Amanita caesarea and remarked that ' as often happens in the Hymenomycetes, the mature spores possess two nuclei'.

Hagerup (1945) investigated the basidial cytology of Lepiota (Limacella) lenticularis and established that the migrating nucleus divided in the basidiospore so that the latter was binucleate at maturity. His findings recall those of Kniep (1928) and Sass (1933) on the related genus Coprinus, and also the tentative claims of Wager (1892) regarding Agaricus (Stropharia) stercorarius.

Kühner (1945a) deplored the fact that mycologists had disregarded the reports by Maire (1902) and others, of a third series of nuclear divisions in the basidia and basidiospores of several species, on the grounds that such behaviour was exceptional. The results of his researches led him to conclude that in the majority of leucosporic Basidiomycetes, a third nuclear division occured either at the base of the sterigmata or within the latter. Four daughter nuclei

then/

then migrated to the basidiospores while the other four remained as residual nuclei within the degenerating basidium. He pointed out that in the leucosporic genera Amanita and Lepiota, and in the chromosporic Basidiomycetes, the third nuclear division took place in the basidiospores so that the latter were binucleate at maturity, while the degenerating basidia were anucleate. In several species with normally uninucleate basidiospores e.g. Hygrophorus hypothejus, Kühner noticed the presence of a nuclear division in several basidiospores. Since the orientation of the spindle was in the same plane as that of the intrasterigmatic division, he speculated on a priori grounds that this behaviour could be accounted for quite simply by a delay in the timing of the third nuclear division.

Kühner (1947) later published a report on the nuclear behaviour observed in a strain of Sistotrema confluens, which departed from the normal in that the basidium bore five to eight basidiospores. He claimed that in this species, only four post-meiotic nuclei were present in the basidium when nuclear migration commenced, but on reaching a sterigma or a basidiospore, each nucleus divided mitotically. The daughter nucleus furthest from the base of the basidium remained in the basidiospore to which it had been directed by the parent nucleus, while the other daughter nucleus left

the/

the basidiospore or sterigma and entered a basidiospore which had not, as yet received a nucleus. Any nuclei in excess of the number of basidiospores produced degenerated in the basidium, since all basidiospores were uninucleate.

The return of a nucleus from a basidiospore to the basidium and its possible migration to a second basidiospore had never before been recorded in mycological literature, and presented yet another complication in the accounts of post-meiotic events in the Basidiomycetes.

Schizophyllum commune was reinvestigated by Ehrlich and McDonough (1949), who were able to add to the information already provided by Essig (1922). They observed karyogmay and meiosis in the basidium and the migration of the four post-meiotic nuclei to the basidiospores. They confirmed Essig's observation that these nuclei now divided in the basidiospores and reported that the two daughter nuclei migrated to the apex of the maturing basidiospore.

Moreau (1953), while discussing heterothallism in the Basidiomycetes, illustrated his findings (Fig. 361) regarding the post-meiotic events in Hypochnus terrestis, the species which had been previously investigated by Kniep (1928). Moreau indicated that the post-meiotic nucleus divided in the young basidiospore as did Kniep, and he thereby provided further evidence to suggest that Harper (1902) had been mistaken/

mistaken in his observations of H. subtilis.

The basidial cytology of Ceratobasidium praticolum was first examined by Hawn and Vanterpool (1953). The basidia of this species have long fingerlike sterigmata, reminiscent of the epibasidia of the Tremellales. Hawn and Vanterpool claimed that the post-meiotic nucleus entered the sterigma and migrated to the tip. Their illustration implies that the nucleus then waits in the tip until the basidiospore differentiates and the sterigma constricts, after which the nucleus passes through this constriction into the basidiospore without dividing. Neither their illustration nor their discussion indicates that the post-meiotic nucleus divides during any subsequent stage of basidiospore development.

Sequeira (1954) in his much quoted report on nuclear phenomena in the basidium and basidiospores of Omphalina flavida suggested that three types of post-meiotic behaviour operated in this species. He described these three cycles as 'typical', occurring in 60-75% of the basidia, 'atypical-A' and 'atypical-B' both of which involved 10-20% of the basidia. The 'typical' cycle involved a post-meiotic mitosis in the basidium, the nuclei dividing asynchronously. Four of the daughter nuclei then migrated to the basidiospores while the remaining four degenerated in the basidium. In the 'atypical-A' cycle, a third nuclear division again took place/

place in the basidium, but in this case, Sequiera claimed that pairs of nuclei migrated to the basidiospores. The 'atypical-B' cycle was characterised by a delay in the timing of the third nuclear division which now took place in the basidiospores.

Sequiera's findings therefore suggest that a third nuclear division always takes place during basidiospore formation in O. flavida, and furthermore, in a given basidiocarp of this species, 60-75% of the basidiospores produced are uninucleate, while the remainder are binucleate.

Almost 80 years of cytological investigations of the Basidiomycetes had now passed, during which time progress in this field had been somewhat disappointing, due mainly to an inability to stain nuclei informatively, particularly during the mitotic cycle. Staining techniques which yielded excellent results when applied to the nuclei of the higher plants generally produced very inadequate results when applied to fungal material. Bakerspigel (1960) pointed out that it was unrewarding to try and demonstrate the chromatin in the fungal nucleus with haematoxylin or other nuclear stains such as aceto-orcein, aceto-carmine and gentian violet. Only the nucleolus and the meiotic prophase nucleus have an affinity for haematoxylin. Consequently, little concrete information could be obtained by studying material stained/

stained with haematoxylin, a stain used by many investigators. Their statements reporting their observation must, in this light, be treated with reserve.

The Feulgen reaction, specific for D.N.A. initially appeared to provide the fungal cytologist with a much improved cytological technique, but the staining reaction was insufficiently intense in the case of fungi to allow satisfactory observation of nuclear phenomena, and modifications of the process did little to improve the situation.

The introduction of the acid-Giemsa technique, developed initially by the bacterial cytologists did, however, dramatically change the course of fungal cytology. Its surpassing qualities were underlined by Saksena (1961) who, while re-investigating the basidial cytology of Ceratobasidium praticolum, employed Haedenhain's haematoxylin, aceto-carmine, Feulgen and acid-Giemsa techniques. He found that the latter provided preparations vastly superior to those obtained by the other three methods. Observation of acid-Giemsa preparations revealed that migration of the post-meiotic nuclei commenced only when the basidiospore primordia had developed, a fact which conflicted with the earlier claims made by Hawn and Vanterpool (1953) concerning this species. Furthermore, Saksena pointed out that this nucleus divided within the basidiospore as the latter matured, a feature

which/

which had been unobserved by the former investigators.

Burnett and Boulter (1963) also used the acid-Giemsa method in their cytological investigation of Mycocalia denudata, and showed that a third nuclear division was also typical of this member of the Gasteromycetes. The site of this division varied, sometimes taking place in the basidium, sometimes in the basidiospore. An analysis of the frequency of occurrence of the different types of behaviour enabled the above authors to determine the genetical basis of this phenomenon.

The acid-Giemsa technique was used by Wilson, Miller and Griffin (1967) in their study of the nuclear behaviour in the basidium of Fomes annosus. While much of their report regarding the meiotic configurations in the basidium is questionable, they nevertheless pointed out that migrant post-meiotic nuclei divide within the basidiospores.

Furtado (1968) employed the acid-Giemsa method in his cytological examination of Exidia nucleata. In a careful study of this species, he established that the basidiospores were uninucleate at maturity, and that the migrating post-meiotic nuclei divided either below or within the sterigmata. One daughter nuclear product then passed into each basidio-spore while the other remained within the corresponding epibasidium and degenerated. His findings repudiate the earlier/

earlier claims made by Whelden (1935) regarding this and three other species of Exidia. It is possible that the latter author, when noting the extremely attenuated nature of the post-meiotic nucleus as it passed through the sterigma, was actually observing the third nuclear division, but failed to interpret his observations as such. His remark, previously quoted, (p.16), in the light of Furtado's findings, would suggest that he was really observing a residual daughter nucleus, and not the migrating parent nucleus.

Lu (1964) employed propionocarmine to stain the nuclei in the basidium and basidiospores of Cyathus stercoreus, and by this means was able to observe a third nuclear division in the developing basidiospore.

One and occasionally two nuclei were observed in the mature basidiospore of Volvariella volvacea by Chang (1969), which suggests that a division may or may not take place in the basidiospores of this species. The basidiospore wall was reported by Chang to be relatively thick and brown in colour. He did not attempt to remove the pigment from the wall before staining, and furthermore, he used haematoxylin to stain the nuclei, a method which yields little information on the mitotic cycle of Basidiomycetes (Bakerspigel, 1960). Heavy pigmentation of the wall, together with poor intensity of staining reaction could easily prevent accurate observation of/

of the nuclear complement of the basidiospore. Thus, if division in the basidiospore were universal, and the two daughter nuclei were to remain side by side, instead of separating to opposite ends of the basidiospore, it could be difficult for the observer to distinguish between uninucleate and binucleate conditions.

Within the last decade, the electron microscope has also been used in ultrastructural studies of the Basidiomycetes. Wells (1965) carried out one such study on the basidium and developing basidiospores of Schizophyllum commune, and was able to confirm the earlier reports by Essig (1922) and Ehrlich and McDonough (1949) that a third nuclear division took place within the basidiospores. This study also showed, as did those of the earlier investigators, that the sister nuclear products migrated to the apex of the basidiospore as the latter matured.

Throughout almost 90 years of cytological investigation of the basidium, there had been a considerable number of publications reporting the post-meiotic events in a Basidiomycete. Many suggested that the 'classical pattern' took place in the species concerned. But later investigations have shown that these reports were either completely wrong, e.g. the early reports concerning Tremella and Ceratobasidium, or highly suspect, e.g. Hypochnus subtilis

and/

and Nolanea cetrata. Very many more publications have indicated that a third nuclear division is a common feature during maturation of the basidiospore. Indeed Kühner's (1945a) remarks strongly imply that the 'classical pattern' is a rarity in the Basidiomycetes.

It has been pointed out above that the post-meiotic nuclei of the boleti were claimed by three authorities, Rosenvinge (1886), Maire (1902) and Levine (1913) to undergo a third nuclear division within the basidiospore, and these observations were confirmed by Duncan (1970) who also employed acid-Giemsa staining in his reinvestigation of the genus. In addition, Duncan's observations revealed the outstanding and hitherto undocumented fact that the basidiospores did not remain binucleate, but instead, reverted to the uninucleate condition by retrogressive migration of one of the daughter nuclear products to the basidium.

The discovery of a previously unreported pattern of behaviour which was of a totally divergent nature from that considered typical of the Basidiomycetes, pointed out the need for a further investigation of post-meiotic events.

The purpose of this investigation was primarily to determine whether or not the pattern of post-meiotic events encountered by Duncan (1970) in the boleti was a feature of genera in the families Gomphidiaceae, Paxillaceae, Hygrophoraceae/

Hygrophoraceae and Russulaceae. When it became evident that many of the reports in the literature concerning post-meiotic events were either incomplete and consequently ambiguous, or totally in error, the investigation was extended to include members of the Cantharellaceae, Polyporaceae, Tricholomataceae, Agaricaceae, Amanitaceae, Coprinaceae, Cortinariaceae, Rhodophyllaceae, Hydnaceae, Thelephoraceae and Tremellaceae. Thus a wide spectrum of Basidiomycetes have been investigated cytologically, and on the basis of the results obtained, it has been possible to confirm or expand certain existing reports, and to refute others. It has also been possible to categorise different patterns of post-meiotic behaviour in the Basidiomycetes, and furthermore, to reach tentative conclusions regarding the taxonomic and phylogenetic significance of these patterns.

Footnote. The species referred to in the Introduction and Discussion are listed in Appendix I together with any current synonyms. The species are assigned to families in accordance with the modern taxomonic views of Singer (1962) and Donk (1964).

MATERIALS AND METHODS

The cytological studies recorded were carried out on members of the Basidiomycetes collected in the vicinity of St. Andrews, Fife, Scotland.

(1) Screening of basidiocarps.

Many species produce basidia with abnormal complements of basidiospores. Such abnormalities may be characteristic of a species, or merely characteristic of individual basidiocarps. It is essential to be aware of such abnormalities before interpreting the post-meiotic events observed in stained preparations. Hence, all basidiocarps collected were screened to detect abnormalities in the basidiospore complement.

Small pieces of lamellae from each basidiocarp were stained with cotton blue in lactophenol and examined under the oil immersion (x90) objective lens to determine the basidiospore complement of the basidia. In those cases where abnormalities were detected, an analysis of the classes of basidiospore complement was carried out using standard sampling techniques, and records retained for future reference.

(2) Fixation methods.

The/

The specimens selected for study were allowed to shed their basidiospores naturally on to clean glass slides. In this way, only mature basidiospores accumulated on the slide. When an adequate basidiospore print had formed, the basidiospores were transferred in a drop of 4% formalin solution to clean slides, which had previously been smeared with Haupt's adhesive. These slides were allowed to dry overnight and then placed in a fixative consisting of a saturated aqueous solution of mercuric chloride containing 1% glacial acetic acid (Burnett and Boulter, 1963) for 24h. They were then washed in running tap water for a further 24h, and finally stained.

Individual lamellae or a small wedge of the pileus were removed from the basidiocarps concerned and fixed in the mercuric chloride-glacial acetic acid mixture for 24h, washed in running tap water for 24h, and finally embedded. Material was fixed in the field to obviate the possibility that the course of post-meiotic events might be changed due to disruption of relevant metabolic activities during transit from the field to the laboratory. A small fixation tube, half-filled with fixative was held under the selected pileus, without disturbing the latter. A wedge of tissue was cut out of the pileus and dropped into the fixative. The remainder of the basidiocarp was placed in a polythene container/

container and returned to the laboratory, where mature, naturally shed basidiospores were collected from the area adjacent to the cut. When very small basidiocarps were chosen, the pileus was halved, one half being placed in the fixative, the other half returned to the laboratory.

(3) Embedding and sectioning methods.

Aquax (Gurr Ltd.), a water soluble polyester wax, was used as an embedding medium. Fixed material was placed in a 50% mixture of aquax and water at 58°C for 24h. It was then transferred to a 6% mixture of glycerol in aquax at the same temperature for a further 24h, this solution being replaced after 12h. A block was made by transferring the material to a fresh solution of 6% glycerol in aquax contained in a mould. The mould was then placed in a freezing cabinet at -12°C. Rapid solidification of the aquax was thus achieved and the amount of crystallization reduced to a minimum. Blocks were wrapped in tin foil, placed in screw-top bottles, and kept under refrigeration (+2°C) until required for sectioning.

Sections were cut using a Hearson Rotatome, the thickness of the section varying with the diameter of the basidia involved. The aim was to cut sections no more than two basidia thick.

Aquax/

Aquax embedded material is much less easily sectioned than paraffin embedded material, since it tends to be more brittle and consequently does not produce convenient ribbons of sections. The plasticity of this wax may be greatly improved by the addition of glycerol. It may be further improved by allowing the block to equilibrate to room temperature overnight, and finally, by breathing heavily on the mounted block each time it is about to pass over the microtome knife. Despite the technical difficulty encountered in sectioning aquax embedded material, aquax is preferred to paraffin wax as an embedding medium, since aquax embedded material yields the superior preparations in conjunction with the acid-Giemsa staining technique. (Duncan, 1970). This may be due to the elimination of alcohols from all stages of processing.

Sections were mounted in a few drops of 4% formalin solution on clean glass slides smeared with Haupt's adhesive, and allowed to dry overnight.

(4) Acid-Giemsa staining technique.

Both basidiospore prints and sectioned lamellae were stained exclusively by the acid-Giemsa technique. The method initially employed was based on that described by Robinow (1942). The slides bearing sections of basidiospores were/

were washed in distilled water at 60°C for 10 min. to remove the aquax and condition the material for hydrolysis in hydrochloric acid at the same temperature. The hydrolysis period ranged from 5-10 min, the optimum period being determined by trial in the case of individual species. The slides were then thoroughly washed to remove all traces of acid and transferred to a 4% solution of Gurr's Improved R66 Giemsa stain in a Sørenson phosphate buffer at pH6.5. Basidiospore prints were stained for 10 min, sections of lamellae for 30 min, after which they were transferred to a dehydrating mixture of acetone and xylene in the proportions 20:1 for 30s, 14:6 for 10s, 6:14 for 10s and finally pure xylene for 10 min. The mounting medium was Euparal.

Giemsa stain is described by Gurr (1965) as a mechanical mixture of the single dyes methylene blue, azur 1 and eosin, in which the uncombined dyes influence the staining potential of each other. Giemsa stain is therefore a mixture of acidic and basic dyes, and it follows that the pH of the diluting buffer solution is important in determining the colour reaction obtained. The fixative used also has a bearing on the ultimate result, the optimum staining pH varying with different fixatives. The aim of the cytologist is to select a pH value at which the eosin, in the presence of azur 1 and methylene blue, is preferentially absorbed by the/

the nucleic proteins while the cytoplasmic proteins fail to absorb stain. Under these conditions, the nuclei appear red against a more or less colourless background, while the nucleoli appear purple, magenta or blue. In all species it is necessary to carry out a series of test stainings in which the stock stain is diluted with buffer solutions spanning a considerable pH range. From the results obtained, the pH range may be narrowed until the pH giving the best results is determined. Early experiences proved that stock stain diluted with a Sørenson phosphate buffer pH6.5 gave excellent results in the case of many species.

Giemsa stain is notorously difficult to prepare (Gurr, 1965). Variation in composition and staining potential is almost inevitable in different batches of this product. Hence the results obtained using one batch of stain are not always reproducible with a later batch, in which case modifications of technique are necessary. Difficulties in this respect were encountered in certain species, particularly with regard to the amount of stain absorbed by the cytoplasm. Not infrequently it was heavily stained blue in colour with consequent masking of the stained nuclei. The staining reaction could be markedly improved by altering the pH of the diluting buffer solution or by differentiating in a citrate buffer of pH5.4. Unfortunately, the use of this buffer/

buffer caused accumulation of water droplets on the slides which could not be removed by the dehydrating process and which caused considerable inconvenience during examination and photography of the preparations. Subsequent experiments, using a number of different buffer solutions, established that a sodium cacodylate - hydrochloric acid buffer (Plumell, 1948) at pH 5.8 produced excellent differentiation and completely eliminated accumulation of water droplets. This buffer was used as a differentiating solution in all subsequent preparations. In cases where differentiation was not required, the slides were washed in a 1:4,000 solution of DC 34 (Welton Laboratories, Ltd.), a non-ionic detergent, before dehydration. This detergent virtually eliminated accumulation of water droplets during dehydration.

Sections prepared by the modified technique frequently lacked intense staining reaction. Trials established that this difficulty could be rectified by hydrolysing material in 60% orthophosphoric acid (H_3PO_4) for 4h at 20°C. It is not immediately obvious why a change of acid should enhance the staining reaction. It has been suggested by Hashim (1953), who observed a similar improvement in the Feulgen nuclear reaction after H_3PO_4 hydrolysis, that the low concentration of water present minimises diffusion of the stainable product. The low temperature of the hydrolysing solution/

solution will also tend to reduce diffusion. It is also possible that the presence of H_3PO_4 modifies the staining entity in such a way that more stain can be absorbed thereby increasing the intensity of reaction. Subsequent hydrolysis was carried out in 60% H_3PO_4 for 4h at room temperature, after which the slides bearing sections or basidiospores were washed thoroughly in several changes of tap-water and finally rinsed in phosphate buffer at pH 6.5. The pH of the buffer was redetermined electrolytically after rinsing. Any lowering of its value indicated traces of acid, in which case the washing procedure was repeated. When no further drift of pH was observed, the slides were transferred to the buffered stain solution and the modified technique applied. Differentiation following staining was necessary in the preparation of material from the majority of species.

(5) Camera lucida drawings.

Camera lucida drawings were made, using a Wild-M20 microscope fitted with x6 eyepieces and a x100 objective lens. The drawing tube had a magnification factor of x1.25, giving a total magnification factor of x750.

(6) Microscope observations and photography.

Light and phase-contrast observations were made using Reichert/

Reichert 'Zetopan' and Baker 'Projectolux' microscopes respectively. Photographs were taken by fitting a Watson 35mm attachment camera to these microscopes. The film used was Ilford 'Microneg', which was developed in Ilford 'ID 2' developer.

POST-MEIOTIC EVENTS

(1) Species investigated.

The species examined are listed in Table I. The family arrangement used follows that of Singer (1962), whilst the specific nomenclature is in accordance with that suggested by Dennis, Orton and Hora (1960), Cunningham (1963) and Coker and Beers (1951). This nomenclature will require revision following the complete publication of the British Fungus Flora (ed. Henderson, Orton and Watling).

(2) Cytological observations.

A detailed examination of the nuclear complement of the developing basidia and basidiospores, and of the mature, freely shed basidiospores of the 27 species listed in Table I, indicates that within this taxonomically wide range of Basidiomycetes, four main patterns of post-meiotic events are encountered. The four patterns all involve three nuclear divisions, meiosis I and II and a post-meiotic mitosis. The patterns may be distinguished from each other on the basis of the following two criteria:

1. The site of the third nuclear division.
2. The fate of the resultant nuclei.

The different patterns will be referred to as Patterns A, B, C, and D in the following account.

TABLE I.

Details of species examined.

Family and Species	Basidiocarps examined	Basidiospore complement	Source of specimens
1. Cantharellaceae.			
Cantharellus cibarius Fr., 1821	16	3-7	Tentsmuir
2. Polyporaceae.			
Polyporus brumalis Pers. ex Fries	4	4	Tentsmuir
3. Hygrophoraceae.			
Hygrophorus nigrescens (Quel.) Quel., 1889	3	4	Tentsmuir
4. Tricholomataceae.			
Collybia confluens (Pers. ex Fr., 1821) Kummer, 1871.	1	4	Tentsmuir
Flammulina velutipes (Curt. ex Fr.) Karst., 1891	3	4	Botanic Gdn, St. Andrews
Mycena alcalina (Fr. ex Fr., 1821) Kummer 1871, s. J. Lange, Konrad & Maubl., Kühn., Pearson non Ricken, Rea	2	4	Tentsmuir
M. swartzii (Fr. ex Fr.) A.H.Smith, 1947	2	95% tetrasporic	Tentsmuir

Oudemansiella mucida (Schrader ex Fr.) Höhnel, 1910	4	4	Spinkey Den, St. Andrews
Panellus mitis (Pers. ex Fr.) Sing., 1936	4	4	Tentsmuir
5. Amanitaceae.			
Amanita rubescens (Fr. ex Pers., 1821) S.F. Gray, 1821	4	4	Peat Inn
A. fulva (Schaeff.) Secr.,1833	1	4	Peat Inn
6. Agaricaceae.			
Lepiota lutea (Bolt.) Quel., (an alien)	1	4	Botanic Gdn. St. Andrews
7. Coprinaceae.			
Coprinus atramentarius (Bull. ex Fr., 1821)	2	4	Botanic Gdn. St. Andrews
8. Cortinariaceae.			
Galerina paludosa (Fr.) Kühn., 1935	1	4	Tentsmuir
9. Rhodophyllaceae.			
Entoloma porphyrophaeum (Fr., 1857) Karst., 1879, s. Ricken non Konrad & Maubl., 1932	1	4	Tentsmuir

Nolanea cetrata (Fr. ex Fr., 1821) Kummer, 1871	2	2	Tentsmuir
N. papillata Bres., 1879	1	4	Tentsmuir
10. Paxillaceae.			
Hygrophoropsis aurantiaca (/von Wulfen/ Fr.) Maire apud Martin-Sans, 1929	3	4	Tentsmuir
Paxillus involutus (Batsch ex Fr., 1821) Fr., 1838	9	4	Tentsmuir
11. Gomphidiaceae.			
Gomphidius rutilus (Schaeff. ex Fr., 1821) Lundell, 1937	18	4	Tentsmuir
12. Russulaceae.			
Russula claroflava Grove, 1888	2	4	Burnside Woods
R. emetica (Schaeff. ex Fr., 1821) S.F. Gray, 1821, s. J.Lange, Sing.	3	4	Burnside Woods
Lactarius rufus (Scop. ex Fr., 1821) Fr., 1838	1	4	Tentsmuir

13. Hydnaceae.			
Auriscalpium vulgare S.F. Gray	4	4	Tentsmuir
14. Thelephoraceae.			
Corticium comedens (Nees) Fries	1	4	Spinkey Den, St. Andrews
Pellicularia otogensis G.H. Cunningham	1	6-8	Culture
15. Tremellaceae.			
Tremella foliacea (Pers.) Fr. non Bref.	1	4	Tentsmuir

Pattern A: The site of the third nuclear division is the apex of the basidium. One of the resultant eight nuclei migrates to each basidiospore. Any nuclei in excess of the number of basidiospores remain in the basidium and degenerate. The mature basidiospores are uninucleate.

Pattern B: The site of the third nuclear division is within the sterigmata. The daughter product distal to the basidium passes into the basidiospore, while the proximal one returns to the basidium. The mature basidium bears four uninucleate basidiospores and contains four residual degenerating nuclei.

Pattern C: The site of the third nuclear division is within the basidiospores. The daughter product distal to the basidium remains within the basidiospore, while the proximal daughter product returns to the basidium. The mature basidium bears four uninucleate basidiospores and contains four degenerating nuclei.

Pattern D: The site of the third nuclear division is within the basidiospores. The daughter products remain within the basidiospores which are therefore binucleate when shed, while the aged basidia are anucleate.

Patterns B and C produce the same end result, although the means by which this is achieved are different.

Observations indicate that with one exception, each species exhibits only one pattern of post-meiotic events. The exception is Mycena swartzii which exhibits Pattern B and C sequences, the frequency of occurrence of the different patterns being ostensibly in the ratio of 4:3. The assessment of frequencies was based on an examination of a single transverse section of a basidiocarp. A more extensive analysis may yield a 1:1 ratio, in accord with genetical expectations.

Nolanea cetrata, a bisporic species, exhibits a modification of Pattern D, in that pairs of post-meiotic nuclei migrate to each basidiospore where they then divide producing tetranucleate basidiospores.

The remaining 25 species entirely conform to Patterns A, B, C, and D, as classified in Table II. The post-meiotic events occurring in individual species are subsequently described in detail under the above headings.

TABLE II

Classification of species on basis of pattern of post-meiotic events.

Pattern	Species
A	Pellicularia otogensis Cantharellus cibarius
B	Collybia confluens Mycena alcalina
C	Polyporus brumalis Panellus mitis Hygrophorus nigrescens Hygrophoropsis aurantiaca Paxillus involutus Gomphidius rutilus Russula claroflava R. emetica Lactarius rufus Auriscalpium vulgare
D	Flammulina velutipes Oudemansiella mucida Amanita rubescens A. fulva

	Lepiota lutea Coprinus atramentarius Galerina paludosa Entoloma porphyrophaeum Nolanea papillata Corticium comedens Tremella foliacea

Pattern A.

(a) Pellicularia otogensis and Cantharellus cibarius.

P. otogensis and C. cibarius are characterised by possession of polymorphic basidia, that is, basidia bearing from two to eight basidiospores. The former species produces six to eight basidiospores, seven-spored and eight-spored basidia being the most frequently occurring types. The latter species has a greater degree of variability, producing basidia bearing two to eight basidiospores. Six-spored basidia were the most frequent type occurring in the specimens examined.

The short club-shaped basidia of P. otogensis are borne in small clusters on aerial hyphae. The young basidium contains a pair of conjugate nuclei which fuse in the mid-basidium (Fig. 1a). Prophase of meiosis apparently lasts for some time, as many basidia are observed in this condition. The later meiotic stages and the subsequent mitotic division were not in fact observed, despite examination of many preparations.

Mature basidia, bearing six, seven or eight uninucleate basidiospores were however frequently observed. Fig. 1e illustrates an anucleate basidium bearing eight uninucleate basidiospores. Fig. 1b illustrates a basidium bearing seven/

seven uninucleate basidiospores, and containing a single residual nucleus.

The freely shed, mature basidiospores are always uninucleate (Fig. 1d), consequently no further division of the nucleus takes place after it has migrated to the basidiospore.

The basidia of C. cibarius are slender and closely appressed to each other. Meiosis I and II take place in the middle of the basidium, the spindle axes lying parallel to the long axis of the basidium. The four meiotic products enter a brief expanded interphase, during which time the nucleolus becomes conspicuous (Fig. 2a). These nuclei then move to the apex of the basidium, where all undergo a third nuclear division. The axes of the mitotic spindles appear to lie somewhat haphazardly in the basidium, being neither parallel to, nor at right angles to the long axis of the latter (Fig. 2b). The sterigmata and basidiospore primordia now develop rapidly. Fig. 2c illustrates a five-spored basidium containing eight highly condensed daughter nuclei prior to migration. A single nucleus passes into each basidiospore where it enters an interphase condition. No subsequent division takes place (Fig. 2e).

Because of the varying number of basidiospores produced per basidium, there may be unrequired nuclei which are destined to remain within the basidium and degenerate

Fig./

Fig. 2d). The difficulties encountered in staining the sectioned lamellae of C. cibarius has prevented a detailed analysis of this situation being made, but were such an analysis to be carried out, it is anticipated that an inverse correlation would exist between the number of basidiospores produced per basidium and the number of residual nuclei present in the aged basidia.

Pattern B.

(a) Mycena alcalina.

(i) 'Normal' sequence of post-meiotic events.

Following meiosis, the four post-meiotic nuclei move towards the centre of the basidium. A typical feature of M. alcalina is that the four, large, post-meiotic nuclei, aligned in the long axis of the basidium, occupy almost the whole volume of the latter (Fig. 3). Basidia are frequently seen in this condition, suggesting that the post-meiotic interphase is a relatively long one. The basidiospores develop towards the end of this stage, and the four expanded post-meiotic nuclei move towards the sterigmata in a tear-drop form, the apex of the drop foremost. This migratory form, which is characteristic of many species, suggests that the four post-meiotic nuclei are pulled through the cytoplasm towards/

towards the sterigmata and basidiospores. They then stream into the sterigmata. When the chromatin is positioned mid-way between the basidiospore and the base of the sterigma, the third nuclear division takes place (Fig. 4). The nucleoli, which have become dis-associated from the post-meiotic nuclei in the early stages of migration may be seen within the basidium (Fig. 5). The daughter product proximal to the basidio-spore passes into the latter as telophase preceeds, while the sister nuclear product moves back into the basidium. The two daughter nuclear products remain connected by the remnants of the spindle for some time (Fig. 6). The basidium bears four uninucleate basidiospores at maturity and contains four residual nuclei in tha apical region (Fig. 7). These residual nuclei are intensely stained, and unlike typical functional nuclei, lack a nucleolus. Both the nuclei within the basidium, and those within the basidiospores become less highly condensed as the basidium matures. The uninucleate basidiospores are shed and the aged basidia, containing the four residual nuclei, degenerate.

(ii) 'Anomalous' behaviour.

Whilst observations indicate that the great majority of aged basidia contain only four residual nuclei, a few/

few basidia have been observed containing five, six, seven and even eight nuclei in diminishing frequency. These basidia exhibit well developed sterigmata and appear to have produced and discharged basidiospores. There is evidence to suggest that following discharge of basidiospores, one or more of the residual nuclei may divide. Fig. 8 illustrates an aged basidium containing three residual nuclei and a fourth one in the final stages of division. Fig. 9 illustrates two residual nuclei and four small, contracted nuclei. The position of the latter would indicate that they had resulted from division of the remaining two residual members of the original four residual nuclei. The fact that basidia containing five residual nuclei are more frequently encountered than basidia containing eight, indicates that all four residual nuclei seldom undergo a further division.

A second abnormality concerned the single observation of a basidium containing five residual nuclei, and bearing only three mature sterigmata, the basidiospores having been shed. The fourth sterigma, although shorter than the others, bore the typical swelling of a basidiospore. Neither this sterigma nor the basidiospore contained a nucleus. This observation suggests that/

that one sterigma and/or basidiospore had failed to develop in the normal manner, and that the nucleus which should have divided on its way to the latter, was forced instead to divide within the basidium.

A third abnormality is illustrated in Fig. 10. In this instance, a single attached basidiospore contains two nuclei. The basidium here is one which is superimposed on the basidiospore-bearing one. Bearing in mind that a binucleate basidiospore has been seen attached to the basidium on three occasions, it appears that either, the post-meiotic nucleus may migrate to, and divide in the basidiospore, or, if division takes place in the sterigma, both daughter products are dragged into the basidiospore during anaphase. No early stages of the third nuclear division have been observed to take place within the basidiospore, which suggests that the latter alternative is the more probable explanation. It is emphasised that all naturally shed basidiospores are uninucleate, and this in turn implies that the binucleate basidiospores either fail to mature and are therefore not discharged, or, retrogressive migration of one daughter nuclear product takes place before basidiospore discharge.

(b) Collybia confluens.

The post-meiotic nuclear events taking place within the basidium/

basidium and developing basidiospores of C. confluens appear to conform exclusively to the above normal sequence of events in M. alcalina and hence are not illustrated.

Pattern C.

(a) Gomphidius rutilus.

(i) 'Normal' behaviour.

Preparations of sectioned lamellae of G. rutilus reveal that in many of the basidiospores attached to basidia, the nucleus is undergoing or has undergone a mitotic division. However, the mature, naturally shed basidiospores are invariably uninucleate. It is evident that additional nuclear events must therefore take place following the third nuclear division.

Meiosis I and II take place in the apex of the basidium, and the four post-meiotic nuclei move to the centre of the basidium where they enter interphase. The sterigmata and basidiospores develop towards the end of interphase. The four post-meiotic nuclei then move towards the sterigmata. It is noteworthy that the migrating post-meiotic nuclei of G. rutilus are cigar-shaped, and quite unlike those of the previously examined species which have a characteristic tear-drop form when migrating (Fig. 11). The post-meiotic nuclei develop

a/

a heterochromatic condition during interphase, and this condition is maintained until completion of migration.

The migrating nucleus becomes highly attenuated as it passes through the sterigma and streams into the basidiospore. Light microscope observations of this stage indicate that the chromatin appears to be in a twin-stranded condition as it enters the basidiospore (Fig. 12), an observation which recalls the claims made by E.J. Duncan and Macdonald (1965) that the chromosomes of Marasmius are joined either in a ring configuration or a double strand, as is the case also in certain higher plants (Wagenaar, 1969). Phase-contrast examinations of this stage establish that the nuclear membrane encompasses the chromatin throughout migration (Fig. 13).

The nucleolus may be seen lagging behind the chromatin but within the nuclear membrane during early stages of migration. Observations of later stages indicate that the nucleolus becomes disassociated from the chromatin as the latter passes into the basidiospore, and is left behind within the sterigma (Fig. 14). The liberation of a nucleolus by its parent nucleus normally occurs at the metaphase/anaphase stage of mitosis in the Basidiomycetes when the nuclear membrane breaks down (Motta,/

(Motta, 1969). The presence of the nucleolus in the sterigma, and the fact that the nuclear membrane is persistent and intact some distance away in the basidiospore, implies that the nucleolus has been prematurely extruded from the post-meiotic nucleus. The nucleolus of G. rutilus is not particularly conspicuous and its staining reaction does not allow its movements to be followed beyond the point at which the chromatin has vacated the basidium.

The migrating nucleus moves towards a subapical point on the basidiospore wall and a fibril connecting it to this point may be detected on occasions by means of phase-contrast (Fig. 15). A similar fibril, having its origin at the opposite extremity of the nucleus, may be observed extending to an undetermined point in the lower region of the basidium (Fig. 16 a and b). The fibrils appear to be unit structures and in general no evidence of radiating subfibrils can be detected. The fact that the migrating nucleus is connected apically and basally to specific sites, is strong circumstantial evidence that there are direct connections to these points. Both sets of fibrils persist until the conclusion of the third nuclear division.

The chromatin begins to contract and condense into

a/

a spherical mass which is centrally situated within the basidiospore. Moreover, it frequently appears to be bipartite before division (Fig. 17). The fibrils referred to above are visible, one running towards the apex of the basidiospore, the other in the opposite direction into the basidium (Fig. 18, a, b, c).

The mitotic spindle which will function in the third nuclear division now becomes visible and is seen to penetrate the chromatin mass (Fig. 19, a, b). The spindle is relatively short at this stage and has distinct terminations presumably in kinetocenters[1]. The nuclear membrane is apparently in the process of degenerating at the stage featured, and a fragment is seen associated with one termination in phase-contrast observations. The fibrils described above extend apically and basally from the ends of the spindle which has an oblique orientation in this species. The third nuclear division now proceeds in synchrony in all four members of each tetrad (Fig. 20).

The inception of anaphase is indicated by the elongation/

[1] Kinetocenter (Grundmann, 1966) is used to denote the pole determinants, in place of more specific and often controversial terms adopted by other authorities. The question of terminology will be discussed later (p.107).

elongation of the spindle. The nuclear membrane is no longer visible. The chromatin separates into its daughter products which move towards opposite poles of the spindle as it elongates asymmetrically. The daughter product distal to the sterigma moves a short distance only towards the attachment point near the apex of the basidiospore (Figs. 20 and 21). In contrast, the sister product migrates in a highly condensed form through the sterigma and plummets down into the basidium for a considerable distance (Figs. 21 and 22).

The chromatin is attached directly or indirectly to the fibril which previously connected the parent nucleus to a point within the lower region of the basidium. There are indications that the nuclear membrane or part thereof is present at a point in advance of the migrating chromatin (Fig. 23). Motta (1969) maintained that resynthesis of the nuclear membrane proceeded from centrosomes sited at the poles of the nuclei. It is therefore provisionally assumed that the chromatin is attached by spindle elements to the kinetocenter which is in turn, connected by the fibril described above to a point in the lower region of the basidium.

The daughter nuclear products remain attached to each other by the remnants of the spindle for some time.

It is noteworthy that the maximum distance separating the daughter products at anaphase is comparable to that observed during the corresponding stage of the final conjugate division in the hyphal tip. These stages are respectively illustrated in Figs. 21 and 32.

During the final stages of the third nuclear division, pigment is laid down in the basidiospore wall, and the daughter nucleus within the basidiospore moves from the subapical position and takes up its final position in the centre of the basidiospore. Most stages of the third nuclear division may be observed relatively frequently in preparations, but the process of retrogressive migration to the basidium is seen less often, which suggests that it takes place very quickly. This fact may explain why the process has gone unnoticed by all authorities prior to Duncan (1970).

The mature basidium bears four uninucleate basidiospores and contains four returned nuclei (Fig. 24). These nuclei move towards the centre of the basidium following the discharge of the basidiospores. They remain as highly condensed entities and their staining reaction is indicative of their degeneration as vacuolation of the basidium proceeds (Fig. 25). These nuclei are totally unlike, and cannot be confused with, the expanded,/

expanded, heterochromatic post-meiotic nuclei of the previous generation. The end result of this sequence of post-meiotic events is the same as that observed in Pattern B.

(ii) 'Anomalous' behaviour.

Observations indicate that most of the basidia in the basidiocarps examined conform exclusively to the sequence of post-meiotic events detailed above. Nevertheless, basidia which did not exhibit the above sequence were noted. These basidia were in a very low frequency, never exceeding 0.1% in any individual basidiocarp.

Meiosis II produces four nuclei (Fig. 27) which normally expand and undergo interphase. In exceptional cases however, one or more of these nuclei may immediately undergo a third nuclear division. Fig. 26, a and b are illustrations at different foci of this behaviour. Fig. 28 illustrates a basidium which has not formed sterigmata, but which contains six nuclei, while Fig. 29 illustrates a basidium containing eight nuclei and bearing sterigmata. The expanded nature of the nuclei present suggests that Fig. 29 represents a later stage in development than that illustrated by Fig. 28. Fig. 30, a and b illustrate, at different foci, a basidium bearing a single anucleate basidiospore. Two

more/

more sterigmata are present from which the basidiospores have been severed during the preparation of the section. At first sight, it appears that one of the post-meiotic nuclei has undergone a third nuclear division within the basidium as in Fig. 26 a and b, but on the other hand, the nuclei are all in exactly the same condition and are equal in size. It may be that they are five of eight nuclei which resulted from division of the post-meiotic nuclei within the basidium, and that Figs. 26, 28, 29 and 30 represent progressively later stages in the same anomalous sequence of events. Fig. 30a indicates that the sterigmata appear to develop normally, but the rounded condition of the nuclei, together with their position near the apex of the basidium is incompatible with normal nuclear migration. It is therefore concluded that when the third nuclear division takes place in the basidium, the daughter nuclei fail to migrate to the basidiospores. The absence of anucleate basidiospores from the basidiospore print substantiates the view that basidia in which the above events take place, do not produce basidiospores.

(b) Paxillus involutus and Hygrophoropsis aurantiaca.

The sequence of post-meiotic events exhibited in the above two species is similar in all major respects, to that/

that observed in G. rutilus.

Observations of the basidia of P. involutus indicate that in this species, the nucleolus is extruded from the migrating nucleus as the latter streams into the basidiospore (Fig. 33). The basidia and basidiospores of H. aurantiaca are smaller than those of P. involutus and consequently, one cannot state that the same nucleolar behaviour takes place. The observation of a common sequence of post-meiotic events provides good a priori reasons for suggesting that the post-meiotic nucleoli of this species will behave as do those of G. rutilus.

As the third nuclear division in the basidiospore proceeds (Figs. 34 and 37), the daughter nuclear product distal to the sterigma moves to an apical position in the basidiospore (Figs. 35 and 38) and remains in this position throughout the subsequent development of the latter. The sister nuclear product migrates retrogressively through the sterigma to the basidium (Fig. 39).

The mature basidium of both species thus bears four uninucleate basidiospores and contains four nuclei (Figs. 36 and 40) which later become closely appressed to the wall of the basidiospore by the development of a large vacuole. The staining characteristics of these nuclei indicate that they are degenerating.

(c)/

(c) Hygrophorus nigrescens.

The post-meiotic nuclear events in this species are essentially similar to those observed in G. rutilus, and are illustrated by Fig. 41, a - e.

This species is a difficult cytological subject because the cytoplasm absorbs a relatively large amount of the eosin component of Giemsa stain, which cannot be removed by the regressive differentiating methods used. The nuclei are sufficiently stained to permit the study of their post-meiotic behaviour. Additional information regarding the behaviour of nucleoli and the possible presence of fibrils could not be discerned.

(d) Russula claroflava, R. emetica and Lactarius rufus.

All three species conform to Pattern C. The post-meiotic events taking place in both species of Russula are illustrated in Figs. 42 - 46. Observations reveal that during the third nuclear division, the daughter nucleus distal to the sterigma moves to the apex of the basidiospore, while the proximal one returns to the basidium. A large vacuole develops within the basidiospore causing the nucleus remaining there to be closely appressed to the wall.

(e) Polyporus brumalis and Panellus mitis.

The nuclei of the above species are stained intensely by the acid-Giemsa method. It has therefore been relatively easy/

easy to determine that Pattern C takes place in both species despite the fact that the basidia and basidiospores are extremely small. This latter feature of the species has made it impossible to obtain information concerning the behaviour of the nucleoli, and to determine whether or not fibrils are involved in the migration processes.

A close scrutiny of the metaphase stage of the third nuclear division indicates that the chromatin appears to be bipartite, a feature also exhibited by the nucleus of G. rutilus. The position of the developing spindle is also visible on occasion. The volume of the basidiospore increases noticeably during metaphase of the third nuclear division. As anaphase of this division proceeds, the daughter product distal to the sterigma moves to the apex of the basidiospore, while the sister product returns to the basidium. The basidiospore nucleus later moves to a final median position. The essential features of the post-meiotic events of P. brumalis are illustrated in Figs. 47 - 50.

(f) Auriscalpium vulgare.

A. vulgare, a member of the Hydnaceae, has the smallest basidia and basidiospores of all 27 species examined. The nuclei stain intensely by the acid-Giemsa method.

Following meiosis I and II, the four post-meiotic nuclei/

nuclei move to the centre of the basidium and enter interphase. They now become very closely associated so that, at first sight, the basidium appears to be uninucleate. Detailed observation reveals the presence of chromosomes and a nucleolus in the fusion nucleus (Fig. 51). The associated post-meiotic nuclei cannot however be confused with the parent fusion nucleus, for although also heterochromatic, individual chromosomes and nucleoli are not apparent (Fig. 52).

The sterigmata and basidiospores develop towards the end of interphase. The post-meiotic nuclei now migrate towards the sterigmata, and become densely staining as they stream in to the basidiospores. The chromatin quickly contracts within the basidiospores and the third nuclear division proceeds. All stages of division in the basidiospore have been observed (Fig. 53) other than retrogressive migration to the basidium of the daughter product proximal to the sterigma. The final distribution of the daughter nuclear products, i.e. four daughter nuclei within the basidiospores, and four daughter nuclei within the ageing basidium has also been observed (Fig. 54). This observation, together with the observation of a single nucleus in the mature, naturally shed basidiospore is proof that retrogressive migration of daughter nuclei takes place following the third nuclear division, and that Pattern C

is operative in this species.

Pattern D.

1. Tetrasporic species.

(a) Coprinus atramentarius.

The basidiospores of this species appear black in the mass due to the presence of pigment in the wall of the basidiospore which completely prevents observation of the contents. This pigment may be removed before hydrolysis and staining, by placing the slides bearing the basidiospores in a saturated aqueous solution of potassium permanganate overnight. Examination of basidiospores whose wall is bleached in this way and which are later stained, reveals the presence of two nuclei lying side by side in a median position.

The basidia of the Coprini mature centripetally, and consequently, a longitudinal section of the whole lamella provides basidia in all stages of development. The illustrations in Fig. 55 are drawn from such a section.

Meiosis I and II take place in the apex of the basidium. The four post-meiotic nuclei then move towards the centre of the basidium and enter interphase (Fig. 55, a), which must be a relatively prolonged stage as many basidia are observed in this condition. The basidiospores develop on short,/

short, truncated sterigmata. The four post-meiotic nuclei migrate towards the sterigmata (Fig. 55, b), and when they reach the base of the latter, the nuclei become tear-drop in form (Fig. 55, c). The chromatin now passes into the basidiospores where the third nuclear division takes place (Fig. 55, d).

Meanwhile pigmentation of the wall of the basidiospore has rapidly developed. A basidiospore may be observed somewhat infrequently, which is underdeveloped as regards pigmentation. It is then possible to observe the third nuclear division taking place. The basidia become highly vacuolate as the basidiospores mature, and any residual cytoplasm is restricted almost exclusively to the sterigmata (Fig. 55, f).

(b) Galerina paludosa.

The post-meiotic nuclear events in G. paludosa are in all essential respects similar to those of C. atramentarius, and are illustrated by Fig. 56, a - f.

(c) Lepiota lutea.

L. lutea also exhibits a similar pattern of post-meiotic events to that in C. atramentarius, (Fig. 57, a - g). Occasionally basidia of this species bear only three basidiospores. Fig. 57, g illustrates such a basidium which has lost one basidiospore. A post-meiotic nucleus has/

has migrated into each of the remaining two basidiospores. The fourth post-meiotic nucleus remains trapped within the basidium.

An examination of the mature basidiospore print of this species reveals that all basidiospores are binucleate when shed, and furthermore, a large central vacuole develops within each causing the cytoplasm to be restricted to a thin zone round the basidiospore wall. Invariably the nuclei lie in the thin band of cytoplasm, one near the apical germ pore, and the other diametrically opposite, near the apiculus (Fig. 57, e).

It has not been possible to determine whether or not the post-meiotic nucleus left behind in a trisporic basidium divides in synchrony with its sister nuclei which by now are within the basidiospores. However, it is almost certain that this trapped nucleus does not migrate later to a basidiospore, since at no time in this species, or in any other normally tetrasporic species has double migration of two post-meiotic nuclei to a single basidiospore been observed; and furthermore, no tetranucleate basidiospores have been found in the mature basidiospore deposit.

(d) Nolanea papillata and Entoloma porphyrophaeum.

N. papillata and E. porphyrophaeum, like the previous species, have pigment present in the mature basidiospores, but/

but in this instance the density is such that it is not necessary to remove the pigment before staining.

Both species exhibit the same pattern of post-meiotic events as C. atramentarius. The post-meiotic events of N. papillata are illustrated in Fig. 58, a - f. The nucleoli of both species, unlike those of the above three species, are extremely large, the fusion nucleolus being particularly conspicuous (Fig. 58, a).

The four post-meiotic nuclei move in a rounded, interphase condition towards the base of the developing sterigmata and basidiospores (Fig. 58, b). Each nucleus becomes tear-drop in shape as it passes into the sterigma. The post-meiotic nucleus then streams into the basidiospore, where it enters metaphase of the third nuclear division (Fig. 58, c). The nucleolus is left behind near the tip of the sterigma. Pigment is laid down in the wall of the basidiospore as the third nuclear division proceeds (Fig. 58, d). The two daughter nuclei remain within the basidiospore which is now shed (Fig. 58, e and f).

(e). Amanita fulva and Amanita rubescens.

Both species differ from N. papillata and E. porphyrophaeum in that the basidiospores are unpigmented when mature. The species do however exhibit the same pattern of post-meiotic events, which is illustrated in

Fig./

Fig. 59, a-d. A. fulva has large, conspicuous nucleoli (Fig. 59,a), but those of A. rubescens are not at all obvious in Giemsa stained material. It would therefore appear that, from the cytological point of view, A. fulva and A. rubescens have very little in common.

(f) Oudemansiella mucida.

O. mucida is a white spored species which exhibits the same sequence of post-meiotic events, (Fig. 60, a-h), as do the species with pigmented basidiospores mentioned above. This species has further cytological features in common with A. fulva, N. papillata and E. porphyrophaeum. The nucleoli are extremely large and conspicuous, even the nuclei in the mature basidiospores may be seen to possess a nucleolus.

The basidiospores of O. mucida become highly vacuolate as they mature, and the nuclei, embedded in the cytoplasm are closely appressed to the wall of the basidiospore. The basidiospore of this species, and to a lesser extent that of A. fulva, has a high turgor pressure, probably due to a high water potential deficit of the abundant mucilage present. On fixation, the basidiospore collapses in many cases and becomes similar in shape to that of the mature basidiospores of species Entoloma and Nolanea.

The/

The post-meiotic nuclei become tear-drop shaped as they approach the sterigmata. Certain preparations reveal that a small body is located at the apex of the nouelus in close association with the nuclear membrane (Fig. 61). A fibril appears to connect this body to a point within the basidiospore.

Fig. 62 illustrates metaphase of the third nuclear division within the basidiospore. The spindle may be seen transversing the chromatin and terminating in a distinct body at either end. These bodies are clearly the pole determinants or kinetocenters. Observation by means of phase-contrast, indicate that the kinetocenters at each pole are connected to loci on the wall of the basidiospore by fibrils.

(g) Flammulina velutipes.

The post-meiotic sequence of nuclear events in this species conforms basically to that described for the above eight species and is illustrated in Fig. 63, a - f. The four post-emiotic nuclei become tear-drop shaped as they begin their migration to the sterigmata. In this respect, the species bears similarities to Mycena alcalina and Collybia/

Collybia confluens, both of which exhibit Pattern B sequence of post-meiotic events.

Examination of the sectioned lamellae occasionally reveals a basidium containing more than four highly condensed nuclei. This is an additional feature which the species has in common with M. alcalina. These basidia occur in such a low frequency that they cannot be regarded statistically as being a significant abnormality in the post-meiotic behaviour of the species.

This anomalous condition could result from either failure of one or more of the migrating nuclei to pass into the sterigmata and their subsequent division within the basidium, or from a mitotic division following immediately after meiosis. All basidiospores present in the mature deposit are binucleate, which indicates that the anomalous basidia probably do not produce functional basidiospores.

(h) Corticium comedens.

Meiosis takes place in the apex of the basidium of C. comedens (Fig. 64, a and b), after which the four post-meiotic nuclei move to the centre of the basidium. As the large sterigmata and allantoid basidiospores develop, the four post-meiotic nuclei become elongated and move towards the former (Fig. 64, c). The actual passage of the nucleus into the basidiospore has not been observed, which/

which suggests that it occurs very rapidly. The post-meiotic nucleus appears to enter mitotic metaphase very soon after its passage into the basidiospore, (Fig.64,d), and the third nuclear division proceeds quickly, resulting in the mature binucleate condition (Fig. 64,e). The basidiospores are binucleate when discharged (Fig. 64,f).

(i) Tremella foliacea.

T. foliacea is the only member of the Heterobasidiomycetidae which has been investigated by the author. Despite examination of a considerable number of sections of the basidiocarp of T. foliacea, basidia bearing mature basidiospores were not observed.

However, it was ascertained that, following meiosis, each compartment of the cruciate basidium develops an epibasidium. The four epibasidia may or may not grow in synchrony. When each is approximately the same length as the hypobasidium, one of the post-meiotic nuclei passes into it. The migrating nucleus is in an interphase condition and has a conspicuous nucleolus. a physical condition which recalls that of the expanded post-meiotic nuclei of the Agaricales examined.

The sterigma and basidiospore primordium develop as the epibasidium penetrates the gelatinous matrix of the basidiocarp. It was not possible to determine/

determine which develops first, but it is evident that both are well differentiated when the post-meiotic nucleus is positioned in the mid-region of the epibasidium (Fig. 65 a). Passage of the post-meiotic nucleus into the basidiospore was not observed.

The mature, freely shed basidiospores are invariably binucleate (Fig. 65 b). It therefore seems probable that the third nuclear division is initiated prior to or during the passage of the post-meiotic nucleus through the sterigma and that this division takes place within the maturing basidiospore. The third nuclear division has not been observed, due to a lack of basidia bearing basidiospores. On the basis of previous examinations, it appears that this division takes place shortly after the passage of the post-meiotic nucleus into the basidiospore.

2. Bisporic species.

(a) Nolanea cetrata.

N. cetrata is a constantly bisporic species which exhibits a modification of Pattern D. In this case, a pair of post-meiotic nuclei migrate to each basidiospore, where both members divide, producing tetranucleate basidiospores. This species provides excellent material for the study of nucleolar behaviour.

A/

A conjugate division takes place in the hyphal tip without the development of a clamp connection. The spindles of this division may lie parallel to or may overlie each other (Fig. 66). The nucleolus of each nucleus is liberated sometime during division.

Following division, a transverse septum is laid down, cutting off the young basidium which contains a conjugate pair of nuclei. These nuclei enlarge and fuse. The fusion nucleus increases involume and moves towards the apex of the basidium. Fig. 67 illustrates the very large furion nucleolus so charasteristic of this and the related species of Nolanea and Entoloma, and also of Oudemansiella mucida and Amanita fulva.

The chromosomes become arranged on the spindle which lies near the apex of the basidium. Meanwhile the liberated nucleolus drifts away into the lower part of the basidium (Fig. 68). The telophase nuclei of meiosis I quickly synthetize a nucleolus. Thes nucleoli are then released during the second division of meiosis (Fig.69).

The four post-meiotic nuclei move to the centre of the basidium and enter interphase (Fig. 70). The nuclei move in tandem towards the developing sterigmata and basidiospores, and/

and become tear-drop shaped only when they reach the base of the former. The nucleolus always occupies a rear position in the migrating nucleus, a feature shared with other species examined. A fibril may be observed connecting each member of the paired nuclei to a point within the sterigma.

The leading member of each pair of post-meiotic nuclei streams into the basidiospore. Its nucleolus becomes disassociated from the chromatin at this point, and falls back towards the base of the sterigma, allowing the second post-meiotic nucleus to pass into the basidiospore (Fig. 71). The nucleolus of this second nucleus is also left behind in the sterigma. Fig. 72 illustrates a sterigma containing a pair of redundant nucleoli. When migration of the post-meiotic nuclei is complete, the basidium bears two binucleate basidiospores. These nuclei now complete a third nuclear division (Fig. 73) without the liberation of nucleoli, since these have already been dispensed with early in migration and now lie within the sterigmata. This situation contrasts with that during the final conjugate nuclear division when the nucleoli lie adjacent to the dividing nuclei (Fig. 66). The nucleoli drift away from the sterigmata into the basidium, as the third nuclear division proceeds in the basidiospores. The mature basidium contains four residual nucleoli (Fig. 72) and bears two tetranucleate basidiospores/

basidiospores (Fig. 74).

Dual pattern behaviour.

(a) Mycena swartzii.

M. swartzii was unique in that it exhibited two of the four patterns of post-meiotic events. This species also had the additional feature that a small percentage (less than 5%) of the basidia were either bisporic or trisporic. The freely shed basidiospores were exclusively uninucleate.

Examination of the sectioned lamellae indicates that in 56% of the tetrasporic basidia, the four post-meiotic nuclei divide shortly after interphase. These basidia exhibit Pattern A behaviour, since the division takes place within the basidium. All four nuclei divide in synchrony and eight highly condensed telophase nuclei are produced. Fig. 75 illustrates a basidium in this condition. Four, only of these nuclei now migrate to the basidiospores, whilst the other four nuclei remain in the basidium (Fig. 76). The absence of binucleate basidiospores from the basidiospore deposit is proof that double migration of pairs of nuclei to the basidiospores does not take place.

In the remaining 44% of the tetrasporic basidia, the third nuclear division is delayed and the four post-meiotic nuclei migrate to the basidiospores (Fig. 77), where they divide in synchrony (Fig. 78). The daughter nuclear product/

product distal to the basidium remains in the basidiospore, while the proximal one migrates retrogressively into the basidium (Fig. 79). Again, the absence of binucleate basidiospores from the mature basidiospore print confirms the interpretation that Fig. 79 illustrates retrogressive migration from the basidiospore and not double migration to the basidiospore. Thus in the above basidia, it is Pattern C which is operative.

In both types of basidia, the end result of the third nuclear division is the same, namely the production of four uninucleate basidiospore and the presence of four degenerating nuclei in the ageing basidium (Fig. 80). The means by which the end results are brought about do however, vary.

In bi- and tri-sporic basidia, the third nuclear division is delayed until two of the post-meiotic nuclei in the first instance, and three in the second instance, migrate to the basidiospores. These nuclei then divide in the basidiospores while the redundant nuclei or nucleus, as the case may be, divide in the basidium (Fig. 81, a and b).

There is no evidence to suggest that in this species double migration of pairs of post-meiotic nuclei to basidiospores takes place when less than four basidiospores are produced by the basidium. Bi-and tri-sporic basidia exhibit a modification of Pattern C.

It/

It is interesting to note that even when one or two of the post-meiotic nuclei are redundant, they nevertheless divide in synchrony with the nuclei in the basidiospores. This fact suggests that the genetic factors determining the timing of the third nuclear division, and consequently the site where it will take place, are determined by the fusion nucleus and not by the individual post-meiotic nuclei.

DISCUSSION

(1) Post-meiotic events - Critical assessment of past accounts

The basidium has been regarded by many cytologists as a functionless entity from the stage at which the post-meiotic nuclei migrate to the basidiospores. The latter have then been looked upon as independent cells. Insufficient attention has therefore been paid to the possibility that further nuclear events involving the basidium may take place.

Basidiospores are all too readily detached from the parent basidium during the preparation of sectioned lamellae. The more mature the basidiospore is, the more easily it is lost. It follows that most basidiospores remaining attached to basidia are at an early stage of development. Thus the sequence of nuclear events observed up to that point in the development of the basidia and basidiospores cannot be regarded as the final state of development. The investigator is not justified in presuming that what is true of the immature basidiospores is equally true of the mature ones. Nowhere is this more clearly seen than in those species which behave according to Pattern C.

Rosenvinge (1886) for instance, examined two species of Boletus and claimed that the developing basidiospore

was/

was binucleate. Later Maire (1902) examined four Hygrocybe spp., two Lactarius spp., four Boletus spp., Hygrophoropsis aurantiaca, Russula lepida and Gomphidius glutinosus, and showed that in 11 of them, the migrating nucleus divided in the basidiospore. He was unable to decide whether or not a similar division took place in R. lepida because of the oil present. Furthermore, he observed that the pigmented and hence mature, basidiospore of G. glutinosus was uninucleate. If both these authorities had examined naturally shed basidiospores form their material they would have realised, as did Duncan (1970) with regard to the boleti, that further nuclear events were yet to take place in many species in which the basidium bore binucleate basidiospores. They would have realised that three theoretical possibilites existed, (1), degeneration of one of the two nuclei present in the basidiospore, or (2), fusion of the two nuclei within the basidiospore, resulting in an anucleate basidium and a diploid life-cycle, or (3), retrogressive migration of one of the basidiospore nuclei, resulting in the basidium containing four degenerating nuclei and a haploid life-cycle. There is no evidence to support the first two possibilities, but Duncan's observations on the boleti and the results presented above do support the interpretation that the third possibility is the rule in the genera Boletus, Russula, Lactarius, Gomphidius, Paxillus, Hygrophoropsis, Hygrophorus,/

Hygrophorus, Panellus, Polyporus and Auriscalpium, and may well apply to other genera yet to be investigated.

Levine (1913), like Rosenvinge and Maire, failed to ascertain the nuclear complement of the mature basidiospores during his study of the boleti. He observed division of the migrating nucleus on the developing basidiospore, and it does appear that he actually observed retrogressive migration (Levine; Pl.7, Fig. 63) in Boletus albellus but failed to interpret his observations as such.

It will be recalled that Bauch (1926) pointed out the presence of two nuclei in what he called the 'mature' basidiospore of Camarophyllus virgineus, and also observed at times, degenerate, 'kollabiert' basidia in an otherwise healthy hymenium. His remarks regarding the staining potential of these basidia and their contents, clearly indicate that he was observing aged and degenerating basidia which had shed their uninucleate basidiospores, but which contained four daughter nuclei which had returned from the latter. If he had examined naturally shed basidiospores of Camarophyllus he would have realised the significance of the 'kollabiert' basidia, and not assumed, as he did, that they were basidia which had failed to produce basidiospores. Bose's (1937) cytolgical examination of members of the Polyporaceae must also be regarded as incomplete for the same/

same reasons.

Ritchie (1941) unquestioningly accepted that the 'classical pattern' of post-meiotic events took place in Russula emetica, and as a result misinterpreted his observations. He featured basidia (Ritchie; Figs. 31, 36) which he claimed, were both in the same stage of development, but owed their differing physical aspect to the different fixatives which he employed. While the latter claim may be correct to some extent, it is evident that a more important factor, that of age, is responsible for their widely differing appearances. Fig. 31 is more likely to be that of an aged, degenerating basidium while Fig. 36 is of an immature developing one. A point in favour of this assertion is the shape of the tips of the sterigmata. The young sterigmata always develop as obtuse humps which later swell out to form the basidiospores, as in Fig. 36. The sterigmata of aged basidia which have shed their basidiospores have acute tips, as in Fig. 31. Similarly, Ritchie's Figs. 30 and 37 are not comparable. The basidium in Fig. 30 has shed its basidiospores, while that in Fig. 37 is in the process of developing them. Likewise, Figs. 33 and 34 cannot be accepted as being of the same stage of development. The basidiospore illustrated in Fig. 34 is an almost mature one in which the sculptured wall has developed, but/

but that shown in Fig. 33 is a highly immature basidiospore which has not synthetized the sculptured wall. In this latter case, the nucleus present is the post-meiotic nucleus migrating to the basidiospore, while that illustrated in Fig. 34, is a nucleus of the next generation on its way back to the basidium. Ritchie failed to note the sister nucleus destined to remain within the basidiospore, near its apex. This nucleus is closely appressed to the wall of the basidiospore as a result of increase in volume of the basidiospore vacuole. Misinterpretation of the facts, together with a disregard of Strasburger's (1884) earlier remark regarding the binucleate condition of the basidiospore of R. rubra, almost certainly led Ritchie to make the erroneous statement previously quoted (Introduction, p.19).

Kühner (1945a), more than any other mycologist, recognised the significance of the third nuclear division observed in the basidiospore by several authorities. He cannot escape criticism however. He paid much attention to the nuclear complement of both the mature basidiospore and the aged basidium. In doing so, he came to the conclusion that in the great majority of the Basidiomycetes in which the basidiospores are white at maturity, a post-meiotic mitosis took place either at the base of the sterigmata or within the latter. He failed to grasp the significance/

significance of the division in the basidiospores which he sometimes observed in species which normally had uninucleate basidiospores, and suggested that it was brought about by a delay in the timing of mitosis. But such an interpretation demands the presence of both uninucleate and binucleate basidiospores in a mature basidiospore print. Kühner's oversight led him to group together species which conform to the Patterns B and C.

In a later paper, Kühner (1947) described division in the sterigmata or basidiospores of the post-meiotic nuclei of Sistotrema confluens, and subsequent retrogressive migration of one of each pair of daughter nuclei to either the basidium or a basidiospore which, as yet, had not received a nucleus. Knowledge of the overall situation regarding the post-meiotic events in the Basidiomycetes would have been considerably advanced if Kühner had correlated the above finding with his previous observations of Hygrophorus, and with the earlier reports of Maire (1902) and Bauch (1926) concerning the related genera Hygrocybe and Camarophyllus.

A great many of Kühner's earlier cytological investigations were centered on the genus Mycena which would appear, in the tetrasporic forms at least, to conform to Pattern B (Kühner, 1938). The realisation that a third nuclear division/

division took place in the sterigmata in members of this genus may well have prejudiced his interpretation of later observations in other genera.

The essential features of Pattern D, namely division of the migrating nucleus in the basidiospore and the absence of nuclei from the degenerating basidium, were also pointed out by Kühner (1945a). He claimed that the features of this pattern were true of the majority of chromosporic species, but in making this expansive statement, he did not, unfortunately, define the limits of his chromosporic group, nor for that matter those of the leucosporic group. His statement, in the light of the author's results requires qualifying. Gomphidius, Paxillus and Boletus all have pigmented basidiospores, but the species of these genera so far investigated all conform to Pattern C, and have uninucleate basidiospores at maturity.

Kühner's claims certainly appear to be true for the Coprini and related genera which have been examined by Wager (1892), Nichols (1904), Kniep (1928), Vokes (1931), Sass (1933), Colson (1935), and Hagerup (1945). The above writers all reported division of the post-meiotic nuclei in the the developing basidiospores, but all assumed that no subsequent development of these nuclei took place until after basidiospore discharge. The coincidence of pigmentation/

pigmentation of the basidiospore wall with later stages of division, was for them an *a priori* reason for making such an assumption. But all conclusions should be founded on established facts, and in this case, the facts can best be obtained from the mature basidiospore print.

The author's recorded observations of the sectioned lamellae and mature basidiospore print of *Coprinus atramentarius*, *Lepiota lutea* and *Galerina paludosa* not only confirm Vokes's (1931) rather incomplete report of post-meiotic events in the first species, but lend weight to the claims made by the six writers referred to above.

Nolanea papillata and *Entoloma porphyrophaeum* both have pigmented basidiospores and conform to Pattern D. The leucosporic species *Amanita rubescens*, *A. fulva* and *Oudemansiella mucida* share this and other cytological features with the first two species. While Kühner (1945a) was aware that the mature basidiospore of *Amanita* was binucleate, he implied that this was an exceptional condition in the leucosporic Agaricales. However this is not entirely true. Rosenvinge (1886) had previously indicated that division of the post-meiotic nuclei took place in the basidiospores of *Collybia velutipes*. The author's recorded observations of the sectioned lamellae and mature basidiospores of this species (now *Flammulina velutipes*) confirm Rosenvinge's claims/

claims but also establish that this species conforms to Pattern D. A similar behaviour has also been established for Corticium comedens, which previously had been investigated by Maire (1902). Maire claimed that the post-meiotic nucleus divided in the basidiospore only when the latter had been discharged, a claim which fits the 'classical pattern'. The results of the present reinvestigation cast doubts on Maire's claim and indicate that this is a further leucosporic agaric which conforms to Pattern D.

It would appear that in all Basidiomycetes in which the basidium contains four post-meiotic nuclei at the time of production of basidiospores, a third nuclear division is initiated within the basidium prior to nuclear migration. In this way, the migrating nuclei appear to overcome the spatial restrictions imposed by the narrow tips of the sterigmata. Indeed, it may be that this phenomenon of a third nuclear division is dictated by the presence of the constricted tips of the sterigmata and may be of universal occurrence in the Basidiomycetes.

Von Istvanffi (1895) illustrated his observations on the developing epibasidium and basidiospore of Tremella lutescens. This illustration (von Istvanffi; Pl. 37, Fig.35) conveys the impression that the post-meiotic nucleus constantly occupies the tip of the growing epibasidium. The author's/

author's observations of the related species T. foliacea establishes that the sterigma and basidiospore are well differentiated at the apex of the epibasidium while the post-meiotic nucleus is in the course of migrating through the latter. The naturally shed basidiospore of this species is binucleate, indicating that a division of this post-meiotic nucleus takes place within the basidiospore, and that this species therefore conforms to Pattern D. Von Istvanffi's illustrations thus convey a misleading impression of the post-meiotic events in T. lutescens.

The timing of sterigma and basidiospore development relative to that of nuclear migration has been shown by Whelden (1934) to be similar in T. mesenterica, T. frondosa, and T. grilletii to that of T. foliacea. Whelden observed that the migrating post-meiotic nucleus of all three species became extremely elongated as it moved towards the sterigma, and he concluded that it did so in order to pass through the sterigma. He stated that no nuclear division took place during the formation of the basidiospores. Examination of highly immature basidiospores may have led to a misconception on Whelden's part of the true sequence of post-meiotic events in the above species. He did not examine mature, naturally shed basidiospores in order to substantiate his claim. He would possibly have revised his/

his ideas on the nuclear behaviour during the development of the basidiospores if such an examination had been made.

Pattern D also operates in a modified form in the bisporic species Nolanea cetrata. In this species, pairs of post-meiotic nuclei migrate in tandem to the basidiospores and then divide, resulting in tetranucleate basidiospores. These observations conflict with the earlier report by Buhr (1932) on the same species. He suggests (Buhr; Fig. 32) that only two of the post-meiotic nuclei migrate to the basidiospores while the other two remain behind in the basidium. It is possible that Buhr observed only the early stage of basidiospore development and failed to see the later stages because of a lack of basidia retaining mature basidiospores. If he had examined the mature, naturally shed basidiospores, he would have realised that further nuclear events were yet to take place.

The author's observation of double migration of the post-meiotic nuclei to the basidiospores and their subsequent division within the latter, establishes that N. cetrata exhibits the same sequence of post-meiotic events as that reported for the bisporic forms of Psalliota campestris (Maire, 1902; Sass, 1929; Colson, 1935; Sarazin, 1938; Evans, 1959), Coprinus ephemerus, Naucoria semiorbicularis, Galera tenera (Sass, 1929), Mycena spp (Smith, 1934, members of the Mycena metata group),/

group) and Aleurodiscus canadensis (Skolko,1944). Only Amanita bisporigera (Lewis, 1906) at first sight, appears to be exceptional in this respect, but a close examination of the report of the post-meiotic events in this species indicates that it is incomplete and that a reinvestigation of the species is highly desirable.

Migration of two nuclei to a single basidiospore could be theoretically possible in the basidial development of Pellicularia otogensis and Cantharellus cibarius, both of which conform to Pattern A. Both species produce more than four but less than eight basidiospores. The present results indicate that double migration does not happen in these species, and that any nuclei in excess of the number of basidiospores formed, simply remain in the basidium and degenerate.

Maire (1902) claimed that in Cantharellus, a second generation of basidiospores could be produced utilising the extra nuclei present. No single authority has since been able to substantiate this claim which must, in the circumstances be regarded with considerable suspicion.

There is clear indication that double migration of nuclei/

nuclei to the basidiospores can only occur in dikaryotic bisporic species exhibiting Pattern D sequence of post-meiotic events, and involves the post-meiotic nuclei exclusively and not the nuclei of a later generation.

Mycena swartzii was unique in its post-meiotic behaviour in that it alone exhibited two pasterns, Pattern A and Pattern C. The only report of a similar phenomenon is the much quoted study by Sequiera (1954) of Omphalia flavida. The genus Omphalia has, in the past, been regarded by taxonomists as overlapping the genus Mycena. This fact, coupled with the knowledge that M. swartzii is referred to by some authorities as O. swartzii (Lange, 1936) renders valid a provisional comparison of the cytology of the two species.

Sequiera described three nuclear cycles which he thought were operative in O. flavida (Introduction,p.22). These cycles may be summarised briefly as follows:-

(a) Typical, involving a post-meiotic division in the basidium followed by single migration of four nuclei to the basidiospores;

(b) Atypical-A, involving a post-meiotic division in the basidium, followed by double migration of the eight nuclei to the basidiospores,/

basidiospores, resulting in binucleate basidiospores;

(c) Atypical-B, involving a post-meiotic division in the basidiospores, resulting in binucleate basidiospores.

It has already been pointed out in the Introduction (p. 22) that a basidiocarp of O. flavida which exhibited all three patterns of post-meiotic nuclear events would shed a mixture of uninucleate and binucleate basidiospores, and furthermore, their frequencies would reflect the sites of the post-meiotic mitosis.

However, Sequiera does not state the nuclear complement of the mature, naturally shed basidiospores, and in the absence of this information, one cannot accept without question, his interpretation of his observations. It is possible that O. flavida, like M. swartzii, invariably produces uninucleate basidiospores, in which case only two patterns of post-meiotic nuclear behaviour would be exhibited by O. flavida as has been demonstrated in M. swartzii. The concept, described by Sequiera, of the 'typical' cycle would still be valid, but the 'atypical-A' and 'atypical-B' cycles would encompass different stages in the single cycle of migration of the post-meiotic nuclei to the basidiospores, their division in the basidiospores, followed by retrogressive migration/

migration to the basidium of the daughter nuclei proximal to the sterigmata.

Sequiera claims that Fig. 18 illustrates double migration to the basidiospore. There is, however, strong evidence against this interpretation. In the first place, it has been pointed out (p. 90) that in all valid reports of double migration, it is only the post-meiotic nuclei which are capable of undergoing double migration, and not the daughter nuclei of the next generation. Secondly, the migrating nucleus streams into the basidiospore in an interphase condition as in G. rutilus, and not as a highly condensed, solid mass of chromatin. Finally, if Sequiera's Fig. 18 is compared with Fig. 79 illustrating M. swartzii, one is struck by the astonishing degree of similarity between them. It seems distinctly possible that Sequiera has misinterpreted his observations and that his Figs. 19, 18, and 16 in that order, illustrate division in the basidiospore of the post-meiotic nucleus, retrogressive migration, and the final distribution of the daughter nuclei. This issue, however, can only be resolved by a determination of the nuclear complement of the naturally shed basidiospores of O. flavida.

The post-meiotic nuclear events observed in M. swartzii also cast doubt on Smith's (1934) interpretation of his findings/

findings in the tetrasporic species M. murina. He claimed that in this species, the four post-meiotic nuclei divide in the sterigmata and the resultant daughter nuclei move in pairs into the basidiospores, producing, he supposed, binucleate basidiospores. The absence of concrete information regarding the nuclear complement of the naturally shed basidiospore prevents a total acceptance of Smith's claims. It is possible that Smith was observing retrogressive migration from the basidiospore, and not double migration to the basidiospore.

While one may or may not agree with a given authority's interpretation of his observations, one must accept two inescapable facts which emerge from all valid reports on the cytology of the developing basidium and basidiospores. Firstly, the evidence reveals that in approximately 100 Basidiomycetes, which range over all taxonomic groups, a third nuclear division takes place either before or during development of the basidiospores. Secondly, there is no valid report of any member of the Basidiomycetes exhibiting the 'classical pattern' of post-meiotic events which has become accepted by mycologists as the unique feature of the group, and which has been constantly purveyed by the authors of general and advanced mycological texts during the last four decades.

The/

The only possible conclusion which can be reached is that the 'classical pattern' of post-meiotic nuclear behaviour in the basidium and basidiospores, involving as it does but two divisions only, namely meiosis I and II, is a totally invalid concept which has been handed down without question to generations of mycologists since the beginning of the century.

(2) Post-meiotic events - Nuclear traction mechanisms.

Wager (1893) was the first authority to comment on the meiotic apparatus of the Basidiomycetes. He observed the spindle in Agaricus stercorarius and claimed that a minute granule was present at each pole, occupying a position similar to that of the centrosomes of higher animals. The apparent absence of astral rays made him hesitate to define these granules as centrosomes, but in his view, they clearly functioned as centrosomes. Wager's opinions were later endorsed by Juel (1897) in his study of meiosis in the basidium of Armillaria mellea.

Maire (1900) in his study of Hypholoma appendiculatum also indicated that each pole of the meiotic spindle was occupied by a body which he called the centrosome. He suggested that in this species, the four centrosomes present at the terminations of the meiotic II spindles became associated with the wall of the basidium and dictated the sites at which the sterigmata would later develop. During the post-meiotic interphase, longitudinal filaments developed from the centrosomes and connected the latter to the post-meiotic nuclei. The post-meiotic nuclei were then drawn by contraction of the fibrils through the sterigmata into the basidiospores where they joined the centrosomes.

Later Maire (1902) suggested that a similar mechanism operated/

operated in other species. Clearly he was uncertain of the origin of the fibrils which connected the centrosomes to nuclei. He stated that in Psathyrella disseminata, the fibrils grew from the centrosomes to the nuclei, that is, they had a de novo origin; but he suggested that in Boletus regius, they were formed by utilisation of the astral rays, that is, they were modifications of pre-existing structures.

Ruhland (1901) was extremely sceptical about previous claims regarding the presence of centrosomes and astral rays in the Basidiomycetes. In his study of H. appendiculatum he admitted that in some cases there was a small swelling at each pole of the spindle, from which radiated a few very short, straight fibrils. But he pointed out that in many other cases, such fibrils were totally absent, and furthermore, the extremely minute size of the swelling, the absence of a hyaline zone around it, and the lack of a corresponding formation closely associated with the interphase nucleus, all argued against the idea of these swellings being centrosomes in the sense originally conveyed by Boveri (1888).

Petri (1902) in his study of Hydnangium carneum claimed that a granule was present at the apex of the sterigma and a fibril connected it to the post-meiotic nucleus. In his opinion, the fibril was part of the nuclear/

nuclear membrane which had been drawn out to form a communication tube connecting the granule directly to the nucleus.

In his study of Hypochnus subtilis, Harper (1902) claimed that fibrils extended from the post-meiotic nucleus up through the sterigma, but he was unable to determine their origin. Van Bambeke (1903) reported that fibrils connected the centrosomes at the apex of each sterigmata to the post-meiotic nuclei in the basidium of Hydnangium carneum, but unlike Petri, he made no claims as to their identity. Fries (1911) described a spindle with centrosomes and astral rays in the basidium of Nidularia pisiformis, and in addition claimed that small granules could be seen on the basidial wall where the sterigmata develop and later at the apices of the latter. He did not identify the granules as centrosomes as did Maire, nor did he comment on the form or function of the fibrils which he observed extending from the base of each sterigmata to the post-meiotic nuclei.

Levine (1913) in reference to the boleti claimed that at both meiosis I and II, the poles of the spindles terminated in small centrosomes from which long, streaming rays extended to the centre of the basidium, a feature previously reported by Maire (1902) in Boletus regius. Levine also

suggested/

suggested that the astral rays of the spindles were attached to the wall of the basidium and separation of the chromosomes was achieved by the contraction of the rays. He also claimed that granules were attached to the basidial wall. The post-meiotic nuclei remained connected to these granules by strands during their migration to the centre of the basidium and throughout the ensuing interphase. In his view the strands were possibly analagous to the astral rays. Levine was uncertain as to the identity of the granules, but suggested that they might be the centrosomes which had become fixed to the wall of the basidium during meiosis II. He agreed with Maire that the position of the centrosomes determined the site of sterigmata production. Moreover, he claimed that the centrosomes maintained their position at the apex of growth of the sterigmata and basidiospores, and that the post-meiotic nuclei were drawn into the developing basidiospores by contraction of the astral ray fibrils.

The nucleus, on entering the basidiospore underwent a third mitotic division. Levine reported that a very narrow spindle could be seen at metaphase and astral rays radiated from the centrosomes at each end of this spindle. He claimed that the nuclear membrane was still present and intact at this stage. Although he observed separation of the daughter nuclear products, he failed to observe

retrogressive/

retrogressive migration of one daughter product.

Vokes (1931) in her study of nuclear migration in Coprinus atramentarius introduced some novel concepts. In her view, the fusion nucleus migrated to the apex of the basidium where it made temporary contact with the basidial wall and in doing so, deposited four tiny hyaline bodies at four points. She claimed that as the fusion nucleus withdrew from the wall, it drew out from the four bodies fine fibrils, which remained attached to the fusion nucleus and later to its four daughter meiotic products. She pointed out that the four hyaline bodies were distinct from the centrosomes of the meiotic spindles, and she was most emphatic that they were laid down by the fusion nucleus some time before meiosis.

In Voke's opinion, the sterigmata arose at the sites occupied by the four hyaline bodies, and as the sterigmata grew, the bodies remained at the apex of growth so that ultimately they occupied an apical position on the wall of each basidiospore. Vokes maintained that growth of the sterigmata and basidiospore as opposed to contraction of fibrils was responsible for drawing the nucleus into the enlarging basidiospore.

In the present examination of the listed Basidiomycetes, and of C. atramentarius in particular, the fusion nucleus was/

was not observed to migrate to the apex of the basidium while in an early prophase condition, and subsequently withdraw from this position as suggested by Vokes. Synapsis always takes place in the centre of the basidium, after which the fusion nucleus enlarges considerably and moves towards the apex of the basidium. Fig. 31 illustrates diakinesis in Gomphidius rutilus, and it may be observed that the highly condensed bivalents are some distance away from the position later occupied by the meiosis I spindle. All recorded observations indicate that the fusion nucleus only occupies the ultimate region of the basidium when in metaphase of meiosis I.

Vokes in addition, claimed that meiosis II may occur in members of a diad at different times. Such behaviour has not been observed in any species examined in the present investigation. The distorted shape of many of the basidia illustrated by Vokes (Figs. 28 and 29), the peculiar alignment of the meiosis II spindles (Figs. 31 and 32) and the extreme asynchrony in the development of the basidiospores are atypical of the Homobasidiomycetidae, and may have arisen from the cytological methods which she employed.

Kühner (1927) in his cytological study of the haplo-parthenogenetic form of Mycena galericulata disagreed with previous authors and claimed that the centrosomes always remained/

remained in contact with the nucleus. In his view, the centrosomes had therefore no influence on the growth of the sterigmata, since each occupied a position on the surface of the nucleus and was responsible for dragging the migrating nuclei towards the sterigmata.

Lander (1935) also claimed that the centrosomes of Pisolithus tinctorius were more closely associated with the post-meiotic nuclei than with the wall of the basidium. She pointed out that a body, suggestive of a centrosome was visible near each post-meiotic nucleus and that strands radiated from each centrosome to each post-meiotic nucleus, which then became stretched towards its centrosome. The centrosomes moved towards the sterigmata dragging the nuclei behind them.

Neither Bose (1937) nor Ritchie (1941) observed granules or centrosomes at the apex of the developing basidiospores in members of the Polyporaceae or in Russula emetica respectively. Bose observed fibrils running from the post-meiotic nuclei to the sterigmata in Polyporus brumalis only, while Ritchie regarded the darkly staining strands occasionally observed running from the post-meiotic nuclei to the apex of the basidium as artifacts arising from fixation.

Wilson and Aist (1967) made a phase-contrast study of the nucleus in the living hypha of Fomes annosus. They claimed/

claimed that a centriole was always associated with the somatic nucleus, and played an active role in moving the nucleus through the cytoplasm, but did not consider the role of the centriole in division. Wilson, Miller and Griffin (1967) studied the basidial cytology of the same species by means of the light microscope. They maintained that the centrioles functioned in aligning the nuclei prior to fusion, in establishing the poles of the meiotic spindles and in moving the post-meiotic nuclei through the sterigmata into the basidiospores.

Lu (1964), in a light microscope study of Cyathus stercoreus claimed that a centriole was always associated with the somatic nucleus, even when the latter migrated through the cytoplasm during mitotic interphase. He also maintained that at the onset of nuclear division in the vegetative hyphae, the centriole of the nucleus destined to divide in the clamp, moved into the latter and drew the nucleus behind it. In Lu's opinion, the centriole was extranuclear, but inseparably connected to the nucleus by an unknown mechanism.

Lu (1967a) later carried out a combined light and electron microscope study of meiosis in Coprinus lagopus and frequently observed a centrosome contained in an invagination of the nuclear membrane during prophase. The ultrastructure of/

of the centrosome was obscure. Although it generally appeared to consist of fibrils, no structural pattern was evident. It was suggested that it may possess a central dense core, surrounded by a more diffuse periphery.

Motta (1969) observed by means of the electron microscope that prior to or concomitant with impending nuclear division in somatic cells, uniformly dense bodies, which he designated centrosomes, appeared in the cytoplasm adjacent to, but not in contact with the nuclear membrane. These were not to be detected in the vicinity of the interphase nuclei. As division proceeded, the spindle became organised between the centrosomes, and following telophase, resynthesis of the nuclear membrane began at the poles of the spindle, in close association with the centrosomes. The centrosome occupied a position deep in an invagination of the nuclear membrane, at the completion of membrane formation. Subsequent expansion of the nucleoplasm brought about a lateral displacement of the centrosome, which simultaneously became modified to a plaque-like structure, appressed to the nuclear membrane. Motta did not comment on the fate or function of the centrosomes during the ensuing interphase, nor did he express any opinions as to whether or not the centrosomes exhibited a continuity throughout subsequent mitotic divisions. In contrast, he stated that he had observed/

observed persistent plaque-like centrosomes in the recently divided meiotic nuclei of A. mellea.

The above electron microscope observations revealed that on the basis of their fine structure, the organelles terminating the spindles of A. mellea and C. lagopus could not be centrioles or centrosomes in the strict sense of these terms. Boveri (1888) originally introduced the term 'centrosome' to describe the hyaline zone which apparently terminated the spindle of the dividing nucleus of Ascaris. He observed a minute granule which he defined as the centriole, within the centrosome. Ultrastructural electron microscope studies have revealed that the spindle terminations of Ascaris, animals in general, and flagellate gametes, exhibit a universal structure. The centriole is always a cylindrical body, consisting of a ring, made up of groups of microtubules, three microtubules being present in each group. Mitochondria, ribosomes and other cytoplasmic inclusions are completely absent from the surrounding centrosome, which suggests that the latter is totally different in structure from cytoplasm. Boveri established that the centrioles of Ascaris replicated at metaphase or anaphase, and that the daughter centrioles, each within a centrosome, moved to opposite extremities of the nucleus towards the end of the ensuing prophase. The microtubules

of/

of the spindle converged towards each daughter centriole as did the dividing chromosomes. Thus the centrioles of animal cells and of flagellate gametes function as kinetocenters (Grundmann, 1966). The term kinetocenter, as used by Grundmann refers to any meiotic or mitotic pole determinant, no matter what its structure, and therefore includes centrioles, centrosomes or any other likewise functioning organelle. In view of the controversial application of certain specialised terms to the pole determinant of the higher fungi, the term kinetocenter will henceforth be adopted to denote the pole determinant in fungi regardless of its ultrastructure.

Many observations have revealed that astral rays, having their origin in kinetocenters radiated far into the cytoplasm of dividing cells. Ultrastructural studies have shown that the astral rays are composed of microtubules (Cohen and Rebhun, 1970). Other studies have shown that secondary microtubules connect the kinetocenters to a localised region of the chromosome termed the kinetochore or centromere (Hughes-Schrader and Ris, 1941). Separation of the daughter chromatids is achieved by division and separation of the kinetochores.

Girbardt (1968) showed in an ultrastructural study, that the moving nucleus of Polystictus versicolor possessed

a/

a kinetocenter or 'activity centre'. The activity centre was located near the periphery of the nucleus, associated with the nuclear membrane, but lacked the typical structure of a centriole. In Girbardt's view, the activity centre corresponded to a kinetochore (= centromere) rather than a centriole and he therefore referred to it as a 'kinetochore equivalent' or 'KCE'. The KCE appeared to be a persistent organelle consisting of two globular poles connected by a dense plate-like middle part. Prior to metaphase, the nuclear membrane disintegrated and the KCE entered the condensing chromatin. The dense middle part was no longer visible, but instead a bundle of growing microtubules seemed to push the globular poles apart. In this way, the Zentralstrang (spindle) was formed. Girbardt expressed the opinion that the nucleus of P. versicolor may consist of a single chromosome which started to divide by the separation of the globular poles to which it was connected. The globular poles could, in this light be regarded as being homologous to the kinetochores (centromeres) present in the chromosomes of higher organisms. It would appear that Girbardt's preference for the term 'kinetochore equivalent' stemmed from such considerations.

Lerbs and Thielke (1969) described a similarly functioning organelle in Coprinus radiatus as a centiole-

like/

like body. This organelle was very similar in appearance to the KCE of Polystictus versicolor observed by Girbardt. It appeared as a unipolar structure in an invagination of the nuclear membrane at prophase I of meiosis. As division proceeded it became bipolar, consisting of two dense globular ends which were connected by a central band. The globular ends moved apart during division, and the spindle fibrils developed between the globules. Meanwhile other fibrils could be observed radiating out into the peripheral cytoplasm.

Recent light and electron microscope studies of the ascus of Ascomycetes illustrate the presence of nuclear associating organelles which function as kinetocenters, but do not exhibit the same ultrastructure as centrioles. Robinow and Catin (1969) reported that the spindle of Aspergillus nidulans was composed of a bundle of fibrils which traversed the nucleus between two dense plaques associated with the nuclear membrane. Aist (1969) described a small flattened kinetocenter which was sited on or near the nuclear membrane of Ceratocystis fagacearum as a centriolar plaque, while Zickler (1970) termed the kinetocenters of Ascobolus immersus, A. stercorarius, Podospora anserina and P. setosa as centrosomal plaques, and showed that they were amorphous bodies without the structure/

structure of centrioles or centrosomes.

Wells (1970) in a study of A. stercorarius described the kinetocenter as a 'centriole-like body'. In his opinion, it was homologous to the 'disque central d'un aster' previously reported in Pustularia cupularis by Schrantz (1967). This latter study had revealed that prior to ascospore formation, the nuclei moved towards the ascus wall and each developed a beak. Following osmium fixation, the kinetocenter was frequently seen capping the beak, being intimately associated with the outer regions of the nuclear membrane. This behaviour on the part of the ascospore nucleus, recalls the form of the migrating post-meiotic nucleus of many Basidiomycetes and, in particular that of Oudemansiella mucida (Fig. 61). It suggests that in the Basidiomycetes a kinetocenter is also located at the apex of the migrating nucleus, as was previously claimed by Kühner (1927). In the light of modern knowledge, the claims of Maire (1900, 1902) and Levine (1913) regarding the location of the kinetocenter subsequent to meiosis and during the ensuing development of the basidiospores, are no longer tenable.

Investigations of the ascus of a number of Ascomycetes reveals that the size of the kinetocenter increases to a maximum prior to the third nuclear division. In addition, numerous/

numerous astral fibrils are conspicuous at this division. They radiate out far into the cytoplasm in all directions from the kinetocenter. Lu (1967b) alone failed to detect fibrils of any description in his study of the ascus of Gelasinospora calospora. He cooled his material to 0°C following hydrolysis at 70°C. Recent reports indicate that submicroscopic fibrils are destroyed by low temperatures.

The only comparable electron microscope study of a member of the Basidiomycetes, is that carried out by Wells (1965), who examined the developing basidium and basidiospores of Schizophyllum commune. He pointed out the presence of unidentified objects in the basidium and basidiospores, but did not observe kinetocenters or fibrils of any description. It is relevant to point out that Wells did not use gluteraldehyde or osmium tetroxide, fixatives which are known to be required for the preservation of microtubules.

Girbardt (1968) used both fixatives in his examination of Polystictus versicolor. His electron microscope observations revealed the presence of many microtubules, radiating far out into the cytoplasm from the globular poles of the KCE. These microtubules were present throughout the entire mitotic cycle except for a brief period prior to metaphase when the KCE entered the condensing chromatin. Neither this study, nor that of Lerbs and Thielke, nor those/

those on the Ascomycetes, located the sites in the cell at which the radiating microtubules terminated. Gordon Carlson (1952) however proved that the spindle of the grasshopper Chorthophage viridifasciata was attached to the plasma membrane by the astral rays. The astral rays of animal cells consist of numerous microtubules and it appears that their function is to anchor the spindle fibrils to the endoplasmic reticulum. The radiating microtubules observed by Girbardt and others may be regarded as serving the same function in nuclear division of the higher fungi.

The results of the above investigations allow one to reach a number of tentative conclusions regarding the nature of the mitotic apparatus in Basidiomycetes. Firstly, it may be concluded that the kinetocenter is a persistent structure which is intimately associated with the nuclear membrane of the interphase nucleus (Wilson and Aist, 1967; Girbardt, 1968). Secondly, replication of the kinetocenter takes place during the process of somatic division (Grundmann, 1966). The daughter kinetocenters are deployed to opposite poles of the nucleus during interphase and form the origins of the spindle fibrils. Thirdly, comparison of the form of the migrating post-meiotic nucleus of many Basidiomycetes and in particular, Oudemansiella mucida with the beaked nucleus present in the ascus of Pustularia cupularis (Schrantz,/

(Schrantz, 1967) suggests that the kinetocenter is located at the apex of the migrating post-meiotic nucleus. Fourthly, microtubules, homologous with the astral ray microtubules of animal cells and flagellate gametes, radiate from the kinetocenter to anchorage points on the endoplasmic reticulum. Furthermore, these microtubules are present throughout the mitotic cycle except possibly, for a brief period prior to metaphase, as Girbardt (1968) claims.

It has frequently been observed that the terminations of the spindles of meiosis II are located close to the wall of the basidium. The studies of Gordon Carlson (1952) render it highly probable that the four kinetocenters present at meiosis II are each anchored by four groups of microtubules to a region of the endoplasmic reticulum adjacent to the wall of the basidium. The four post-meiotic nuclear products each become closely associated with a kinetocenter and enter interphase. Sterigmata and basidiospores are initiated at the end of this interphase. Maire (1902) and Levine (1913) both claimed that initiation occurred at those sites on the wall of the basidium to which the kinetocenters were attached during and subsequent to meiosis II. In the light of the recent investigations summarised above, this claim is no longer tenable in its original form, and requires modification. It is proposed that/

that the development of sterigmata and basidiospores is initiated at anchorage sites adjacent to the wall of the basidium. As each sterigma and basidiospore develop, each anchorage point is maintained at or near the apex of growth and remains connected by a group of microtubules to the corresponding kinetocenter now located on the membrane of the interphase post-meiotic nucleus within the basidium.

The nature of the fibril connecting the migrating post-meiotic nucleus of Gomphidius rutilus to a sub-apical point on the wall of the basidiospore may be deduced in the light of these conclusions. This fibril is probably composed of a group of microtubules, although appearing as a unit structure in the light microscope. Moreover, these microtubules probably originate in the kinetocenter located on the membrane of the post-meiotic nucleus and extend to a site on the endoplasmic reticulum at the apex of the basidiospore.

The presence of a fibril extending from the rear of the migrating nucleus to an undetermined point in the lower region of the basidium, has not been previously documented. This observation indicates that deployment of the daughter kinetocenters to opposite poles of the nucleus takes place prior to or concomitant with the onset of migration. The marked elongation of the nucleus suggests that the lower fibril/

fibril impedes migration as it might do if it were attached to a fixed site in the lower part of the basidium. Deployment of daughter kinetocenters to opposite poles of the nucleus, and the presence of fibrils which may be homologous to the astral rays, do not necessarily imply that a mitotic spindle has been formed. In G. rutilus, the situation regarding the deployment of kinetocenters and presence of fibrils is as described above, but there is no evidence of the spindle whilst the nucleus is in the process of migration. The spindle becomes evident in the basidiospore when metaphase is about to take place. In the case of animal cells and of flagellate gametes, synthesis of the spindle takes place concurrently with deployment of the kinetocenters, but it may be that in the Basidiomycetes, the pattern is modified to facilitate migration.

Coiling of fibrils has been claimed to be accomplished by ATP-activated contraction of the microtubules (Robards, 1970). It is possible that such a mechanism is operative within the basidiospore. Contraction of the microtubules comprising the apical fibril would result in the migrating nucleus being drawn into the basidiospore. Correspondingly, the microtubules of the basal fibril may be induced to elongate, or their length may be increased by intussusception of new material. Subsequent contraction

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of these microtubules and further, but limited contraction of those of the apical fibril, would cause separation of the kinetocenters, elongation of the spindle and separation of the chromosomes as proposed by Levine (1913). The daughter nuclear product distal to the sterigma would be drawn towards the subapical point near the wall of the basidiospore, while the proximal daughter nuclear product would be drawn towards the anchorage point in the lower region of the basidium. Anaphase of the third nuclear division of G. rutilus is markedly asymmetrical. A similar asymmetry was observed by Girbardt (1968) during anaphase in division of the nucleus within the clamp of Polystictus versicolor. It must be borne in mind that the apical fibril of the post-meiotic nucleus of G. rutilus contracted during migration and relaxed only slightly at metaphase of the third nuclear division. It can therefore only contract to a limited extent during anaphase. In contrast, the basal fibril does not contract until anaphase. In addition, movement of the daughter product distal to the sterigma is limited by the wall of the basidiospore, while movement of the proximal daughter product is comparatively unrestricted. Anaphase separation of the daughter products of the third nuclear division is remarkably wide, but no more so than that observed during the final conjugate nuclear division in the/

the hyphal tip.

The post-meiotic nuclei of species conforming to Pattern D sequence of events, appear to remain rounded during the initial stages of migration and only develop a tear-drop form as they enter the sterigmata. This observation suggests that the initial stages of migration are not rapid and may be brought about by growth of the sterigmata and basidiospores as claimed by Vokes (1931). The presence of the tear-drop form implies that during the later stages of migration, the nucleus is actively pulled through the sterigma into the basidiospore. This form of the nucleus is brought about by a pulling force exerted at the apex, in the absence of an impeding force exerted at the opposite pole of the nucleus. Following migration, the post-meiotic nucleus divides in the basidiospore, and both daughter products remain within the latter. Therefore it is postulated that in this group of Basidiomycetes, deployment of the daughter kinetocenters takes place after the completion of migration. Thus both anchorage points for the spindle would be laid down within the basidiospore as has been established in Oudemansiella mucida (Fig. 62). It is in this respect that Pattern D differs from Pattern C. Consequently, in Pattern D retrogressive migration of daughter products of the third nuclear division cannot take place.

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The migrating nucleus of species conforming to Pattern B sequence of post-meiotic events divides in the sterigma. In this case deployment of kinetocenters and development of the spindle must take place prior to or concomitant with migration. The similarity in the final distribution of the daughter nuclear products suggests that Patterns B and C may be the same in all respects save for the timing of division. It appears that the apical fibril does not reach its maximum contraction before division in Pattern B. The position of the post-meiotic nucleus at metaphase of the third nuclear division would indicate that at this stage the apical and basal fibrils are approximately equal in length. Consequently, the third nuclear division in Pattern B is a symmetrical event, unlike that of Pattern C.

Pattern A species exhibit a totally different behaviour from species conforming to Patterns B, C, and D. It will be recalled that in Cantharellus cibarius, the meiotic spindles lie in the long axis of the basidium, while the spindles of the third nuclear division are formed in the apex of the latter. The orientation of these spindles is haphazard. The author's interpretation of the views of Maire (1902) and Levine (1913) regarding the initiation of sterigmata at anchorage sites of the spindles present at meiosis II have been outlined previously (p.114). If this

view/

view is correct, it would appear that in C. cibarius, it is the anchorage sites present during the third nuclear division which are involved in the initiation process. The observed reduction in the number of basidiospores from the theoretical maximum of eight is possibly due to failure of one or more of these anchorage sites to initiate a sterigma and basidiospore. A detailed examination at both light and electron microscope level may indicate the method by which sterigmata and basidiospores are initiated in those species whose basidia bear more than four basidiospores.

Fibrils, homologous to astral rays of animal cells, may function during conjugate nuclear division of the somatic nuclei of Basidiomycetes and in particular during the final conjugate division in the hyphal tip. Fig. 32 (G. rutilus) illustrates anaphase separation of this division. The conjugate daughter products destined to become the nuclei of the basidium are widely separated from their sister products, and are located relatively near the apex of the cell. It is possible that fibrils, connecting the kinetocenters at the termination of each spindle to the apex of the cell persist, so that the prefusion nuclei, and later the fusion nucleus, are connected to the apex of the basidium.

It is agreed by all authorities that fusion of the conjugate nuclei within the basidium involves fusion of their nuclear/

nuclear membranes, fusion of the nucleoli, and association of the sister chromosomes, but nothing is known of the behaviour of the kinetocenters at this time. They probably become associated on the membrane of the fusion nucleus. In all species exhibiting Pattern B, C and D sequence of post-meiotic events, the fusion nucleus moves to the apex of the basidium, where the spindle of meiosis I develops at right angles to the long axis of the basidium. Nothing is known of the mechanism by which migration is achieved, but it may be engineered by the fibrils referred to above.

It has already been noted that replication of kinetocenters takes place some considerable time before nuclear division. The two nuclei which fuse in the basidium will, therefore, each possess paired kinetocenters. Hence, no replication of the latter is necessary during meiosis I to provide the four kinetocenters required at meiosis II. The spindles of this division are also formed in the apex of the basidium, again at right angles to the long axis. They may be parallel to or at right angles to each other, but their orientation relative to the spindle of meiosis I is unknown.

Nolanea cetrata is a constantly bisporic species in which pairs of post-meiotic nuclei migrate to each basidiospore/

basidiospore where they divide, producing tetranucleate basidiospores. The observation of two fibrils running into each sterigma suggests that, in this species, pairs of adjacent anchorage points are responsible for the initiation of each sterigma and basidiospore. Each pair of fibrils would have its apical termination at a point near the apex of the basidiospore, but the basal terminations would be at the kinetocenter of two separate nuclei. Contraction of these fibrils would cause pairs of post-meiotic nuclei to be drawn into each basidiospore.

The organelles implicated in nuclear traction are beyond the resolution of the light microscope, and much of the above discussion is therefore highly speculative in the absence of observations provided by the electron microscope.

The author's observations clearly indicate that a third nuclear division during the development of the basidium and the basidiospores is a universal feature in the Basidiomycetes, and may be brought about by the spatial restrictions imposed upon the migrating nuclei by the sterigmata tips. The daughter nuclear products of Pattern A species are drawn into the basidiospores in a telophase condition, while those of Pattern B species pass into the basidiospore in an anaphase condition. In both cases,

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the chromatin is in a highly condensed condition, and nucleoli are absent. Observations of migration of the post-meiotic nuclei of Pattern C and D species, reveal that the chromatin is in a granular condition typical of the diffuse interphase nucleus. Close scrutiny of this stage of development in G. rutilus shows that the nuclear membrane is present during and subsequent to migration. Examination of developing basidia and basidiospores of G. rutilus (Pattern C) and N. cetrata (Pattern D) establishes beyond doubt that the nucleolus of the migrating nucleus does not pass into the basidiospore, but remains behind within the basidium. In Pattern C and D species therefore, the nucleolus disassociates from the migrating nucleus while the latter is in an interphase/prophase condition. Girbardt (1968), using the light microscope reported that the nucleolus of Polystictus versicolor dissolved in the cytoplasm during prophase although the contracting chromatin was still enclosed in a membrane. Motta (1969), as a result of his electron microscope observations claimed that the nucleolus and nuclear membrane of the dividing nucleus of Armillaria mellea disappeared at late metaphase or early anaphase. It would appear from the above observations that in Pattern C and D species, the nucleolus becomes prematurely disassociated from the nucleus as the latter passes through

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the sterigma into the basidiospore. It is probable that in all species of Basidiomycetes in which the basidium contains four nuclei at the time of production of basidiospores, the nuclei which pass into the latter lack nucleoli. One can only conclude that the nucleolus cannot pass through the tip of the sterigma and must be dispensed with, prior to or during migration. This requirement may be achieved in one of two ways; either, the migrating nucleus can divide before reaching the restricting tip, in which case a highly condensed daughter product lacking a nucleolus passes into the basidiospore and there synthetizes a nucleolus; or, the migrating nucleus can extrude its nucleolus before passing through the sterigma. The nucleus must then complete mitosis in order that a new nucleolus may be synthetized within the basidiospore. The synthesis of a nucleolus is no doubt required to provide a fully functional nucleus which can control the later stages of maturation of the basidiospore.

(3) Post-meiotic events - Taxonomic and phylogenetic significance.

The presence of a third nuclear division during formation of basidiospores appears to be a basic feature of all members of the Basidiomycetes. The recognition that the site of the third nuclear division differs in different species, and that the fate of the resultant daughter products differs likewise, could be of considerable value as taxonomic criteria in any revision of the Basidiomycetes.

Two patterns of post-meiotic events have been reported within the Tremellales. Pattern B operates in the species of Exidia which have been examined, the third nuclear division taking place in the sterigmata (Furtado 1969), while Pattern D occurs in Tremella species, the third nuclear division taking place within the basidiospore. Thus, the available cytological data regarding post-meiotic events in these two genera, substantiates the decision to separate them on taxonomic data.

In contrast, similar evidence indicates that the Tricholomataceae are at present a heterogeneous family. Mycena and Collybia conform to Pattern B, while Flammulina conforms to Pattern D and Panellus conforms to Pattern C. A detailed cytological examination of the post-meiotic events/

events in members of this relatively large family could possibly contribute valuable information in any future revision of the group.

The observation of Pattern D in species of Amanita, Psalliota, Lepiota, Coprinus, Stropharia, Galerina, Nolanea and Entoloma strengthens the view that these genera are closely related. The striking cytological similarities exhibited by Oudemansiella mucida and Amanita fulva suggest a closer relationship of the former species to members of the Amanitaceae than to members of the Tricholomataceae with which it is at present classified. Similarly, Panellus which conforms to Pattern C has much more in common with the Polyporaceae from the cytological point of view, than with the Tricholomataceae, while Schizophyllum, which exhibits Pattern D would appear to have nothing in common with the Polyporaceae.

The presence of Pattern C in members of the Polyporaceae and Boletaceae may indicate ancestral connections between these families. Singer (1962) believes, that the Boletaceae, Gomphidiaceae and Paxillaceae are related families. The fact that members of the Russulaceae, Hygrophoraceae and Hydnaceae also conform to Pattern C may indicate a more extensive relationship than has hitherto been realised.

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The pattern of post-meiotic events occurring in individual species may also clarify the limits of existing genera. Mycena, Collybia and Omphalina are regarded at present as being closely related and even overlapping genera. The genus Mycena, contains at least three different cytological groupings; the 'haploparthenogenetic' species (Kühner, 1927; Smith 1934), Pattern B species such as M. alcalina, and dual pattern species such as M. swartzii. Dual pattern behaviour however, may yet be found to be a feature of Omphalina. Information relating to the post-meiotic events in all Mycena, Collybia and Omphalina species may prove to be of considerable value in any future taxonomic revision of these difficult genera.

The phylogenetic implications of different patterns of post-meiotic events may also be viewed in the light of Juel's (1898) assertions regarding the Stichobasidiae and Chiastobasidiae. He observed that in the Stichobasidiae, the meiotic spindles are aligned in the long axis of the basidium, while the spindles of the third nuclear division are at right angles to the latter. In members of the Chiastobasidiae however, it is the meiotic spindles which occupy this latter position, while those of the third nuclear division lie in the long axis of the basidium or basidio-spore. The alignment of the spindles of meiosis I and II and/

and of the third nuclear division in the Stichobasidiae is similar to that observed in the three nuclear divisions in the ascus of the Ascomycetes, and suggests that this group bear a closer relationship to a possible ascomycete ancestor than do the Chiastobasidiae.

Kühner (1945b) maintained that within the Agaricales, two categories only, of post-meiotic events took place. In species belonging to the first category, the third nuclear division took place in the sterigmata, while in those of the second category, the third nuclear division took place within the basidiospore. Species conforming to category one were regarded by Kühner as being primitive, while those of category two were advanced. Kühner was unaware of retrogressive migration however, and consequently his category one is a heterogeneous grouping.

One can nevertheless accept the general basis of his reasoning. Pattern A species would be regarded as the most primitive types since the third nuclear division takes place within the basidium as it does in the ascus of the Ascomycetes. Pattern B species, exhibiting division in the sterigmata would be more advanced, while patterns C and D would be regarded as having evolved from Pattern B. Both branches of the Basidiomycetes show this evolutionary trend. Fig. 82 illustrates a hypothetical phylogenetic classification of the/

the Basidiomycetes based upon Juel's assertions (1898) and the author's observation of four patterns of post-meiotic behaviour within this taxon.

The value of the present cytological findings to the taxonomist cannot be finally assessed until the pattern of post-meiotic events occurring in the majority of the Basidiomycetes has been determined. Such a survey is clearly an almost impossible task, but examination of the type species of individual genera would be a valuable and rewarding undertaking, and would provide a firm basis for future comparisons.

SUMMARY

The results of research over several decades into the cytology of basidiospore production has led to the emergence of a concept of post-meiotic events in the basidiomycetes which has been termed the 'classical pattern' by the author. The essential features of the 'classical pattern' are karyogamy, followed by meiosis I and II in the basidium. The four post-meiotic nuclei produced migrate to the basidiospores, which are discharged in a uninucleate condition. While several reports of basidial cytology appear at first sight to conform to the 'classical pattern' (e.g. Harper, 1902; Whelden, 1934; Ritchie, 1941), others reveal that a third nuclear division occurred in the formation of the basidiospores (e.g. Maire, 1902; Kühner, 1945). Maire (1902) and Levine (1913) observed that a third nuclear division occurred in the post-meiotic events of species of Boletus. Many years later, Duncan (1970) confirmed these observations, and in addition, was able to show that one daughter nuclear product from each basidiospore migrated retrogressively to the basidium during the final stages of the third nuclear division. The mature basidium of Boletus spp. bears four uninucleate basidiospores and contains four nuclei which degenerate. The present study was initiated in an attempt to find out whether or not species/

species within families related to the Boletaceae also exhibited this pattern of post-meiotic events. The post-meiotic behaviour of members of the Gomphidiaceae, Paxillaceae, Hygrophoraceae, and Russulaceae was first investigated. When it became clear that many reports in the literature concerning post-meiotic events were incomplete or erroneous, the study was expanded to include members of the Cantharellaceae, Polyporaceae, Tricholomataceae, Agaricaceae, Amanitaceae, Coprinaceae, Cortinariaceae, Rhodophyllaceae, Hydnaceae, Thelephoraceae and Tremellaceae.

Progress in the cytological study of the Basidiomycetes has undoubtedly been hindered by the inability to stain the nuclei informatively at all stages of the mitotic cycle. Bakerspigel (1960) pointed out how unrewarding was any attempt to stain fungal nuclei by standard staining techniques. The introduction of the acid-Giemsa technique however, provided a new, efficient method by means of which the cytologist could obtain information on nuclear behaviour in the Basidiomycetes. The staining schedule originally outlined by Robinow (1942) for the bacteria has been modified by the author to provide a method by which the nuclei of the Basidiomycetes are intensely stained at all stages of the mitotic cycle.

A detailed cytological investigation of 27 members of/

of the Basidiomycetes, using the acid-Giemsa staining method indicates that in all cases, a third nuclear division takes place during the formation of the basidiospores. Furthermore, the post-meiotic events fall into four patterns which may be distinguished from each other on the basis of two criteria.

1. The site of the third nuclear division.
2. The fate of the resultant nuclei.

The four patterns have been designated as Pattern A, B, C, D, and their features may be summarised as follows:-

Pattern A. The third nuclear division takes place in the basidium, and one of the resultant eight nuclei migrates to each basidiospore, any nuclei in excess of basidiospores degenerating in the basidium.

Pattern B. The third nuclear division takes place in the sterigmata. The four daughter nuclear products distal to the basidium pass into the four basidiospores, and the remaining four daughter products return to the basidium and degenerate there.

Pattern C. The third nuclear division takes place in the basidiospores. The four daughter nuclear products distal to the basidium remain in the four/

four basidiospores, while their sister products migrate retrogressively to the basidium where they degenerate.

Pattern D. The third nuclear division takes place in the basidiospores. The daughter nuclear products remain in the four basidiospores which are thus binucleate when discharged.

A perusal of the existing cytological reports has been made in the light of the above findings. The present investigation has provided evidence which substantiates several of these reports, but which completely refutes many others. It has been possible to conclude that the 'classical pattern' of post-meiotic events, regarded by mycologists as a major feature of the Basidiomycetes, is a totally invalid view which has apparently arisen through misconceptions regarding Dangeard's (1895) investigations.

Detailed observations of Gomphidius rutilus and Oudemansiella mucida make it possible to postulate the means by which the post-meiotic nuclei migrate to the basidiospores. It is tentatively concluded that a kinetocenter (Grundmann, 1966) is permanently associated with the post-meiotic nuclei or their daughter products, and that this organelle is attached to the apex of the developing basidiospore by a fibril which draws the nucleus/

nucleus into the basidiospore by contraction. The fibril is thought to be homologous to the astral rays. In Pattern B and C species, deployment of the daughter kinetocenters is believed to take place prior to or during migration of the post-meiotic nuclei. The apical daughter kinetocenters of the post-meiotic nuclei are attached by fibrils to the wall of the basidiospores, while the basal daughter kinetocenters are attached by fibrils to loci in the lower region of the basidium. In Pattern D it is the view that deployment of the daughter kinetocenters is delayed until migration of the post-meiotic nuclei to the basidiospores is completed.

The post-meiotic nuclei of G. rutilus and Nolanea cetrata have been observed to extrude their nucleoli prematurely during their passage through the sterigmata. This observation implies that the nucleolus of the post-meiotic nucleus cannot pass through the sterigma and must therefore be dispensed with.

The establishment of different patterns of post-meiotic behaviour in the Basidiomycetes may provide new taxonomic criteria of value at generic and family level. The phylogenetic relationships of a number of genera have been evaluated on the basis of Juel's (1898) assertions regarding/

regarding the orientation of the axes of the meiotic spindles and the pattern of post-meiotic events typical of the individual genera.

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APPENDIX I.

Species examined by previous authors together with current synomyms.

Species previously examined.	Cytological investigator.	Current nomenclature and taxonomic position.
		Polyporaceae.
Fomes annosus	Wilson, Miller & Griffin, 1967	Heterobasidion annosa (Fr.)Bref.
Polyporus adustus	Bose, 1937	Bjerkandera adusta (Fr.)Karsten
P. versicolor	"	Coriolus versicolor (Fr.) Quél.
		Schizophyllaceae.
Schizophyllum commune	Essig, 1922, Ehrlich & McDonough, 1949, Wells, 1965	Schizophyllum commune Fr.
		Hygrophoraceae.
Hygrophorus agathosmus	Maire, 1902	Hygrophorus agathosmus (Fr. ex Secr.)Fr.
Godfrinia cerácea	"	H. ceraceus (Wulf. ex Fr.)Fr.
G. conica	"	H. conicus (Scop. ex Fr.) Kummer
Hygrophorus hypothejus	" Kühner, 1945	H. hypothejus (Fr. ex Fr.) Fr.
H. miniatus	Maire, 1902	H. miniatus Fr.
Camarophyllus virgineus	Bauch, 1926	H. virgineus (Wulf. ex Fr.) Fr.

		Tricholomataceae.
Collybia maculata	Rosenvinge, 1886	Collybia maculata (Alb. & Schw. ex Fr.) Kummer
C. velutipes	"	Flammulina velutipes (Curt. ex Fr.) Karst.
Mycena clavicularis	Smith, 1934	Mycena clavicularis Fries
M. immaculata	"	M. gracilis (Quél.) Kuhn.
M. graveolens	"	M. iodiolens Lundell
M. margaritospora	"	M. margaritospora J. Lange
M. mirata	"	M. mirata (Peck) Sacc.
M. murina	"	M. stannea (Fr.) Quel.
M. viscosa	"	M. viscosa (Secr.) Maire
Omphalia flavida	Seguiera, 1954	M. citricolor (Berk. & Curt.) Sacc.
Tricholoma virgatum	Rosenvinge, 1886	Tricholoma virgatum (Fr. ex Fr.) Kummer
		Amanitaceae.
Amanita bisporigera	Lewis, 1906	Amanita bisporigera Atk.
A. caesarea	Ritchie, 1948	A. caesarea (Scop. ex Fr.) Pers. ex Schw.
A. porphyria	Rosenvinge, 1886	A. porphyria(Alb. & Schw. ex Fr.) Secr.

Lepiota(Limacella) lenticularis	Hagerup, 1945	Limacella guttata (Pers. ex Fr.) Konrad & Maubl.
Volvariella volvacea	Chang, 1969	Volvariella volvacea (Bull. ex Fr.) Sing.
		Agaricaceae.
Psalliota campestris f. bispora	Maire, 1902, Sass, 1929, Colson, 1935, Sarazin, 1938, Evans, 1959	Agaricus bisporus (J.Lange) Pilat
		Coprinaceae.
Coprinus atramentarius	Vokes, 1931	Coprinus atramentarius (Bull. ex Fr.) Fr.
C. narcoticus	Kniep, 1928	C. narcoticus (Batsch ex Fr.) Fr.
C. ephemerus f. bisporus	Sass, 1929	C. sassii M. Lange & A.H.Smith
C. stercorarius	Kniep, 1928, Sass, 1929	C. stercorarius (Bull.)Fr.
C. sterquilinus	Kniep, 1928, Sass, 1929	C. sterquilinus (Fr.)Fr.
Hypholoma appendiculatum	Ruhland, 1901	Psathyrella candolleana (Fr.)Maire

Galera tenera f. bispora	Sass, 1929	Bolbitaceae. Conocybe tenera (Schaeff. ex Fr.) Kühn.
Naurcoria semiorbicularis f. bispora	"	Agrocybe semiorbicularis (Bull. ex St. Amans) Fayod
Hypholoma fasciculare	Wakayama, 1930	Strophariaceae. Hypholoma fasciculare (Huds. ex Fr.) Kummer
H. perplexum	Nichols, 1904	H. perplexum
Agaricus (Stropharia) stercorarius	Wager, 1892	Stropharia semiglobata (Batsch. ex Fr.) Quél.
Cortinarius cinnamomeus	Wakayama, 1930	Cortinariaceae. Cortinarius cinnamomeus (L. exFr.) Fr.
Nolanea cetrata	Buhr, 1932	Rhodophyllaceae. Nolanea cetrata (Fr. ex Fr.) Kummer
Hygrophoropsis aurantiaca	Maire, 1902	Paxillaceae. Hygrophoropsis aurantiaca (Fr. ex von Wulf.) Maire apud Martin-Sans

		Gomphidiaceae.
Gomphidius glutinosus	Maire, 1902	Gomphidius glutinosus(Schaeff. ex Fr.) Fr.
		Boletaceae.
Boletus tessellatus	"	Boletus crocipodius Letellier
B. edulis	Rosënvinge, 1886	B. edulis Bull. ex Fr.
B. flavus	Maire, 1902	B. luteus L. ex Fr.
B. regius	"	B. regius Krombh.
B. scaber	"	B. scaber Bull. ex Fr.
B. variegatus	Rosenvinge, 1886	B. variegatus Sow. ex Fr.
		Russulaceae.
Lactarius deliciosus	Maire, 1902	Lactarius deliciosus (L. ex Fr.) S.F.Gray
L. piperatus	"	L. piperatus (Scop. ex Fr.)S.F.Gray
Russula emetica	Ritchie, 1941	Russula emetica (Schaeff. ex Fr.) S.F.Gray
R. lepida	Maire, 1902	R. lepida Fr.
R. rubra	Strasburger, 1884	R. rubra (Fr. ex Lam.) Fr.

Cantharellus cibarius	Maire, 1902	Cantharellaceae. Cantharellus cibarius Fr.
Hydnangium carneum	Petri, 1902	Hydnangiaceae.
Aleurodiscus canadensis	Skolko, 194	Aleurodiscús canadensis
Hypochnus terrestis	Kniep, 1928 Moreau, 1953	Thelophora terrestis Ehrh. ex Fr.
Coniophora cerebella	Kemper, 1937	Coniophoraceae. Coniophora puteana (Schum.)Karst.
Peniophora ludoviciana	Biggs, 1938	Peniophora ludoviciana
Vuilleminia comedens	Maire, 1902	Vuilleminia comedens (Fr.)Maire
Sistotrema confluens	Kühner, 1947	Corticiaceae. Sistotrema confluens Pers.
Ceratobasidium praticolum	Saksena,1961	Tulasnellaceae. Thanatephorus praticola(Kotila) Talbot
Ceratobasidium praticolum	Hawn & Vanterpool, 1953	T. aff. cucumeris (Frank)Donk
Hypochnus subtilis	Harper, 1902	True identity unknown.

		Nidulariaceae.
Cyathus stercoreus	Lu, 1964	Cyathus stercoreus (Schw.)de Toni
Mycocalia denudata	Burnett & Boulter, 1963	Nidularia denudata (Fr.)Nordholm
Nidularia pisiformis	Fries, 1911	N. pulvinata (Schw.)Fr.
		Pisolithiaceae.
Pisolithus tinctorus	Lander, 1935	Pisolithus tinctorus (Mich. ex Pers.)Coker & Couch
		Tremellaceae.
Exidia glandulosa	Whelden, 1935	Exidia glandulosa (Bull.)Fr.
E. recisa	"	E. recisa (Ditm. ex S.F.Gray)Fr.
E. saccharina	"	E. saccharina Fr.
E. nucleata	Whelden, 1935, Furtado, 1968	Myxarium hyalinum (Pers.)Donk
Tremella frondosa	Whelden, 1934	Tremella frondosa Fr.
T. grilletii	"	T. grilletii Boud.
T. lutescens	von Istvanffi, 1895	T. lutescens (Pers. ex Pers)Fr.
T. mesenterica	Whelden, 1934	T. mesenterica Retz. ex Hook.

Melampsora bigelowii	Savile, 1939	Melampsoraceae. Melampsora bigelowii Thum.
Puccinia malvacearum	Savile, 1939, Allen, 1933	Pucciniaceae. Puccinia malvacearum Mont.
Uromyces lezpedezae-procumbentis	Savile, 1939	Uromyces lezpedezae-procumbentis (Schw.)Curt.

AIEN APIΣTEYEIN

POST-MEIOTIC EVENTS IN THE BASIDIOMYCETES.

by

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Volume II.

A thesis submitted to the University of St. Andrews for the Degree of Doctor of Philosophy.

Department of Botany,
University of St. Andrews.

May, 1971.

Th 5805

Pellicularia otogensis.

Fig. 1a. Fusion nucleus. Prophase of meiosis I.

Fig. 1b. Mature basidium bearing seven uninucleate basidiospores. Note residual nucleus within basidium.

Fig. 1c. Aged, anucleate basidium.

Fig. 1d. Mature, naturally shed basidiospores.

Fig. 1e. Mature basidium bearing eight basidiospores.

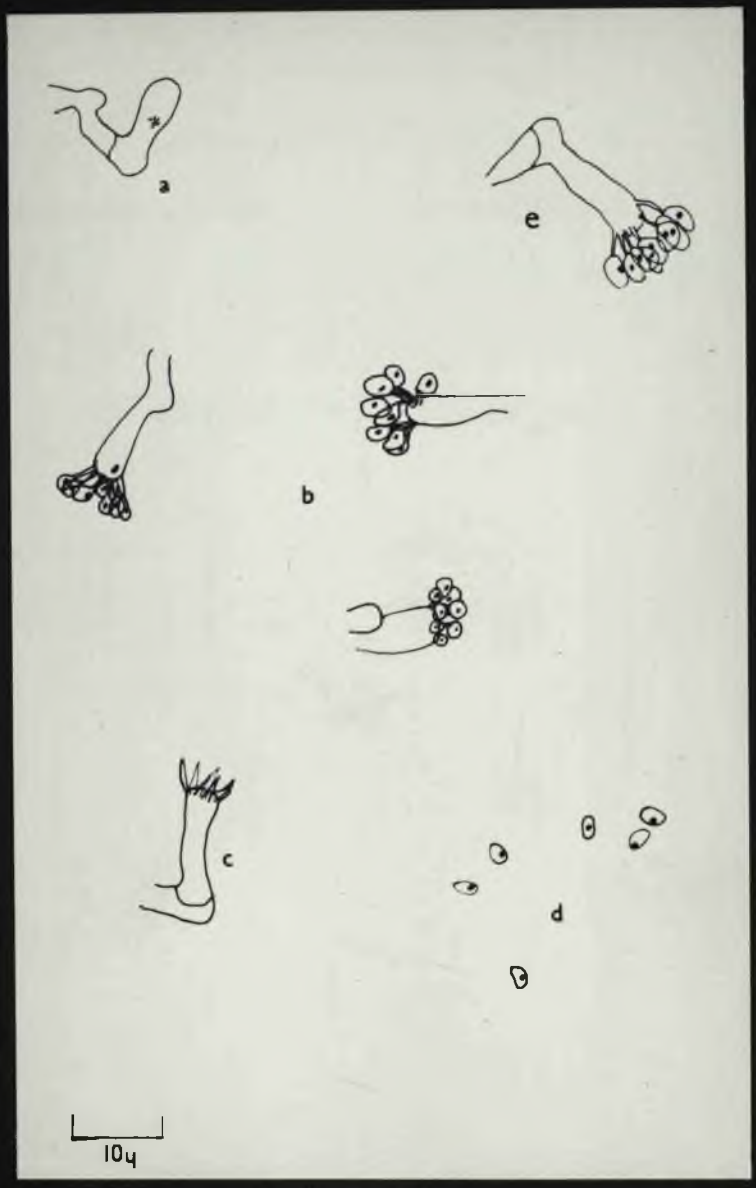

Fig.1

Cantharellus cibarius.

Fig. 2a. Post-meiotic interphase. Note conspicuous nucleolus in each nucleus.

Fig. 2b. Third nuclear division. Axes of division indeterminate.

Fig. 2c. Third nuclear division. Eight daughter nuclei within basidium developing five basidiospores.

Fig. 2d. Bi-sporic basidium. Nucleus adjacent to attached basidiospore may be about to enter latter.

Fig. 2e. Mature, naturally shed basidiospores.

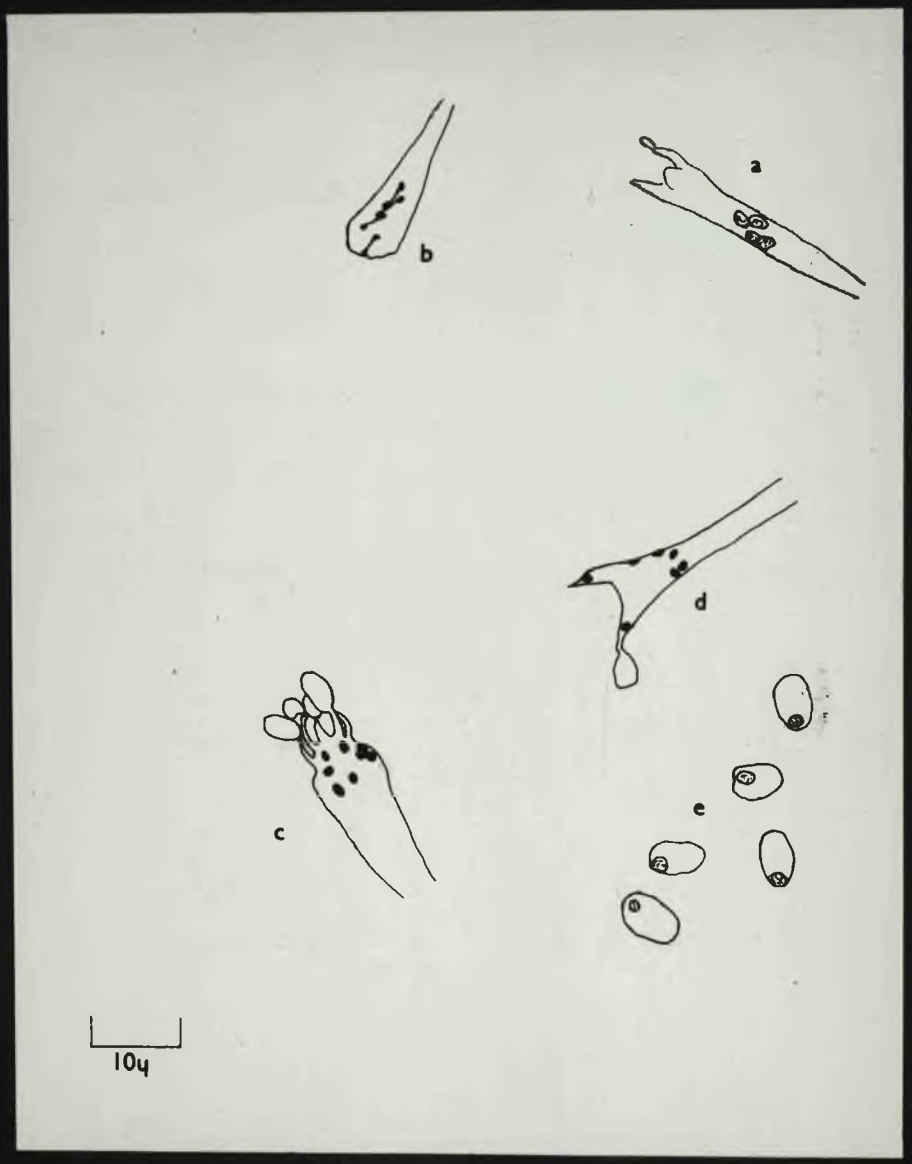

Fig.2

Mycena alcalina.

Fig. 3. Post-meiotic interphase. The four nuclei aligned in long axis of basidium. (x 3,000).

Fig. 4. Third nuclear division. Stages in anaphase. (x 3,000).

Fig. 5. Third nuclear division. Nucleoli (arrowed), disassociated from dividing nuclei, within basidium. (x3,000).

Fig. 6. Third nuclear division. Late anaphase. Remnants of spindle remain evident between daughter products. (x 3,000).

Fig.3

Fig.4

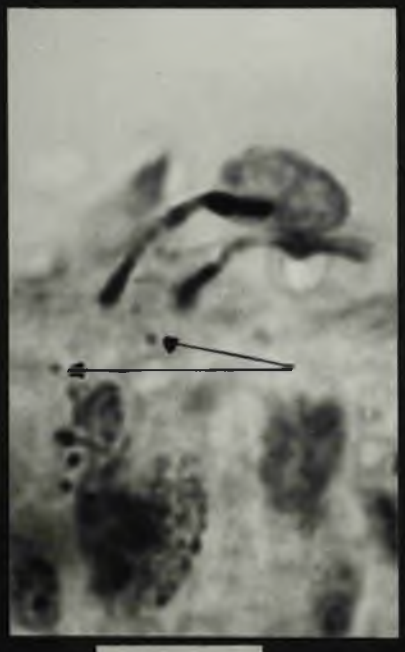

Fig.5

Fig.6

Mycena alcalina.

Fig. 7. Third nuclear division completed. Basidium contains four nuclei and bears four uninucleate basidiospores. (x3,000).

Fig. 8. Asynchronous division of one residual nucleus within basidium following discharge of basidiospores. (x3,000).

Fig. 9. Asynchronous division of two residual nuclei within basidium following dischage of basidiospores. Note prominent sterigma on right. (x3,000).

Fig. 10. Third nuclear division. Anaphase. Anomalous behaviour involving presence of both daughter products within basidiospore. (x 3,000).

Fig.7

Fig.10

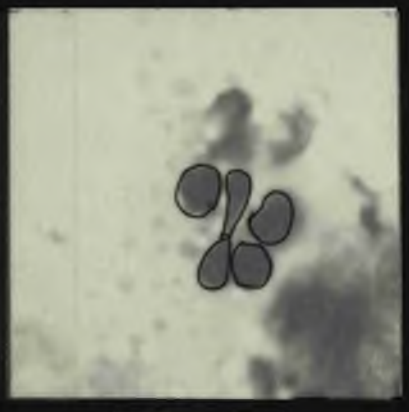

Fig.8

Fig.9

Gomphidius rutilus.

Fig. 11. Migration of post-meiotic nuclei to basidiospores. Note cigar-shape of nuclei and heterochromatic condition. (x 2,000).

Fig. 12. Migration of post-meiotic nucleus into basidiospore. Note twin-stranded condition of chromatin. (x 3,000).

Fig.11

Fig.12

Gomphidius rutilus.

Fig. 13. Post-meiotic nucleus within basidiospore. Nuclear membrane (nm) encompassing chromatin (c) still intact. Phase-contrast observation. (x 3,000).

Fig. 14. Post-meiotic nucleus within basidiospore. Extruded nucleolus evident within sterigma. (x 3,000).

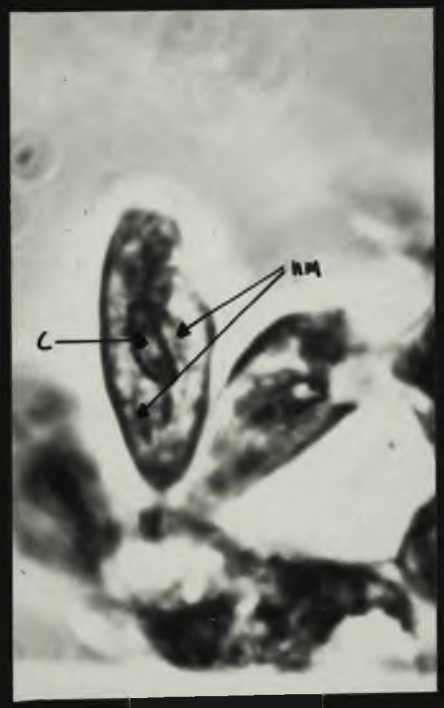

Fig.13

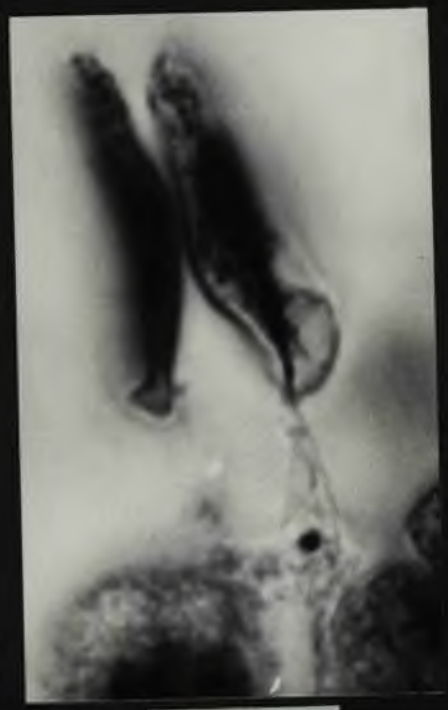

Fig.14

Gomphidius rutilus.

Fig. 15. Migration of post-meiotic nucleus. Fibril (arrowed) connects migrating nucleus to sub-apical point on wall of basidiospore. Phase-contrast observation. (x3,000).

Fig. 16, a,b,. Migration of post-meiotic nucleus. Fibrils (arrowed) connecting migrating nuclei to undetermined loci in lower region of basidium. Fibrils delineated in Fig. 16b. Phase-contrast observation. (x3,000).

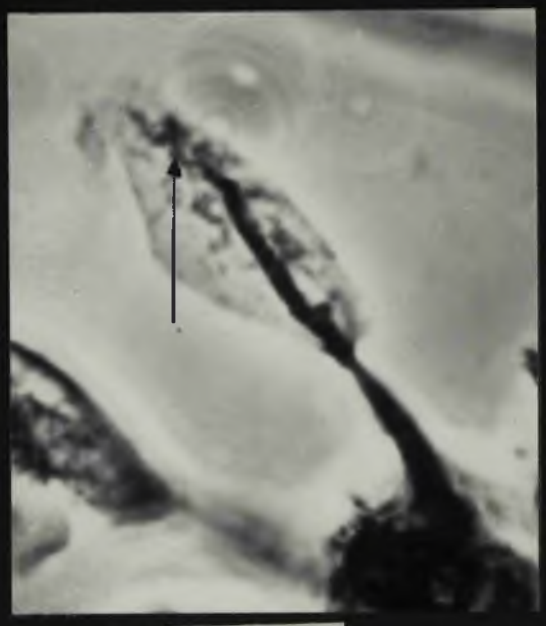

Fig.15

Fig.16a

Fig.16b

Gomphidius rutilus.

Fig. 17. Migration of post-meiotic nuclei to basidiospores completed. Only two basidiospores remain attached to anucleate basidium. (x 2,000).

Fig. 18,a,b,c. Post-meiotic nucleus connected by fibrils (arrowed) to sub-apical locus on basidiospore wall and to undetermined locus within basidium. Fibrils delineated in Fig. 18c. (x3,000).

Fig.17

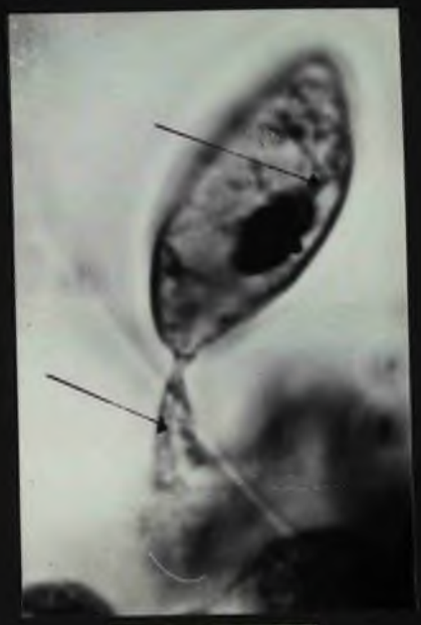

Fig.18a

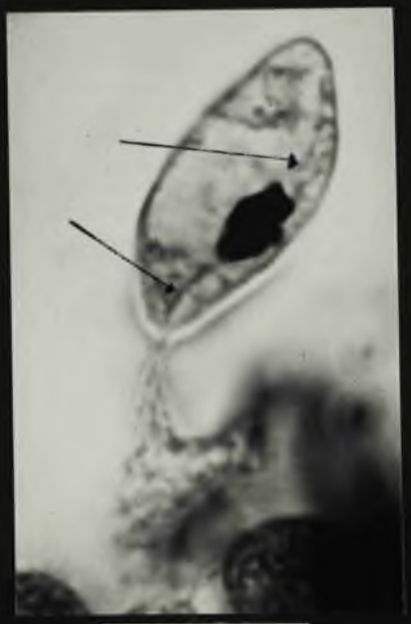

Fig.18b

Fig.18c

Gomphidius rutilus.

Fig. 22. Third nuclear division in basidiospore. Late anaphase. Daughter product from one of the missing basidiospores moves rapidly downwards into basidium. (x 3,000).

Fig. 23. Third nuclear division in basidiospore. Late anaphase. Nuclear membrane, or part thereof, evident in advance of chromatin which is connected to point on membrane. Fibril (arrowed) extends from latter to point within basidium. Phase-contrast observation. (x 3,000).

Fig.22

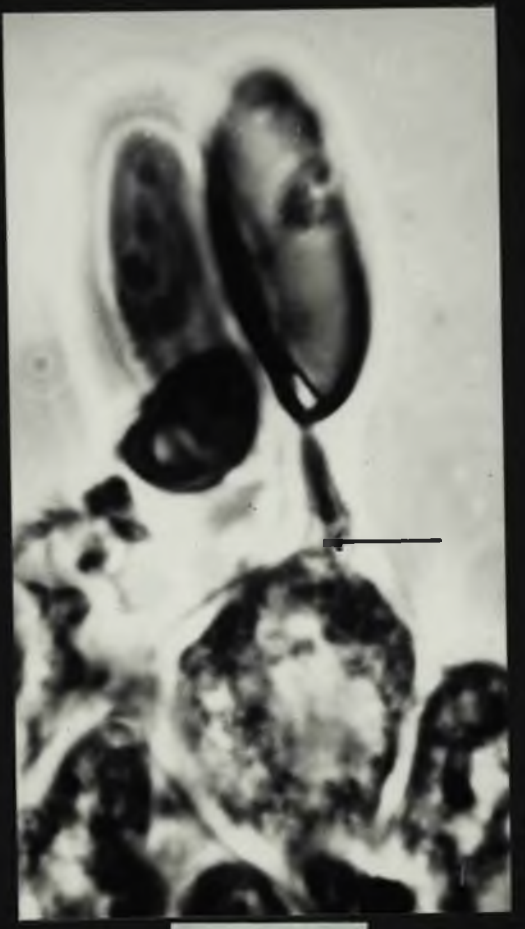

Fig.23

Gomphidius rutilus.

Fig.24. Retrogressive migration complete. Basidium contains four nuclei and bears four (three detached) uninucleate basidiospores. (x 3,000).

Fig. 25. Basidiospores discharged. Four degenerating nuclei remain in highly vacuolate basidium. (x 3,000).

Fig.24

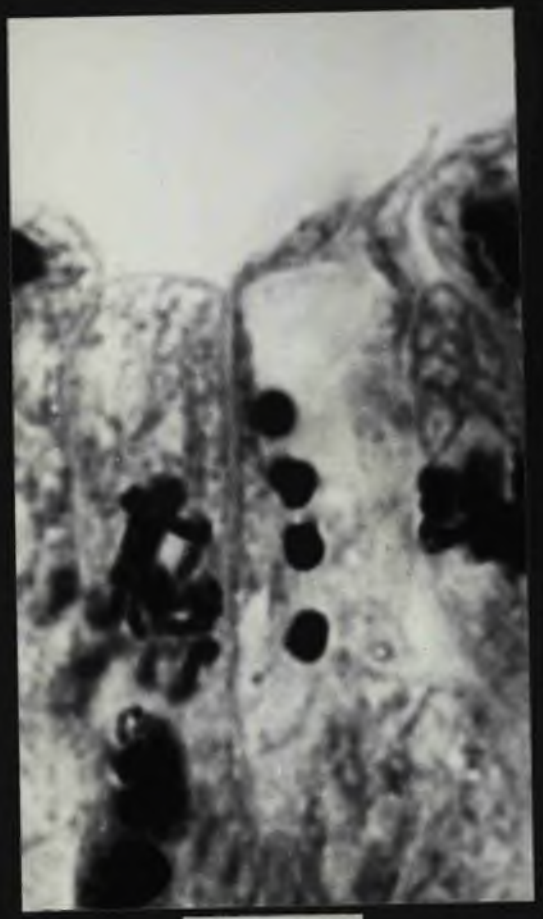

Fig.25

Gomphidius rutilus.

Fig. 26,a,b,. Third nuclear division in basidium. Anomalous behaviour involving division of one of the post-meiotic nuclei . Photographed at different fosal levels. (x 3,000).

Fig. 27. Final stages of meiosisII.(x 3,000).

Fig. 28. Third nuclear division in basidium. Six nuclei present. Basidium does not bear sterigmata. (x3,000).

Fig. 29. Third nuclear division in basidium. Eight expanded nuclei present. Basidium bears sterigmata. (x3,000).

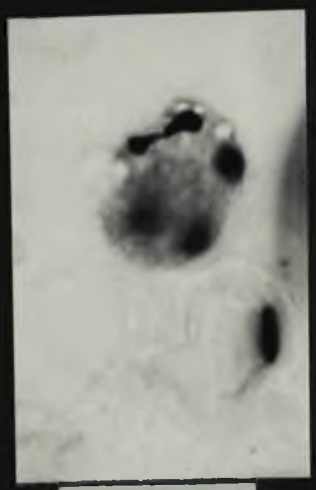

Fig.26a

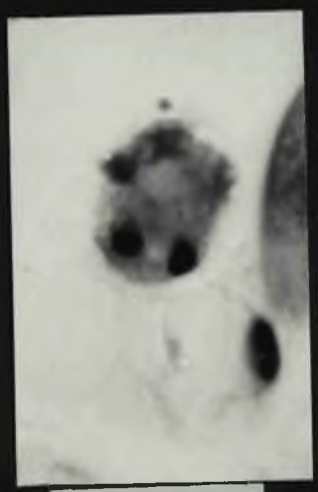

Fig.26b

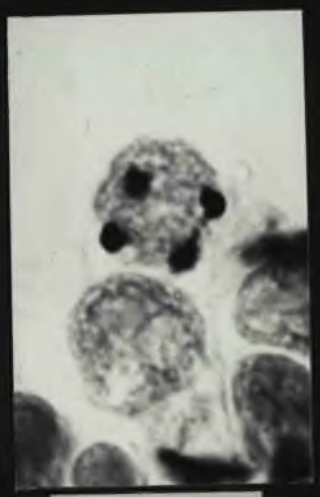

Fig.27

Fig.28

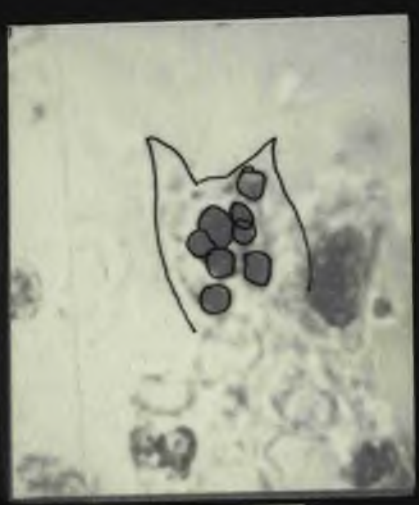

Fig.29

Gomphidius rutilus.

Fig. 30, a,b. Third nuclear division in basidium. Three sterigmata present. Five nuclei in expanded condition in evidence. Photographed at different focal levels. (x3,000).

Fig. 31. Diakinesis. (x 3,000).

Fig. 32. Final conjugate nuclear division in hyphal tip. (x 3,000).

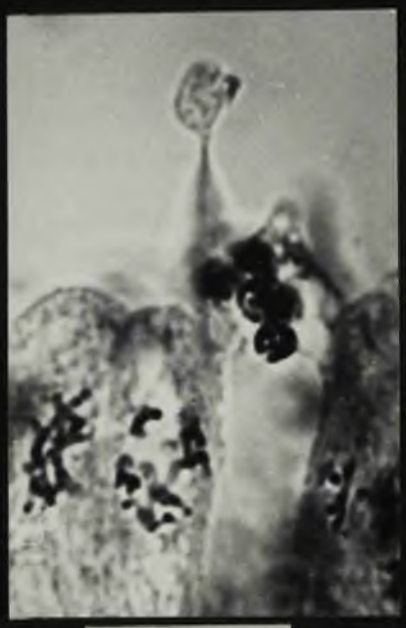

Fig.30a

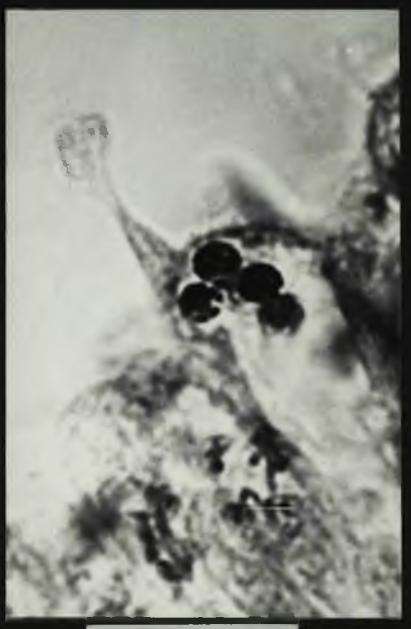

Fig.30b

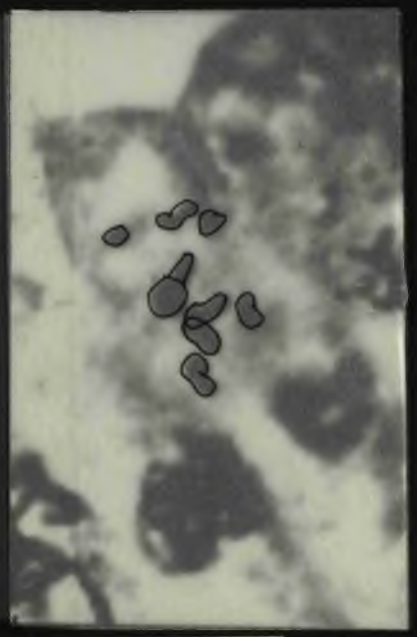

Fig.31

Fig.32

Paxillus involutus.

Fig. 33. Migration of post-meiotic nuclei to basidiospores. Three nucleoli (arrowed) extruded from post-meiotic nuclei evident in basidium. (x 3,000).

Fig. 34. Third nuclear division in basidiospore. Metaphase-anaphase. Two basidiospores only present. (x 3,000).

Fig. 35. Third nuclear division in basidiospore. Late anaphase. Divisional figure in longitudinal axis of basidiospore. Remnants of spindle connect daughter products in basidiospore on right. (x 3,000).

Fig. 36. Final distribution of daughter nuclear products. Mature basidium bearing one of its complement of four uninucleate basidiospores and containing four nuclei which have completed retrogressive migration. (x 3,000).

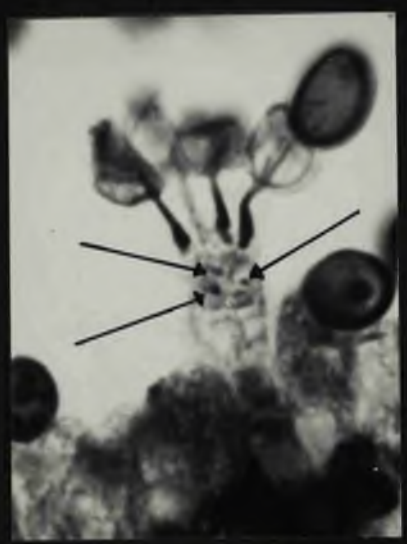

Fig.33

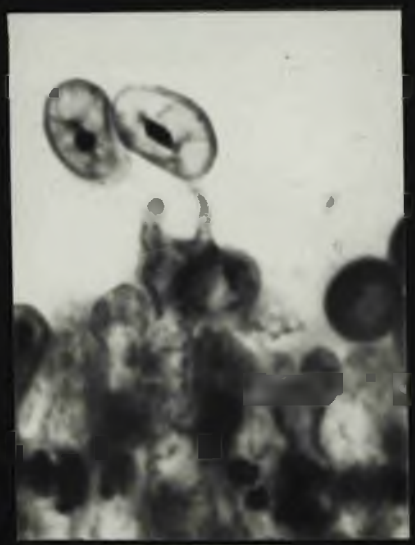

Fig.34

Fig.35

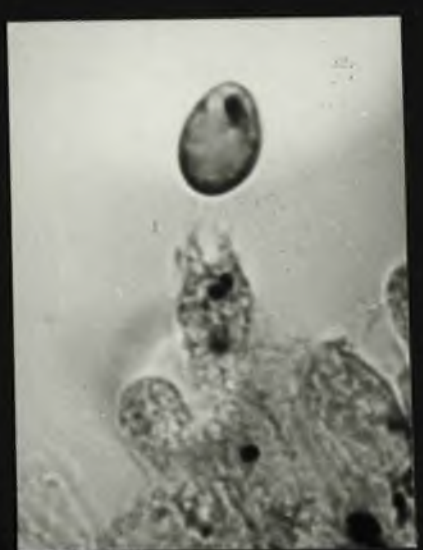

Fig.36

Hygrophoropsis aurantiaca.

Fig. 37. Third nuclear division in basidiospore. Metaphase-anaphase. Note presence of (i), upper fibril (arrowed) connecting nucleus to point at apex of wall of basidiospore, and (ii), lower fibril (arrowed) running in direction of sterigma. (x 3,000).

Fig. 38. Third nuclear division in basidiospore. Late anaphase. Divisional figure in longitudinal axis of basidiospore. Remnants of spindle connect daughter products. (x3,000).

Fig. 39. Third nuclear division. Retrogressive migration of daughter products proximal to sterigmata. One daughter product within basidium. (x 3,000)

Fig. 40. Final distribution of daughter nuclear products. Vacuolate basidium bears four uninucleate basidiospores and contains four nuclei which have completed retrogressive migration. (x3,000)

Fig.37

Fig.38

Fig.39

Fig.40

Hygrophorus nigrescens.

Fig. 41,a. Section of hymenium illustrating (i), final conjugate nuclear division, (ii), basidium containing pair of conjugate nuclei, (iii), basidia containing fusion nucleus in prophase.

Fig. 41,b. Migration of post-meiotic nuclei to basidiospores.

Fig. 41,c. Third nuclear division in basidiospores. Anaphase.

Fig. 41,d. Aged basidium containing four retrogressive nuclei now degenerating,

Fig. 41,e. Mature, naturally shed basidiospores.

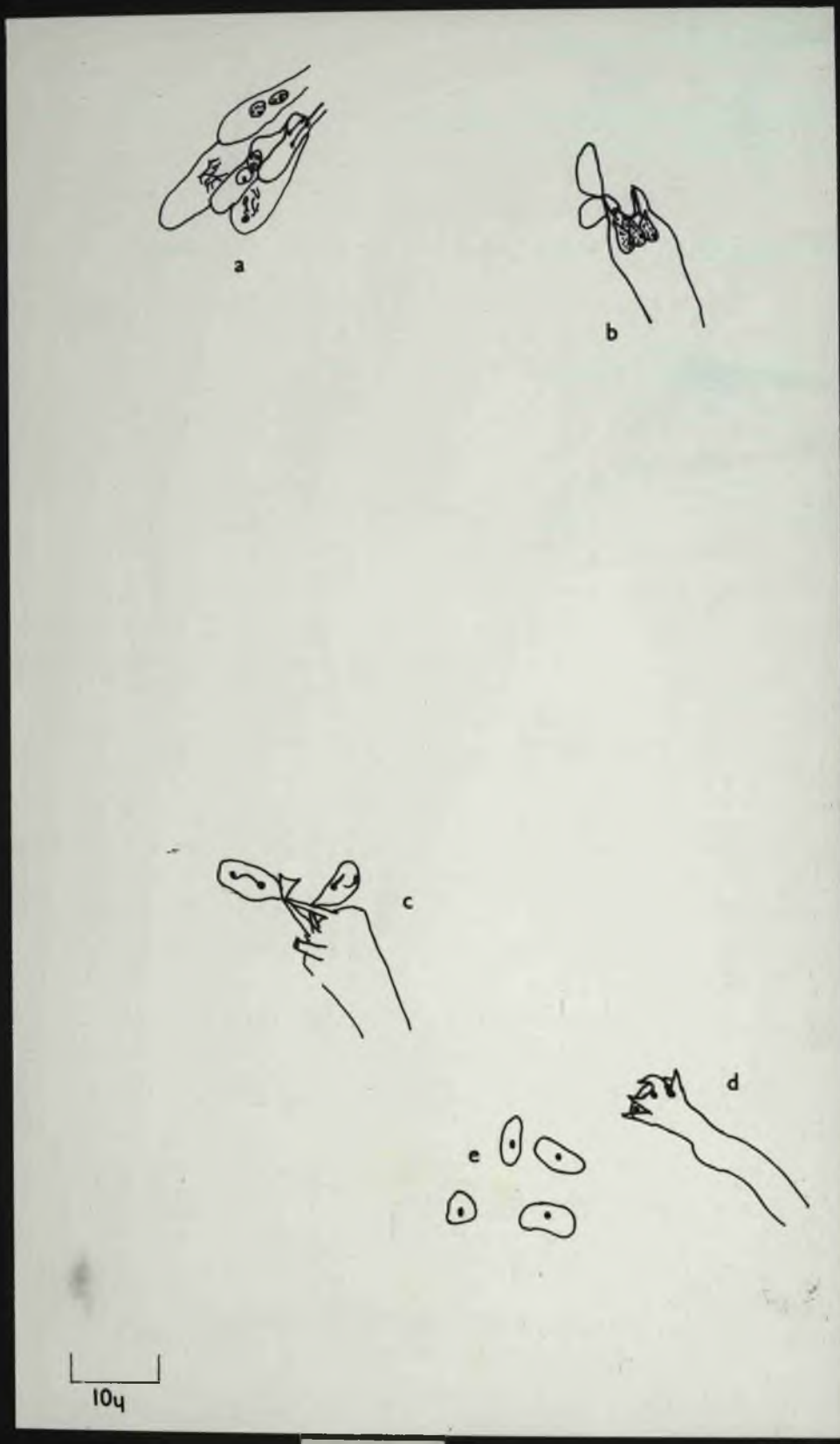

Fig.41

Russula claroflava.

Fig. 42. Migration of post-meiotic nucleus to basidiospore. (x 3,000).

Fig. 43. Third nuclear division in basidiospore. Note anucleate basidium. Only two basidiospores attached. (x3,000).

Fig. 44. Third nuclear division in basidiospore. Anaphase. Only two basidiospores attached. (x 3,000).

Fig. 45. Third nuclear division completed. Basidium bears four uninucleate basidiospores and contains four nuclei which have completed retrogressive migration. (x 3,000).

Fig.42

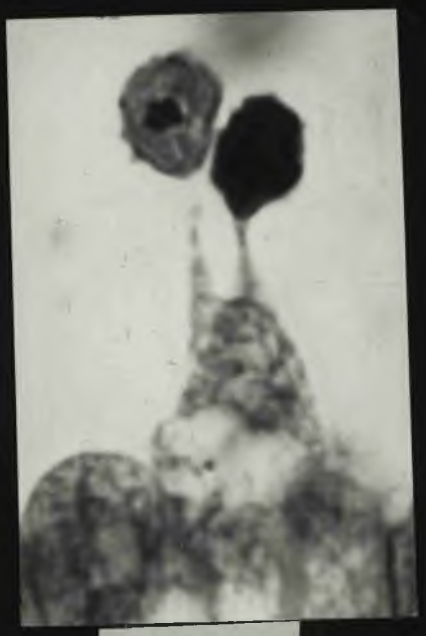

Fig.43

Fig.44

Fig.45

Russula emetica.

Fig. 46,a. Four post-meiotic nuclei in interphase.

Fig. 46,b. Migration of post-meiotic nuclei to basidiospores.

Fig. 46,c. Third nuclear division in basidiospores. Retrogressive migration of daughter nuclear products to basidium.

Fig. 46,d. Third nuclear division completed. Final distribution of daughter nuclear products. Two basidia illustrated.

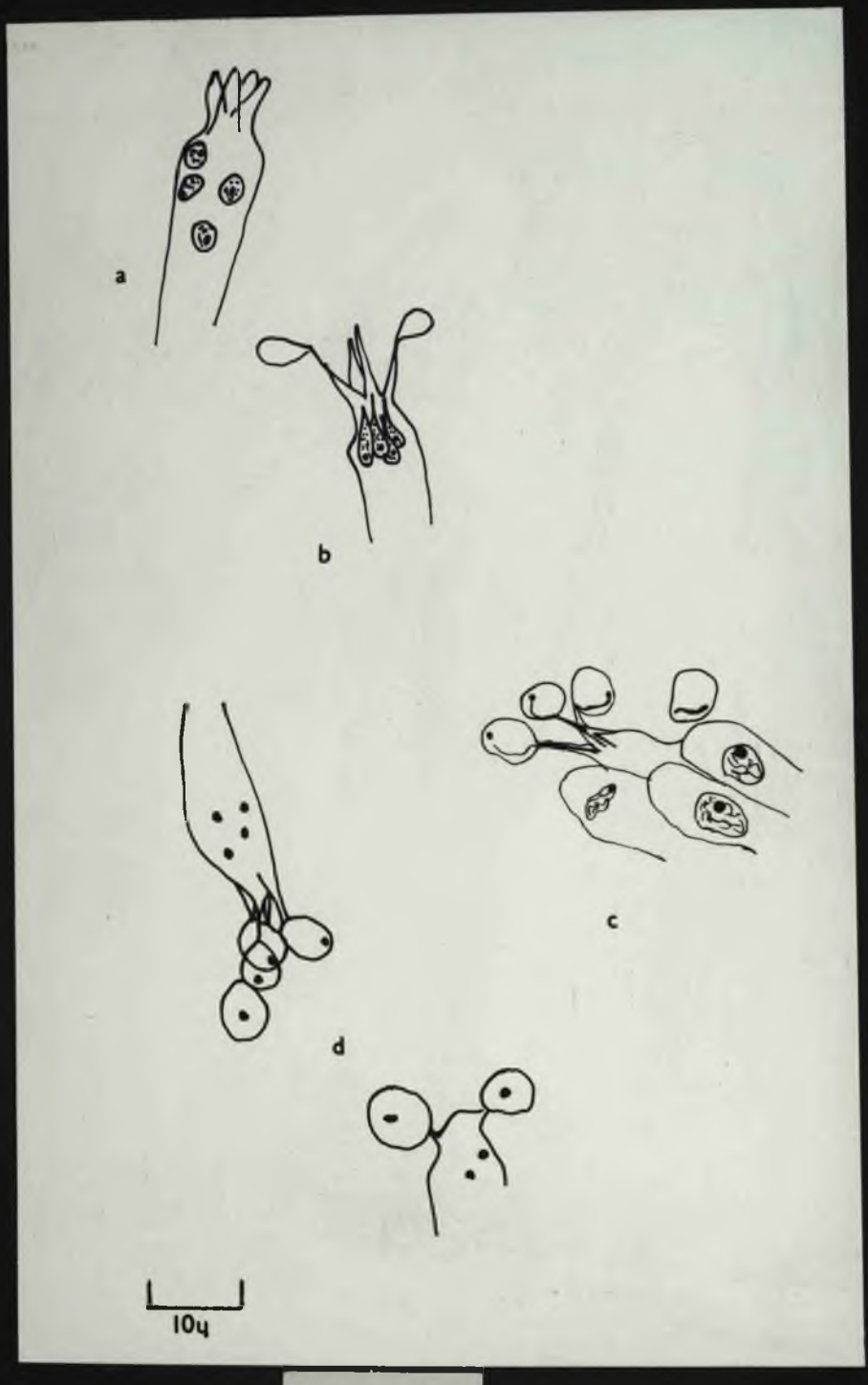

Fig.46

Polyporus brumalis.

Fig. 47. Early stage in migration of post-meiotic nuclei to basidiospores. Chromatin in particulate condition. (x 3,000).

Fig. 48. Later stage in migration of post-meiotic nuclei to basidiospores. Chromatin now highly condensed. (x 3,000).

Fig. 49. Third nuclear division in basidiospore. Metaphase- anaphase. (x 3,000).

Fig. 50. Third nuclear division completed. Basidium bears four uninucleate basidiospores and contains four nuclei which have completed retrogressive migration. (x 3,000).

Fig.47

Fig.48

Fig.49

Fig.50

Coprinus atramentarius.

Fig. 55,a. Post-meiotic nuclei in interphase.

Fig. 55,b. Post-meiotic nuclei migrating towards basidiospores, early stage. Two basidia illustrated.

Fig. 55,c. Post-meiotic nuclei migrating towards basidiospores, later stage. Nuclei now in tear-drop form. Two basidia illustrated.

Fig. 55,d. Third nuclear division in basidiospores. Anaphase.

Fig. 55, e. Mature, naturally shed binucleate basidiospores.

Fig. 55,f. Aged basidia following discharge of basidiospores.

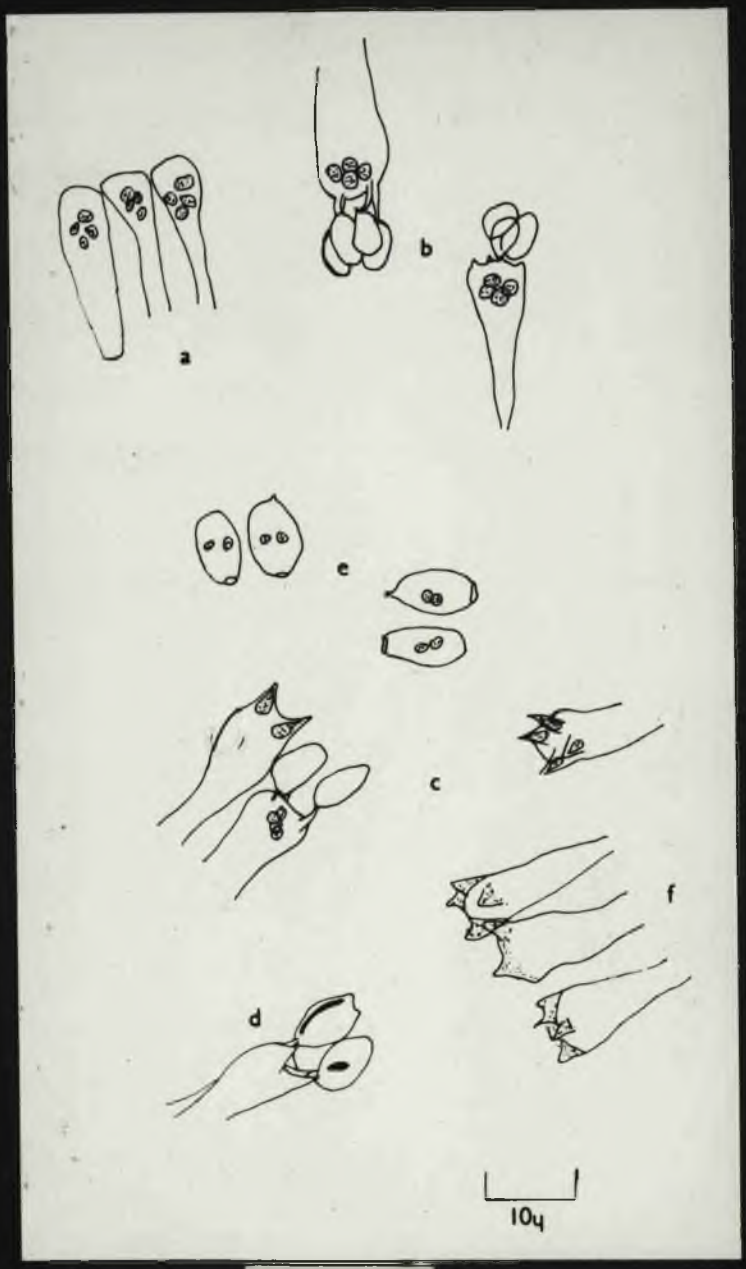

Fig.55

Galerina paludosa.

Fig. 56,a. Section of hymenium illustrating (i), basidium containing fusion nucleus in prophase, (ii),basidium containing four post-meiotic nuclei in interphase, (iii),basidium containing fusion nucleus in late prophase.

Fig. 56,b. Migration of post-meiotic nuclei to basidiospores.

Fig. 56,c. Third nuclear division in basidiospores. Metaphase.

Fig. 56,d. Third nuclear division in basidiospore (arrowed). Anaphase.

Fig. 56, e. Third nuclear division in basidiospore. Telophase.

Fig. 56, f. Mature, naturally shed, binucleate basidiospores.

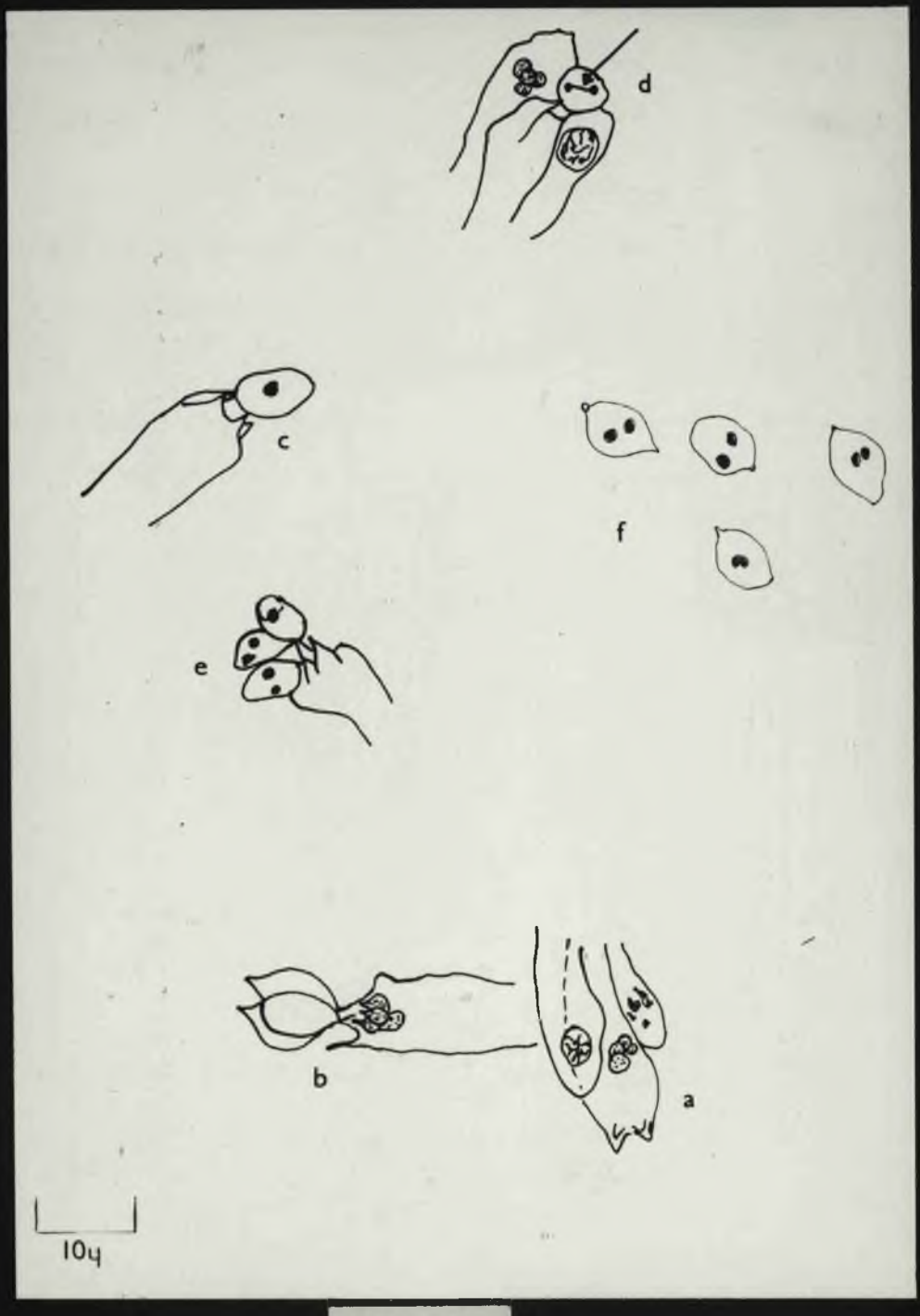

Fig.56

Lepiota lutea.

Fig. 57,a. Final stages of migration of post-meiotic nuclei to basidiospores.

Fig. 57,b. Third nuclear division in basidiospores. Metaphase.

Fig. 57,c. Third nuclear division in basidiospores. Anaphase.

Fig. 57,d. Third nucleaf division in basidiospores completed.

Fig. 57, e. Mature, naturally shed, binucleate basidiospores.

Fig, 57, f. Aged, anucleate basidium.

Fig. 57,g. Trisporic basidium, one basidiospore missing. Migration of post-meiotic nuclei to the basidiospores, the fourth nucleus remaining trapped within the basidium.

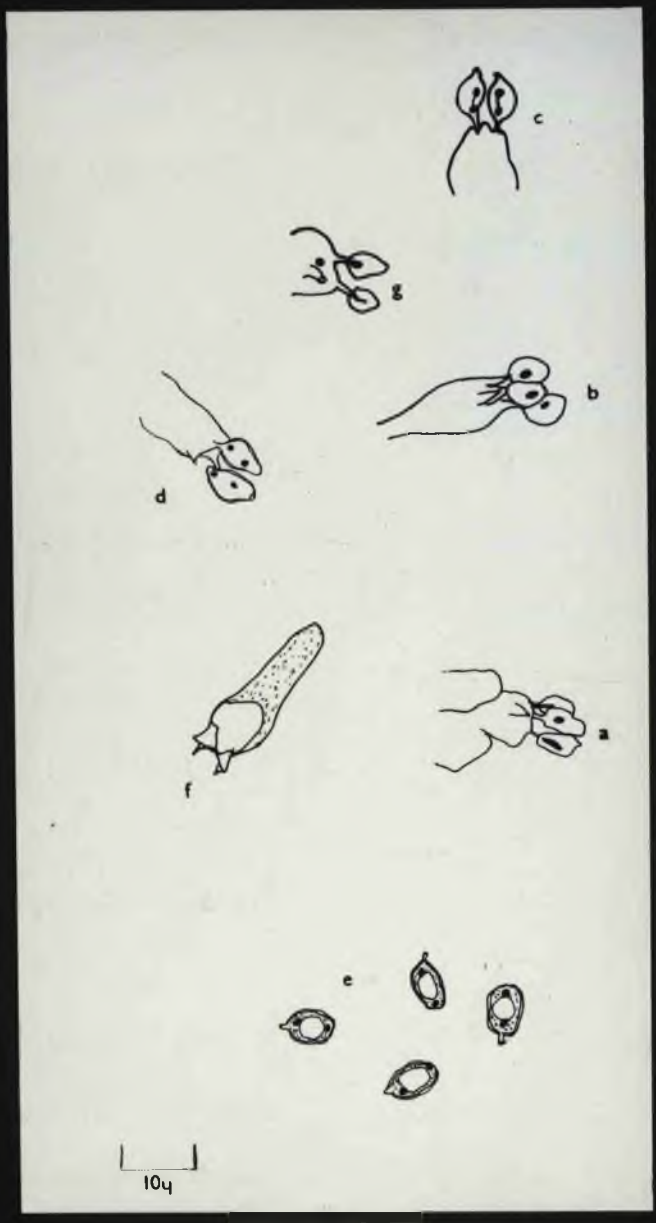

Fig.57

Nolanea papillata.

Fig. 58,a. Section of hymenium showing (i), basidium containing four post-meiotic nuclei, (ii), basidium containing fusion nucleus in prophase, (iii), metaphase of meiosis I near apex of basidium. Note conspicuous nucleoli.

Fig. 58,b. Migration of the four post-meiotic nuclei to basidiospores.

Fig. 58,c. Third nuclear division in basidiospore. Metaphase. Note presence of nucleoli in sterigmata.

Fig. 58,d. Third nuclear division in basidiospore. Metaphase- anaphase.

Fig. 58,e. Third nuclear division in basidiospore completed.

Fig. 58,f. Mature, naturally shed, binucleate basidiospores.

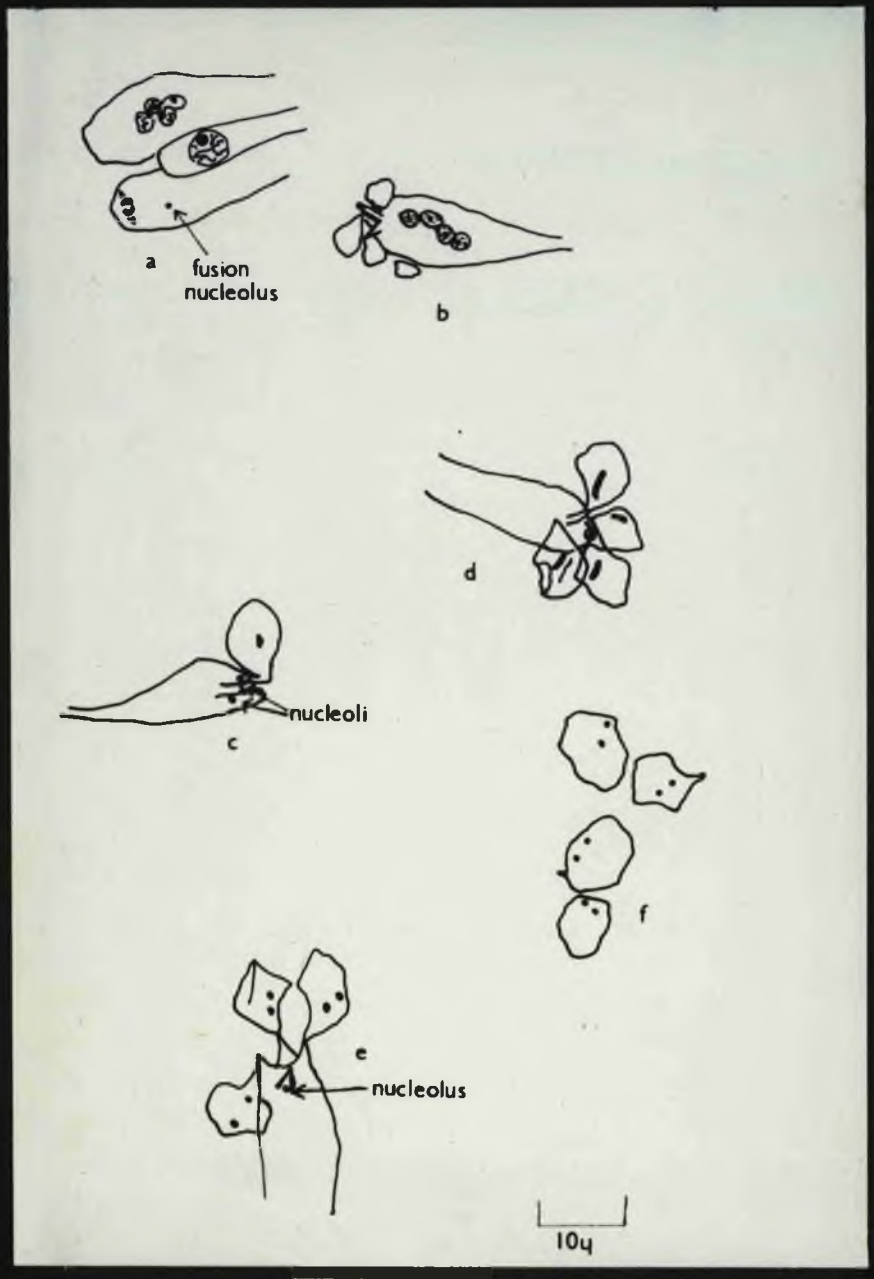

Fig.58

Amanita fulva.

Fig. 59,a. Section of hymenium illustrating (i)basidia containing fusion nucleus in prophase, (ii), metaphase of meiosis I near apex of basidium. Note conspicuous nucleoli.

Fig. 59,b. Basidium containing four post-meiotic nuclei in interphase.

Fig. 59,c. Third nuclear division in basidiospores. Anaphase.

Fig. 59,d. Aged, anucleate basidium. Mature, naturally shed, binucleate basidiospores.

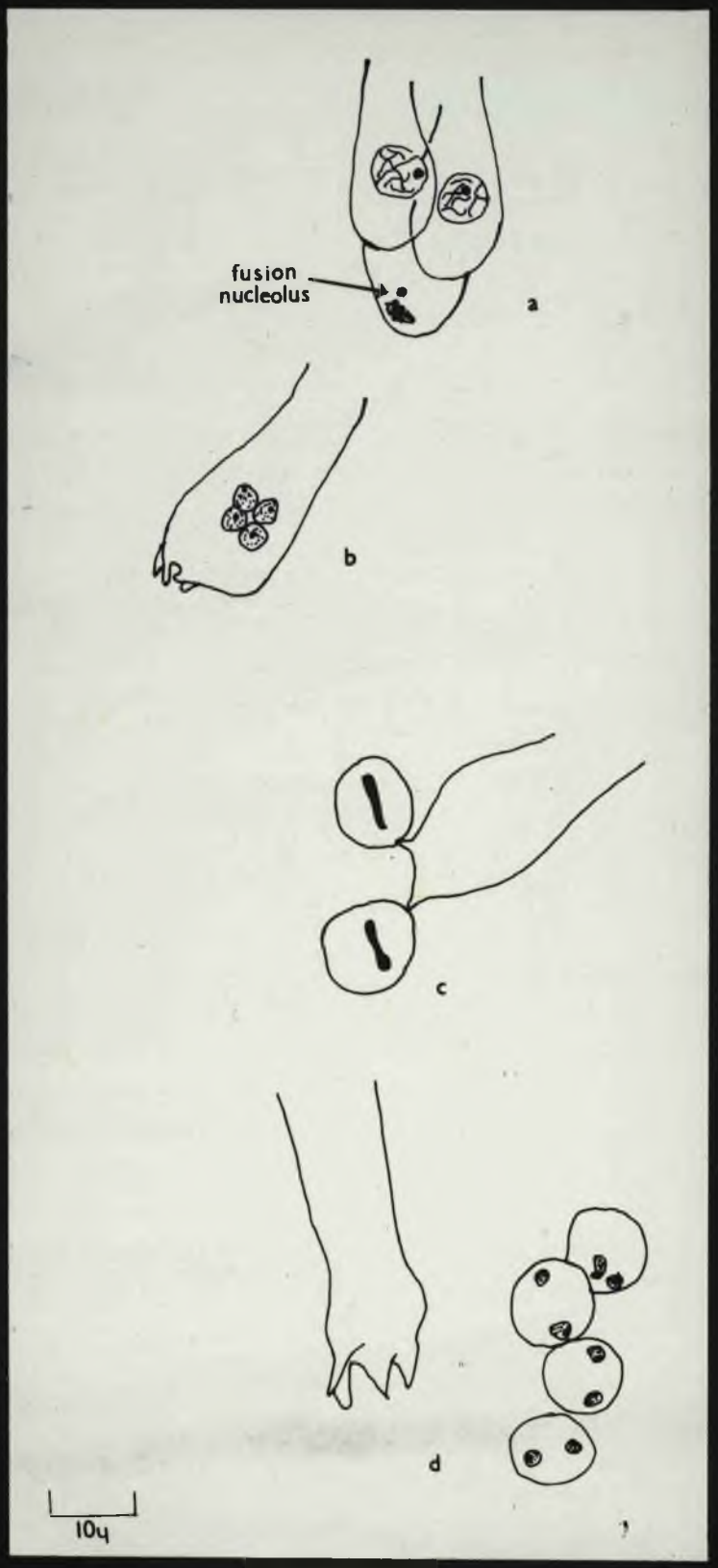

Fig.59

Oudemansiella mucida.

Fig.60,a. Young basidia containing conjugate nuclei. Note conspicuous nucleoli.

Fig. 60,b. Basidium containing fusion nucleus. Fusion nucleolus conspicuous.

Fig. 60,c. Metaphase of meiosis I near apex of basidium. Fusion nucleolus disassociated from chromatin.

Fig. 60d. Four post-meiotic nuclei in interphase.

Fig. 60,e. Migration of postmeiotic nuclei to basidiospores.

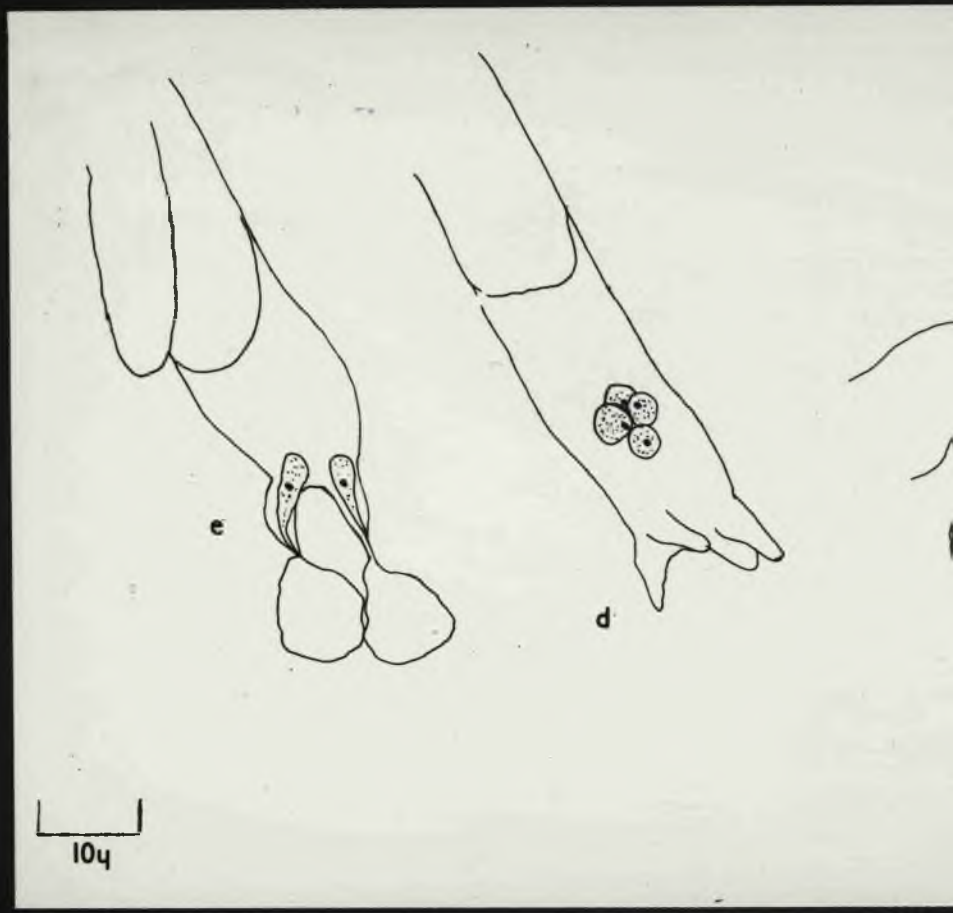

Fig.60

fusion
nucleolus
c
b
a

Oudemansiella mucida.

Fig. 60,f. Third nuclear division in basidiospores. Anaphase.

Fig. 60,g. Aged, anucleate basidium. Basidiospores discharged.

Fig. 60,h. Mature, naturally shed, binucleate basidiospores.

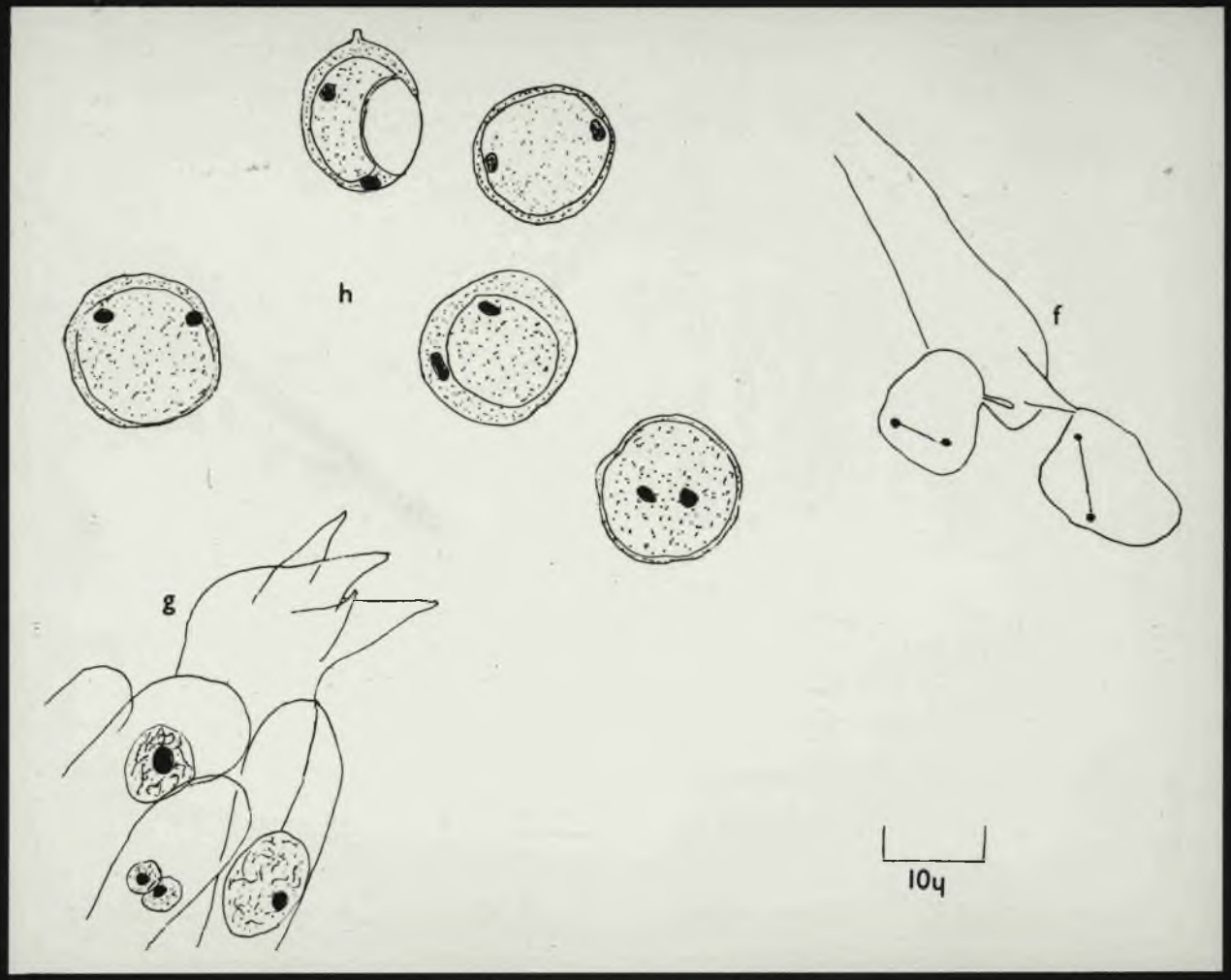

Fig.60

Oudemansiella mucida.

Fig. 61. Migration of post-meiotic nuclei to basidiospores. Note (i), kinetocenter (k) at apex of nucleus, (ii), fibril (f), connecting kinetocenter to locus within basidiospore, (iii), conspicuous nucleoli (n). (x 3,000).

Fig. 62. Third nuclear division in basidiospore. Note (i), kinetocenter (k) terminating spindle, (ii), fibril (f) connecting kinetocenter to wall of basidiospore. (x 3,000).

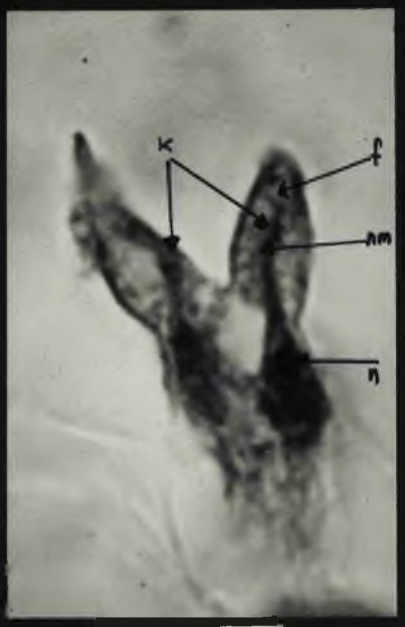

Fig.61

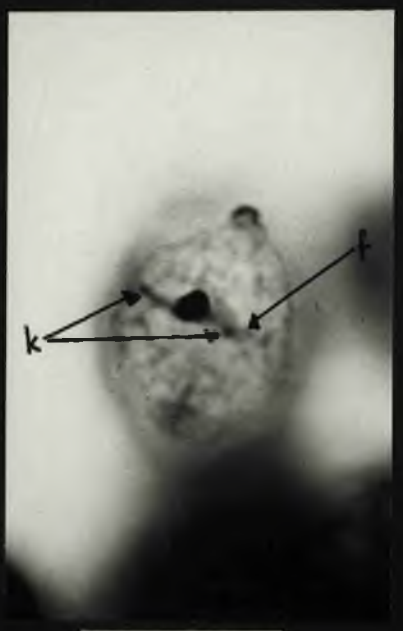

Fig.62

Flammulina velutipes.

Fig. 63,a. Final conjugate nuclear division in hyphal tip.

Fig. 63,b. Migration of post-meiotic nuclei to basidiospores.

Fig. 63,c. Third nuclear division in basidiospore. Metaphase.

Fig. 63,d. Third nuclear division in basidiospore. Anaphase.

Fig. 63,e. Third nuclear division in basidiospore completed.

Fig. 63, f. Mature, naturally shed, binucleate basidiospores.

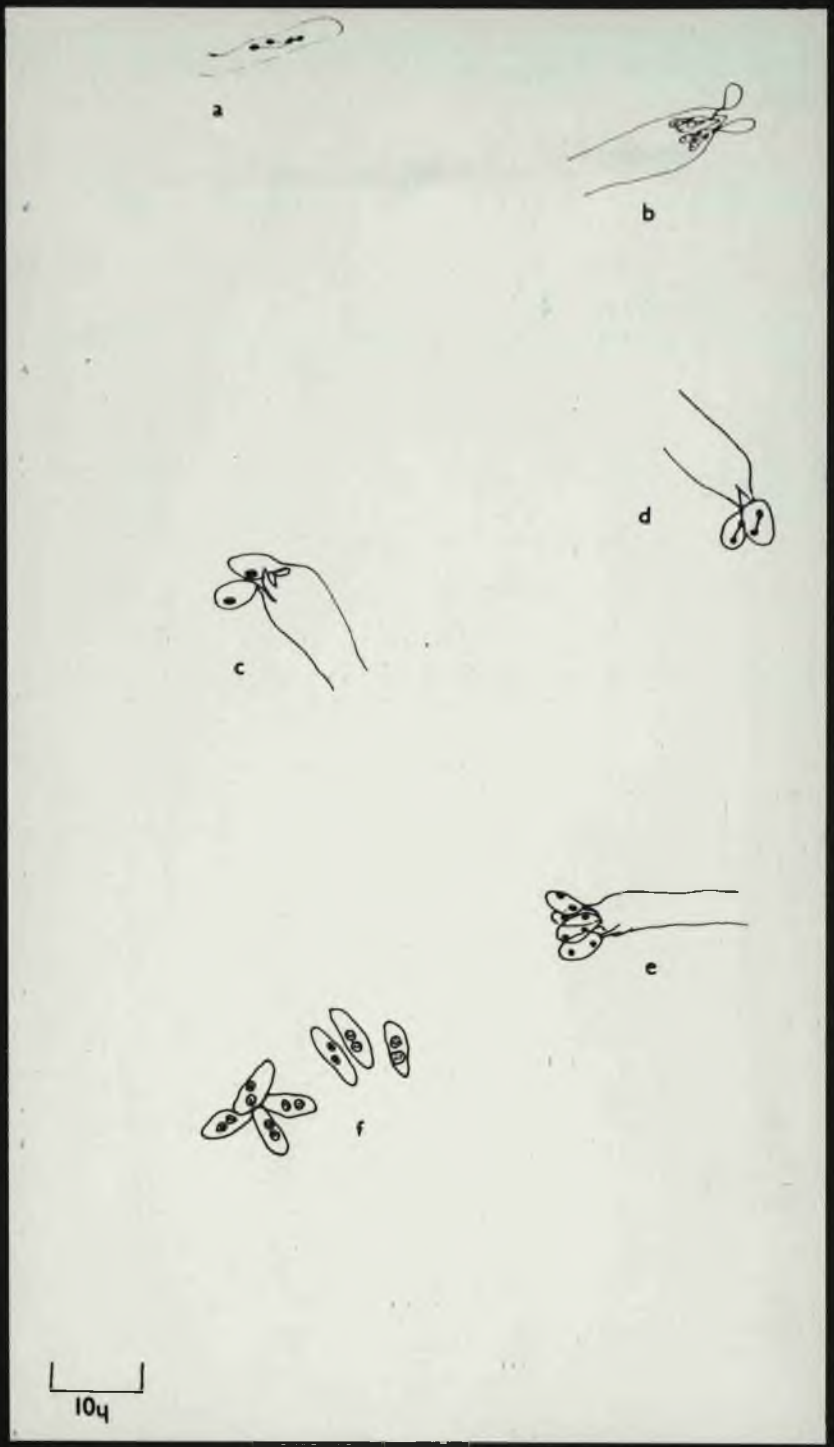

Fig.63

Corticium comedens.

Fig, 64,a. Meiosis I near apex of basidium.

Fig. 64,b. Meiosis II near apex of basidium.

Fig. 64,c. Four post-meiotic nuclei migrating towards sterigmata.

Fig. 64, d. Third nuclear division in basidiospore. Metaphase.

Fig. 64,e. Third nuclear division in basidiospore. Anaphase.

Fig. 64, f. Mature, naturally shed, binucleate basidiospores.

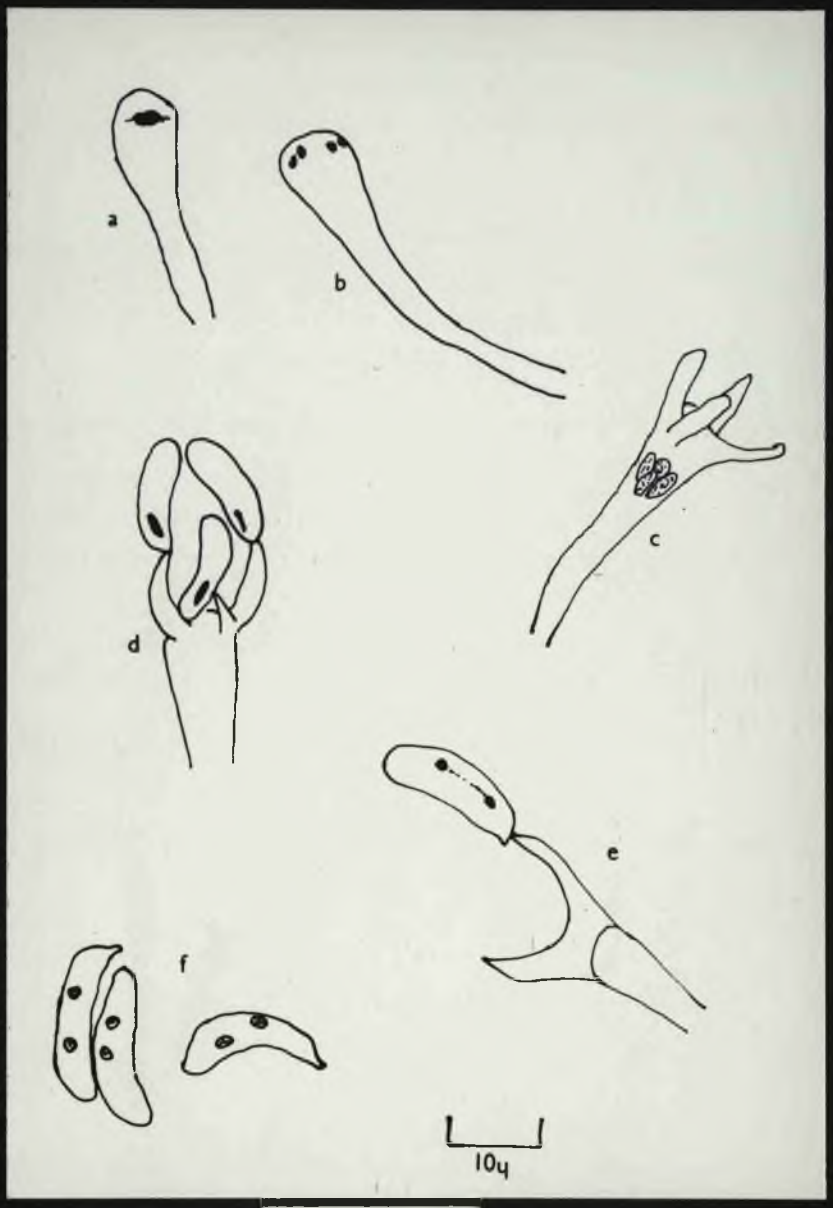

Fig.64

Tremella foliacea.

Fig. 65,a. Post-meiotic nucleus in interphase within epibasidium. Note presence of basidiospore and sterigma.

Fig. 65,b. Mature, naturally shed, binucleate basidiospores.

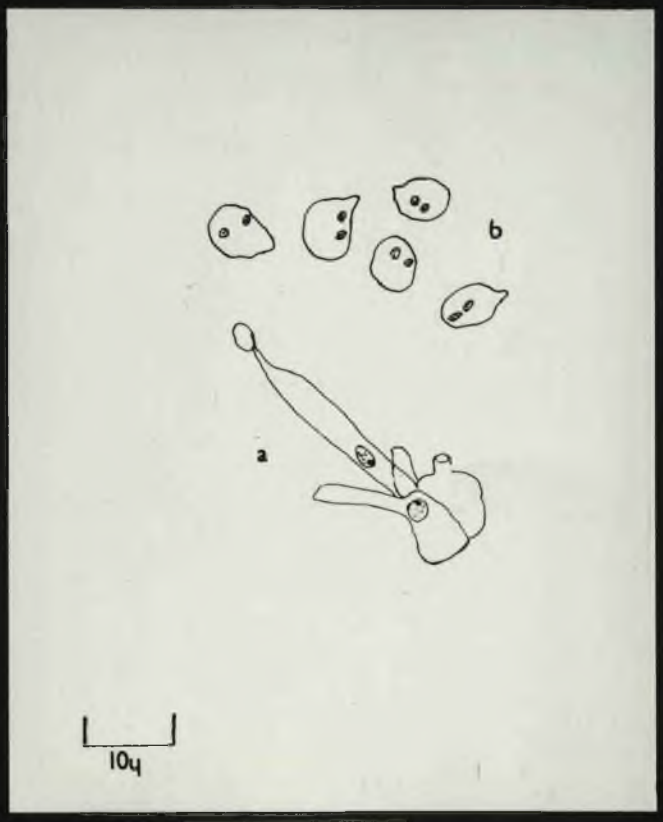

Fig.65

Nolanea cetrata.

Fig. 66. Final conjugate nuclear division in hyphal tip. Note nucleolus disassociated from dividing chromatin. (x 3,000).

Fig. 67. Fusion nucleus. Prophase. Note large fusion nucleolus. (x 3,000).

Fig. 68. Metaphase of meiosis I. Fusion nucleolus disassociated from chromatin. (x 3,000).

Fig. 69. Metaphase of meiosis II. Nucleoli disassociated from chromatin. (x 3,000).

Fig. 70. Four post-meiotic nuclei in interphase. Each nucleus contains a conspicuous nucleolus. (x 3,000).

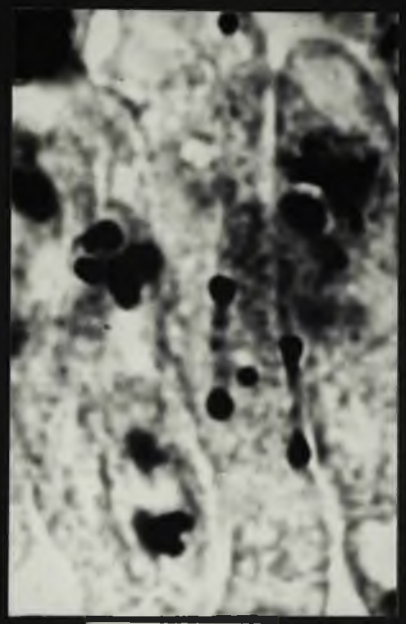

Fig.66

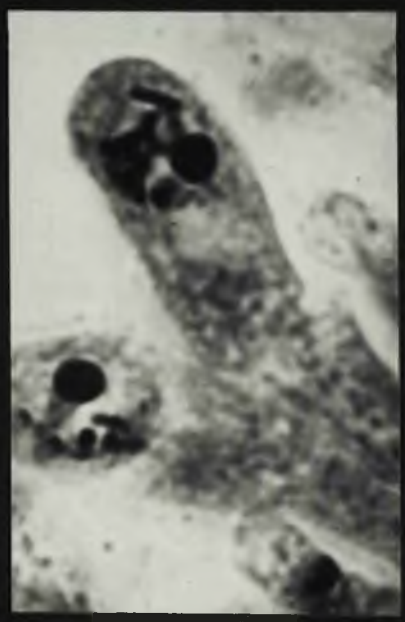

Fig.67

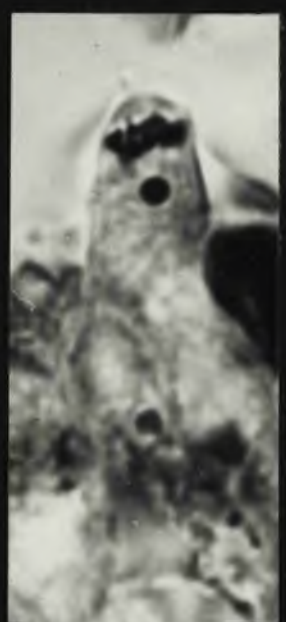

Fig.68

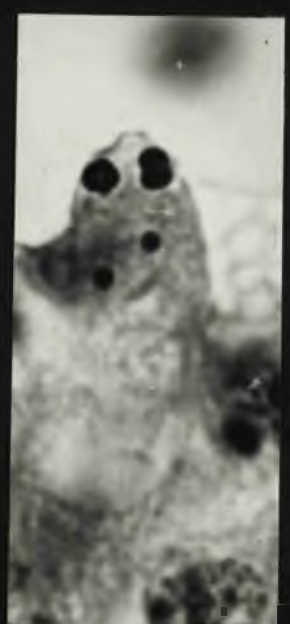

Fig.69

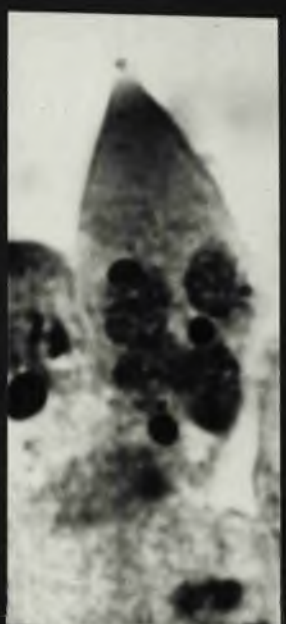

Fig.70

Nolanea cetrata.

Fig. 71. Migration of post-meiotic nuclei to basidiospores. One member of each pair has passed into a basidiospore(both of which are detached) and its nucleolus is left behind in sterigma. Second member of each pair about to pass into basidiospore. (x 3,000).

Fig. 72. Pairs of extruded nucleoli moving into basidium from sterigmata. Basidiospores detached. (x 3,000).

Fig. 73. Third nuclear division in basidiospore. Anaphase. Note, no nucleoli adjacent to chromatin. (x 3,000).

Fig. 74. Mature, tetranucleate basidiospore. (x 3,000).

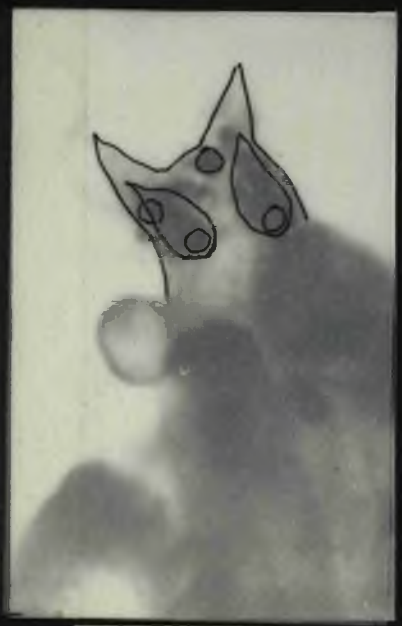

Fig.71

Fig.72

Fig.73

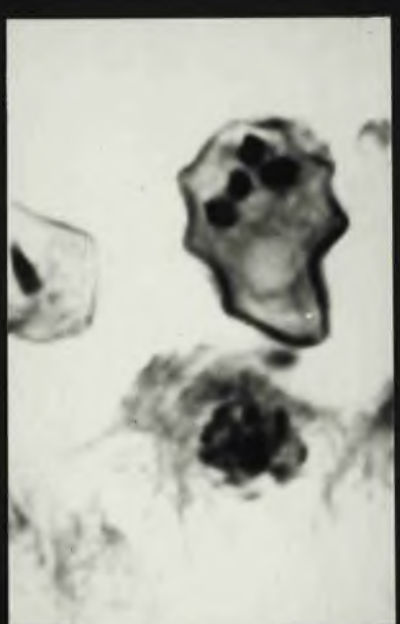

Fig.74

Mycena swartzii.

Fig. 79. Third nuclear division in basidiospores. Retrogressive migration of daughter nuclear product of lower basidiospore to basidium. Corresponding daughter product of upper basidiospore yet to undergo retrogressive migration. (x 3,000).

Fig. 80. Third nuclear division completed. Final distribution of daughter nuclei. Two basidia at similar stage of development. (x,3,000).

Fig. 81,a,b. Bisporic basidium. Third nuclear division in basidiospores. In Fig. 81a the two post-meiotic nuclei which migrated to the basidiospores have divided, and one daughter nucleus is in process of retrogressive migration. In Fig. 81b,the two redundant nuclei can be seen dividing in synchrony with those in the basidiospores. (x3,000).

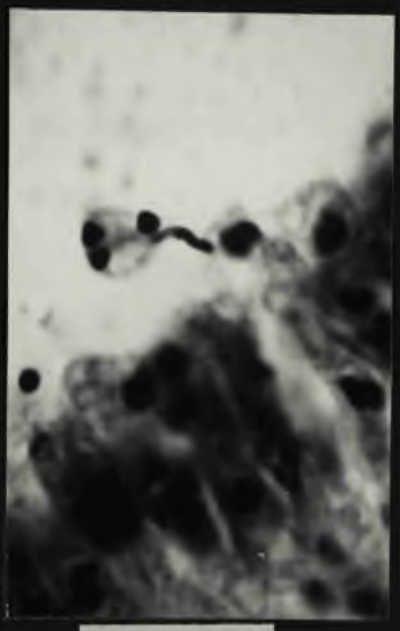

Fig.79

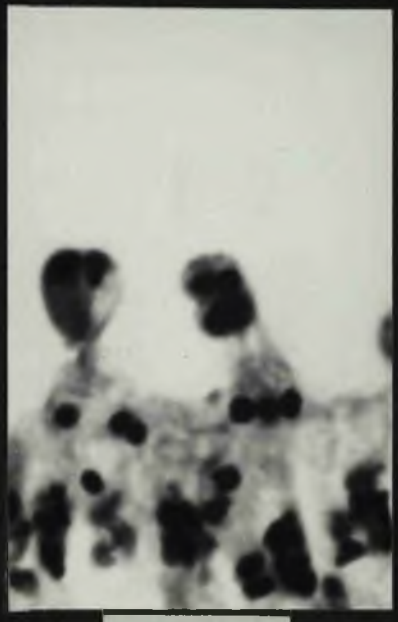

Fig.80

Fig.81a

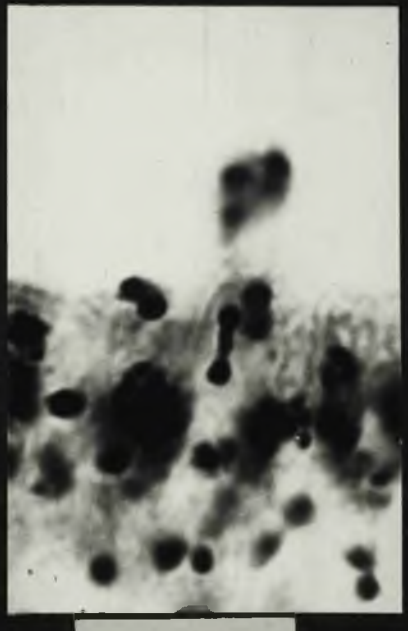

Fig.81b

Fig. 82. Hypothetical phylogenetic classification of the Basidiomycetes based upon Juel (1898) and observed patterns of post-meiotic behaviour.

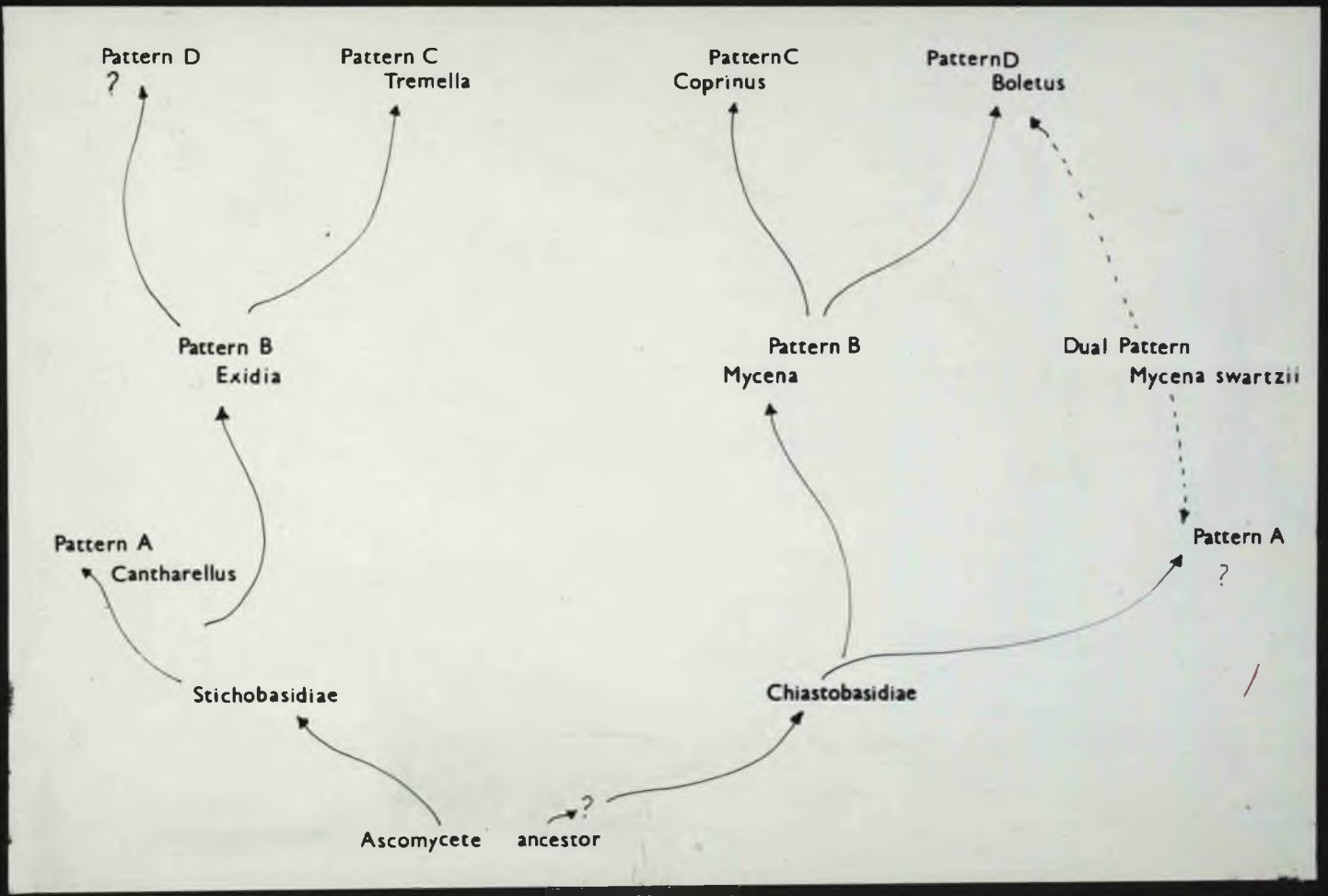
Pattern D
?
Pattern C
Tremella
Pattern B
Exidia
Pattern A
Cantharellus
Stichobasidiae
Ascomycete
?
ancestor
PatternC
Coprinus
PatternD
Boletus
Pattern B
Mycena
Dual Pattern
Mycena swartzii
Pattern A
?
Chiastobasidiae

Fig.82