

NUCLEAR BEHAVIOUR IN *LENZITES REPANDA* (MONT.) FR.

BY

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(with 3 Text-figures)

In the present paper the nuclear phenomena in the life-cycle of *Lenzites repanda* (Mont.) Fr. have been investigated in detail.

The synkaryon formed by nuclear fusion within the binucleate basidium undergoes typical meiotic division. During prophase I, the chromatin reticulum appears together with a prominent nucleolus. This reticulum then resolves into minute chromosomes which form bivalents with their respective homologues ($n=5$). At metaphase I the intranuclear spindle in association with polar centrosomes becomes clearly visible. The orientation of the spindle within the basidium, however, follows no definite order. After the completion of meiotic cycle, a third homotypic division follows giving rise to eight nucleate basidium. A group of four nuclei migrates into four basidiospores, one in each, and the other group of four nuclei degenerates within the basidium.

The primary mycelium originating from a single basidiospore or the secondary one formed as a result of pairing between two primary mycelia of opposite sex are composed of cells containing nuclei of "compact and homogenous" type. The interphase nucleus is frequently associated with a centrosome. These nuclei divide typically by mitosis.

INTRODUCTION

An investigation in the nuclear behaviour in the life-cycle of fungus helps to understand thoroughly the other aspects of its biology. Olive (1953, 1965) in his outstanding review has already presented all the previous works in this line of research which includes almost all groups of fungi.

The present study is an attempt to follow the series of investigation in this line of research that has so far been carried out by Banerjee and his co-workers (1955, 1956, 1957, 1960, 1961, 1962, 1966a, 1966b, 1967, 1969). Those contributions include karyological phenomena in the life-cycles of several species of Thelephoraceae, Polyporaceae and Agaricaceae. From the available literature it appears that no work has yet been done on *Lenzites repanda* (Mont.) Fr. in so far as karyological aspects are concerned.

MATERIAL AND METHODS

The basidiocarps of *Lenzites repanda* (Mont) Fr. were collected fresh from Calcutta and suburbs during the months of July to September, 1965, while growing saprophytically on heavily soaked logs of *Mangifera indica* Linn.

Fresh basidiocarps were cut into small pieces and fixed in different fixatives, namely, 'Sass', 'Carnoy', 'Bouin-Allen' and 'Formal-acetic-alcohol'. The fixation was followed by washing where necessary. Following Ehrlich and McDonough (1959) the materials were partially dehydrated by passing through specific grades of *n*-butanol to avoid difficulties in sectioning. The cut pieces were finally embedded in paraffin. Microtome sections, 5 μ thick, were found to be suitable for this purpose.

In order to study the nuclear phenomena in spores, in germinating spores and in primary and secondary mycelia, the agar film technique of Kniep (1913) modified by Banerjee and Mukherjee (1955) was employed. Thin films of 2% cleared sterile agar were drawn on the upper surface of several grease free sterile slides. Spore deposits were taken aseptically on each of them from pieces of heavily sporulating basidiocarps. Some of the slides were immediately fixed and the rest were incubated at 30°C. in darkness. After the germination of the spores different sets of slides were treated with fixing fluids at intervals of 4 hours, washed where necessary gradually dehydrated in ethanol upto 70% and stored in it.

Similarly, prepared agar films were inoculated with the primary mycelium and secondary mycelium separately on one side and incubated aseptically at 25°C. in moist chambers. As soon as the advancing zone of each type of mycelium reaches the middle portion of the film, the slides were fixed and stored as before.

The staining was done with Heidenhein's Iron-alum Haematoxylin, Crystal violet, Feulgen's reagents, and Methyl-green-pyronin for making permanent preparations. Squash techniques with Aceto-Carmine, Aceto-Orcein, and HCl-Giemsa were also tried. Most satisfactory preparations were obtained with Iron-Haematoxylin and Crystal violet following fixation with 'Sass' and Bouin-Allen'. Crystal violet yielded brilliant preparations but faded gradually in course of time. Repeated trials with Methyl-green-pyronin failed to produce differential staining. Half-an-hour mordanting in 4% Iron-alum solution and staining for one hour in Haematoxylin followed by differentiation in saturated solution of Iron-alum yielded best and permanent result.

OBSERVATIONS

The sequence of events in the nuclear cycle of *L. repanda* has been described from the hymenial layer. The short, terminal, binucleate hyphal cells of the inner surface of each pour tube produce basidia forming the hymenium. The basidia

are parallelly arranged with one another and are at different stages of development. Each slightly swollen terminal cell is thin-walled, binucleate, filled with dense, vacuolated protoplasm, and bears a basal simple clamp-connexion. The two nuclei are placed one above the other at about the midway along its long axis (Text-fig 1, a). They are condensed and of the 'homogenous' type (Pinto-Lopes, 1949). Each nucleus consists of a prominent, central spherical chromatin body, a hyaline surrounding zone of karyolymph and a nuclear membrane ($1.35-1.0 \mu$ in diameter).

With the onset of karyogamy the paired nuclei gradually come closer to each other and the outer walls fuse (Text-fig. 1, a'-e). At first the two chromatin bodies remain side by side within the common wall (Text-fig 1, b-b'). Finally, they unite to form a common stainable mass (Text-fig. 1, c). Neither chromosomes nor several chromatin granules are visible at this stage. The synkaryon thus formed is larger in size ($1.9-2.7 \mu$ in diam.), than the fusing nuclei and it remains near about the middle part of the basidium. Structurally, this nucleus is identical with the nuclei of the terminal cells as described before. The dikaryophasic condition of the basidium thus ends and the short deplophasic stage begins.

The differentiation in shape and size of the basidia continues with the nuclear changes. The synkaryon next migrates to the upper part of the basidium. With the inception of meiosis the metabolic nucleus is at the interphase of the nuclear cycle. It enlarges considerably ($2.7-3 \mu$), nearly converging the greater part of the apical region of the basidium (Text-fig. 1, e'). The chromatin body increases rapidly in size almost occupying the space within the nucleus leaving the nuclear membrane and a narrow zone of karyolymph visible. It also takes up the stains intensely. At this stage, sometime a conspicuous stainable minute spherical body is found to remain closely associated with the nucleus just outside the nuclear membrane ($0.3-1.35 \mu$ diameter). It is the centrosome, whose presence in other basidiomycetes has been reported by previous workers like Maire (1902), Levine (1913), Kuhner (1927), Wakayama (1930, 1932), Goto (1936), Olive (1953), Ward and Ciurysek (1961), Robinow and Bakerspigel (1965), Aist and Wilson (1967), and Motta (1967).

With the onset of prophase the nucleus enlarges ($2.7-3 \mu$ in diameter) further and it can be recognised by its excentric position and remarkable reduction in the size of its chromatin body. The hyaline space within the nucleus appears to be traversed by a delicate reticulum and a nucleolus becomes distinct (Text-fig. 1, d-f). A number of heterochromatin granules are also visible occupying different positions on the reticulum.

As the prophase advances the reticulum resolves into chromosomes which undergo further contraction. The chromosomes are irregularly distributed within the nuclear membrane and are widely spaced (Text-fig. 1, f'). With further condensation and contraction, the chromosomes are seen migrating towards the

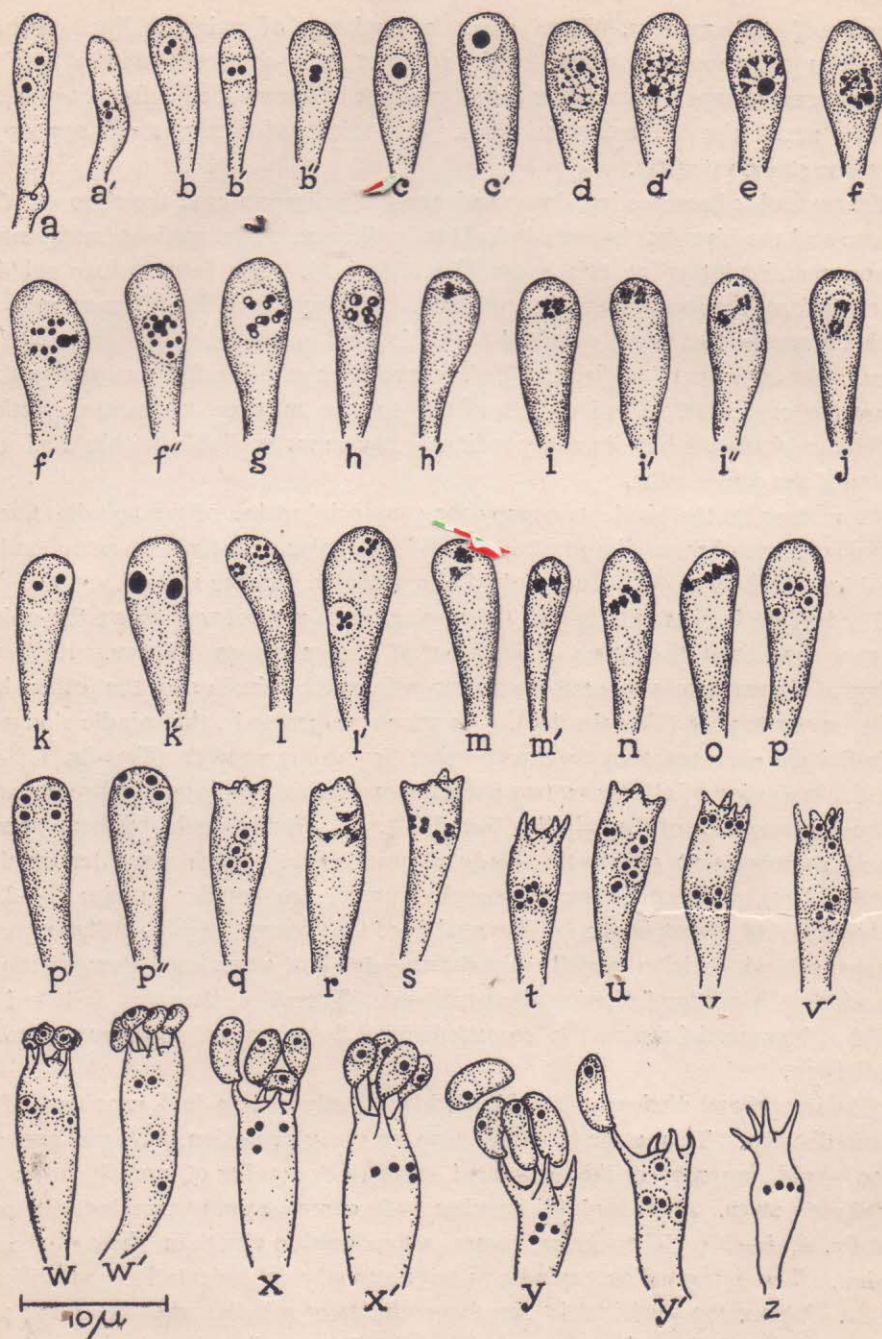
periphery of the nucleus. They show a tendency of pairing. Each pair of homologous chromosomes come to lie adjacent to each other (Text-fig. 1, f-f"). Whether actual synapsis takes place or not can not be ascertained definitely due to extremely small size of the chromosomes. The thickened chromosomes appear to take up the stains intensely.

The nucleolus becomes less dense and gradually disappears at the close of the prophase and the bivalents become individually distinct. The nuclear membrane, however, remains intact at this stage (Text-fig. 1, g). The intranuclear spindle appears before the beginning of metaphase. The bundle of fine fibres appears to originate from the two densely stained bodies located in the cytoplasm and outside the nuclear membrane (Text-fig. 1, h'). Their relation with the fibres suggests that they are the centrioles. The long axis of the spindle may be transverse, vertical and oblique within the basidium depending on the space available within and the position of the centrosome.

At metaphase the bivalents occupy the equatorial region of the spindle fibres. The nuclear membrane disappears. From the polar view they are peripheral in disposition (Text-fig. 1, h). The haploid chromosome number is $n = 5$.

The basidia further enlarge and their apical parts project well above the apices of young basidia. Anaphasic disjunction of chromosomes follows. Half the number of chromosomes move towards one pole of the spindle and the other half to the opposite pole (Text-fig. 1, i). As anaphase proceeds, the spindle elongates and individual chromosomes are drawn apart from one another (Text-fig. 1, i'-i"). With the beginning of telophase two irregular masses of aggregated chromosomes occupy the two poles of the spindle (Text-fig. 1, j). Finally, each of them rounds up and a prominent chromatin body appears within each daughter nucleus (Text-fig. 1, k). The newly formed daughter nuclei are smaller in size ($1-2.4 \mu$ in diameter) and mirror image of the nuclei of the young basidia. These nuclei undergo second division usually simultaneously but sometimes they divide in succession. This second division is equational. Therefore the steps followed by these daughter nuclei during the second division do not exactly correspond to the first division.

The interphase nucleus (Text-fig. 1, k') directly enters into prophase of the second division. This stage is rather rare in the preparation. This is probably due to rapid progress of the divisional stages. A number of nuclei shows the metaphase stage. The haploid number of chromosomes remains the same (Text-fig. 1, l-l"). The chromosomes are reduced in size than those of the first division. The intranuclear spindle varies greatly in orientation in different basidia. When the division is simultaneous, two spindles may be at the same level or they are diversely arranged (Text-fig. 1, m-m'). Anaphasic separation then takes place. Sister chromatids are drawn apart towards opposite poles. The process, beginning from the telophase stage to the reorganisation (Text-fig. 1, n-o)



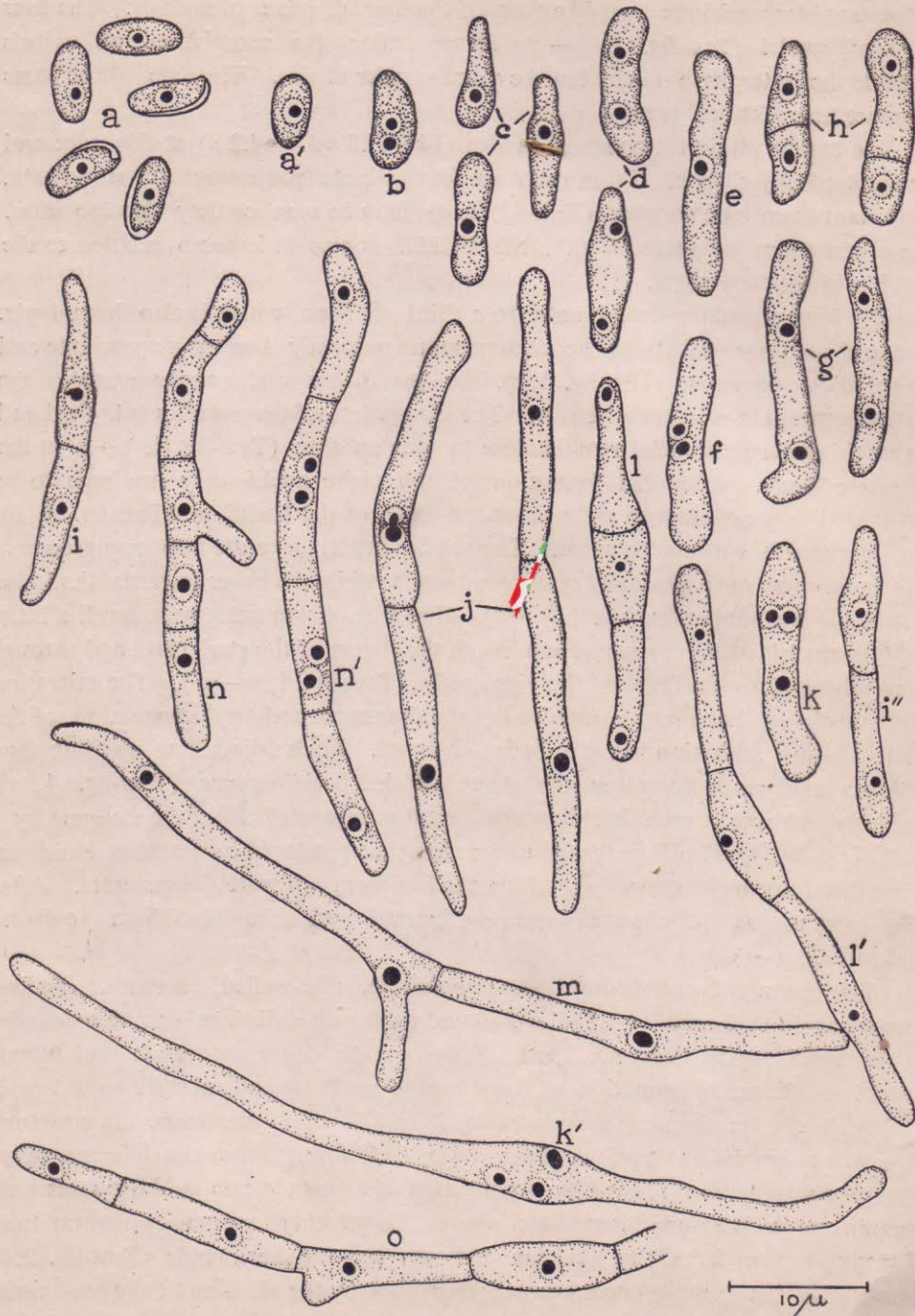
TEXT-FIG. 1. Nuclear phenomena in the basidia of *Lenzites repanda* (Mont.) Fr. (vide TEXT).

of the daughter nuclei are just like that of the first division of meiosis. The four daughter nuclei (Text-fig. 1, p-p'') formed after the completion of meiosis resemble the interphase nuclei but they are smaller in size. They are of the same 'compact homogenous' type.

The basidia attain their maximum size ($14-17.5 \times 3.4-4.2 \mu$) at this stage and become typically clavate. From the apex four minute projections or sterigmata, equidistant from one another (Text-fig. 1, q) may be seen or they develop later. The distribution of nuclei within the basidia appear to have no relation to the position of the sterigmata.

The four daughter nuclei undergo a third division which is also homotypic. Due to the successive division the chromosomes naturally become very minute and the spindles too small (Text-fig. 1, r). In ~~metaphase~~ and anaphase stages the chromosomes are still recognisable. The elongation of the spindles is limited and the resulting pairs of nuclei remain close to one another (Text-fig. 1, s-t) at the telophase stage. After the formation of the eight nuclei they are seen to be distributed in the cytoplasm throughout the body of the basidium (Text-fig. 1, u). The sterigmata further elongate. The eight nuclei separate into two groups of four each, one group gradually moving upwards, while the other towards the lower part of the basidium (Text-fig. 1, v-v'). The tips of the sterigmata swell a little, the four nuclei of the upper group reach the bases of the sterigmata and through them migrate into the four swollen tips singly (Text-fig. 1, w-w'). The migration of the nuclei is, however, successive or simultaneous and any attenuation of the nuclei during migration has not been observed. Each uninucleate swelling then enlarges and gradually takes the shape of the basidiospores (Text-fig. 1, x). During development each basidiospore becomes separated from the sterigma by a wall (Text-fig. 1, x'). Four basidiospores are thus produced by a mature basidium. The other four nuclei remaining within the basidium ultimately degenerate. After the spores are discharged the sterigmata and the basidium collapse (Text-fig. 1, y-z).

The mature basidiospores are uninucleate, thin-walled, smooth, hyaline, somewhat boat-shaped ($4-5.5 \times 1.5-3 \mu$) and each with a distinct excentric apiculus at the basal region (Text-fig. 2, a). They contain dense cytoplasm and minute vacuoles. Before germination a basidiospore swells up and enlarges considerably ($5-7 \times 2-3 \mu$). Its nucleus also increases in size ($2-3 \mu$ in diameter). In structural details it resembles any nucleus of the young basidium. From the different stages of spore-germination it has been found that the uninucleate basidiospores may remain uninucleate and germinate as such producing one or two germ tubes ($2-4 \times 2 \mu$) either from the apicular end or from the both ends (Text-fig. 2, c). Sometimes the basidiospores become binucleate by the division of the pre-existing nucleus (Text-fig. 2, b) and then germination begins in a similar fashion (Text-fig. 2, d). The position of the nucleus in uninucleate spores is not



TEXT-FIG. 2. Nuclear phenomena in the basidiospores and germinating basidiospores of *Lenzites repanda* (Mont.) Fr. (vide TEXT).

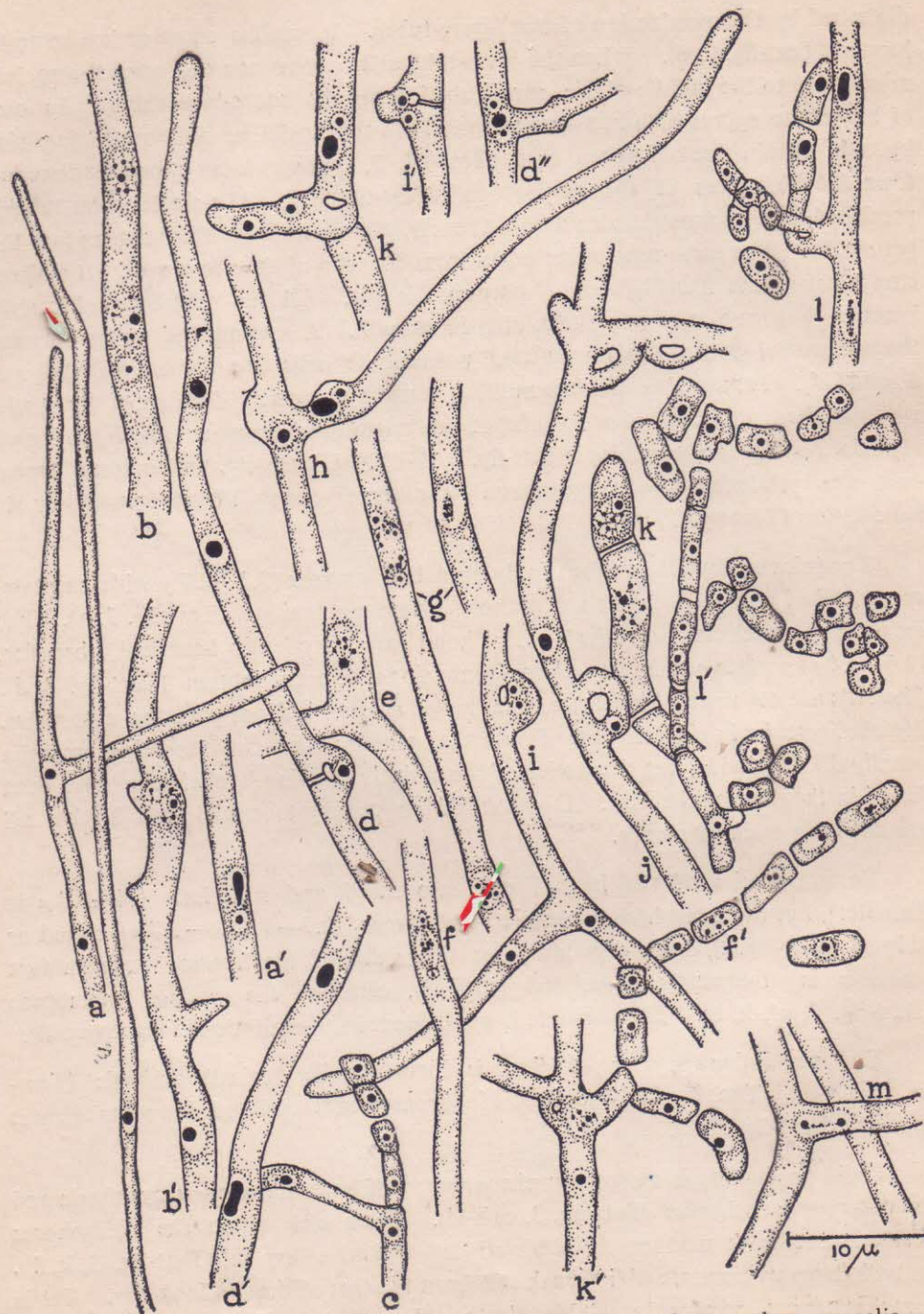
disturbed by the production of two germ-tubes. It remains more or less centrally located (Text-fig. 2, e). When the germ-tube arises from one end only, the nucleus migrates upto the middle of the germ-tube leaving the spore-case empty. In case of binucleate spores with two germ-tubes, both the nuclei, at first remaining side by side within the old spore case (Text-fig. 2, f) migrate in opposite direction towards the apices of the germ-tubes (Text-fig. 2, g). If a single germ-tube is produced, one nucleus remains within the spore-case and the other moves into the germ-tube. The germ-tube never arises from the side of the spore-wall. It widens, elongates and the identity of the spore-case is lost. All the germ-lings ultimately become binucleate at certain stage of their growth. A septum then arises between the two nuclei and in this way two uninucleate cells are formed (Text-fig. 2, h, and i-i'). It may also become multinucleate by repeated division of the nuclei without the formation of septa and at the same time growing rapidly in length ($16-24 \times 2 \mu$) before branching (Text-fig. 2, k-k'). Ultimately, uninucleate hyphal cells are produced by laying down transverse walls simultaneously or in succession (Text-fig. 2, m-o).

The primary mycelium is sparingly branched, narrow ($0.3-2.7 \mu$ wide), septate, composed of uninucleate cells and without clamp-connexions (Text-fig. 3, a). The secondary mycelium, on the other hand are frequently branched, broader, ($0.6-3.4 \mu$) septate and having clamp-connexions at each septum (Text-fig. 3, d). The cells are constantly binucleate, thin walled and with dense granular cytoplasm. Metabolic nuclei in elder hyphae of both the primary and the secondary mycelia usually do not divide, but the nuclei of the actively growing hyphal cells have been found in the divisional stages. Division of the conjugate nuclei in a hyphal cell are not always simultaneous.

Resting nuclei of the hyphal cells are small and spherical ($1.4-2.7 \mu$ in diameter), but the interphase nuclei are much larger ($2.7-4 \times 1.4-2.7 \mu$), round or oblong (Text-fig. 3, d-d') depending on the width of the hyphae. A prominent centriole is frequently associated with it, outside the nuclear membrane (Text-fig. 3, a', k, h). The centriole is not evident in the mitotically inactive cells.

The vegetative nuclear division is found to be typically mitotic (Text-fig. 3, b, e-g). The haploid number of chromosomes counted in polar view is 5 ($n=5$) (Text-fig. 3, f').

Oidia production is a common phenomenon of both the monokaryophasic and the dikaryophasic hyphae (Text-fig. 3, c, k-l'). The nuclei within the oidiophores divide in a similar manner as observed in vegetative hyphae (Text-fig. 3, k, f'). In both cases uninucleate oidia break out from the tips of the oidiophores. But in some instances occurrence of binucleate oidia (Text-fig. 3, l) from dikaryotic mycelium was also observed (Vandendries and Martens, 1932).



TEXT FIG. 3. Nuclear phenomena in the primary and secondary mycelia of *Lenzites repanda* (Mont.) Fr. (vide TEXT).

DISCUSSION

The nuclear phenomena within the basidia of *L. repanda* show striking similarity with those of previous investigations carried out by Banerjee *et al.* (1955, 1956, 1957, 1960, 1961, 1962, 1966a, 1966b, 1967, 1969). The formation of a synkaryon within originally dikaryotic basidium initiates a short diplophase. This large interphase nucleus with a prominent nucleolus then enters the meiotic cycle. The successive stages of prophase I have not, however, been possible to distinguish in a well defined order. Of them, the formation of chromatin reticulum with distinct chromosomes eventually resolving into definite minute chromosomes, the delayed formation of bivalents and the stage of diakinesis have been observed with sufficient accuracy. The appearance of chromocentres on the chromatin reticulum has also been observed by Wakayama (1930, 1932), and Banerjee *et al.* (1955, 1956, 1957, 1960, 1961, 1962, 1966a, 1966b, 1967, 1969). The spindles are intranuclear in origin in all cases. The association of polar centrosomes with the spindles and with the 'interphase' nuclei has been clearly demonstrated in most cases. The number of haploid chromosomes of *L. repanda* has been counted to be $n=5$.

After completion of the meiotic cycle the diploid nucleus gives rise to four haploid daughter nuclei. Following another homotypic division the basidium becomes eight nucleate. This type of division of the tetracyte nuclei has also been observed by Goto (1936), Biggs (1938), Kuhner (1947), and Banerjee *et al.* (1956, 1960, 1961, 1966a, 1966b, 1967, 1969). The significance of this duplication can not, however, be explained at the present state of our knowledge.

Though no difference of opinion exists regarding the nature of meiotic division of the synkaryon within the basidium yet the mitotic division of the vegetative nuclei has been questioned by many investigators like Robinow *et al.* (1957, 1962, 1965), Bakerspigel (1959), Lamour (1958), Widra (1959), Brushaler, Wilson and Aist (1967), Saksena (1961b) and others. At the same time, the existence of typical mitotic division in the vegetative cells has been supported by Olive (1953), McGinnis (1953), Knox-Davies and Dickson (1960), Ward and Ciurysek (1961), Motta (1967), and others.

The present observations regarding nuclear division in the vegetative phase strongly support the view that the division is typically mitotic.

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