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CONTRIBUTIONS TO THE CYTOLOGY OF HYMENOMYCETES: IV. CYTOLOGICAL STUDIES IN *LENTINUS SUBNUDUS* BERK. AND *LENTINUS PRAERIGIDUS* BERK.

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(with 2 Text-Figures)

Nuclear phenomena in the life-cycles of *Lentinus subnudus* and *Lentinus praerigidus* have been worked out and found to be in full agreement with their 'heterothallic' and sexually 'bipolar' natures.

Heidenhein's Iron-Alum-Haematoxylin staining yielded satisfactory preparations when used after fixation with standard killing and fixing fluids.

In both the species each basidiospore, when first formed, receives only one nucleus which prior to germination may or may not divide again. It germinates to produce a septate primary mycelium of uninucleate cells and without clamp-connexions.

Secondary mycelia, of both binucleate (conjugate pair) and multinucleate hyphal cells, in both the species are obtained by pairing, compatible primary mycelia. The clamp-connexions, however, occur much infrequently.

In both the species, the young badidium is always binucleate and the nuclei soon fuse to form a large fusion-nucleus. This fusion-nucleus divides reductionally to produce four haploid nuclei. The orientation of the first-division-spindle is transverse in *L. subnudus* and longitudinal in *L. praerigidus* while that of the spindles during second-division is extremely variable. Each of the tetracyte nuclei finally enters a spore. The basidium, after spore-discharge, becomes empty and gradually collapses.

The haploid and diploid chromosome numbers in both the species have been found to be 3 and 6 respectively.

Introduction

In the past quite a large number of Agaricaceae have been cytologically investigated by Sass (1929), Wakayama (1930) and others, and Olive (1953) has given an excellent review on the subject. From the available records it is evident that, so far as the genus *Lentinus* is concerned, very little cytological details are known, *Lentinus variabilis* being the only species which has been investigated by Kühner (1928). The present communication reports on the karyological phenomena in the life-cycles of *Lentinus subnudus* Berk. and *Lentinus praerigidus* Berk., the two noteworthy species of Agaricaceae of subtropical India, and forms a part of the series of investigations taken up by Banerjee and his co-workers (1955, 1956).

In conformity with the modern tendency of seeking a cytological basis of the sexual behaviour of Hymenomycetes, the object of this investigation, apart from the karyological study of the life-cycles in the main, is to look for a correlation of the data obtained by the writers (1956) during their studies on heterothallism.

Material and methods

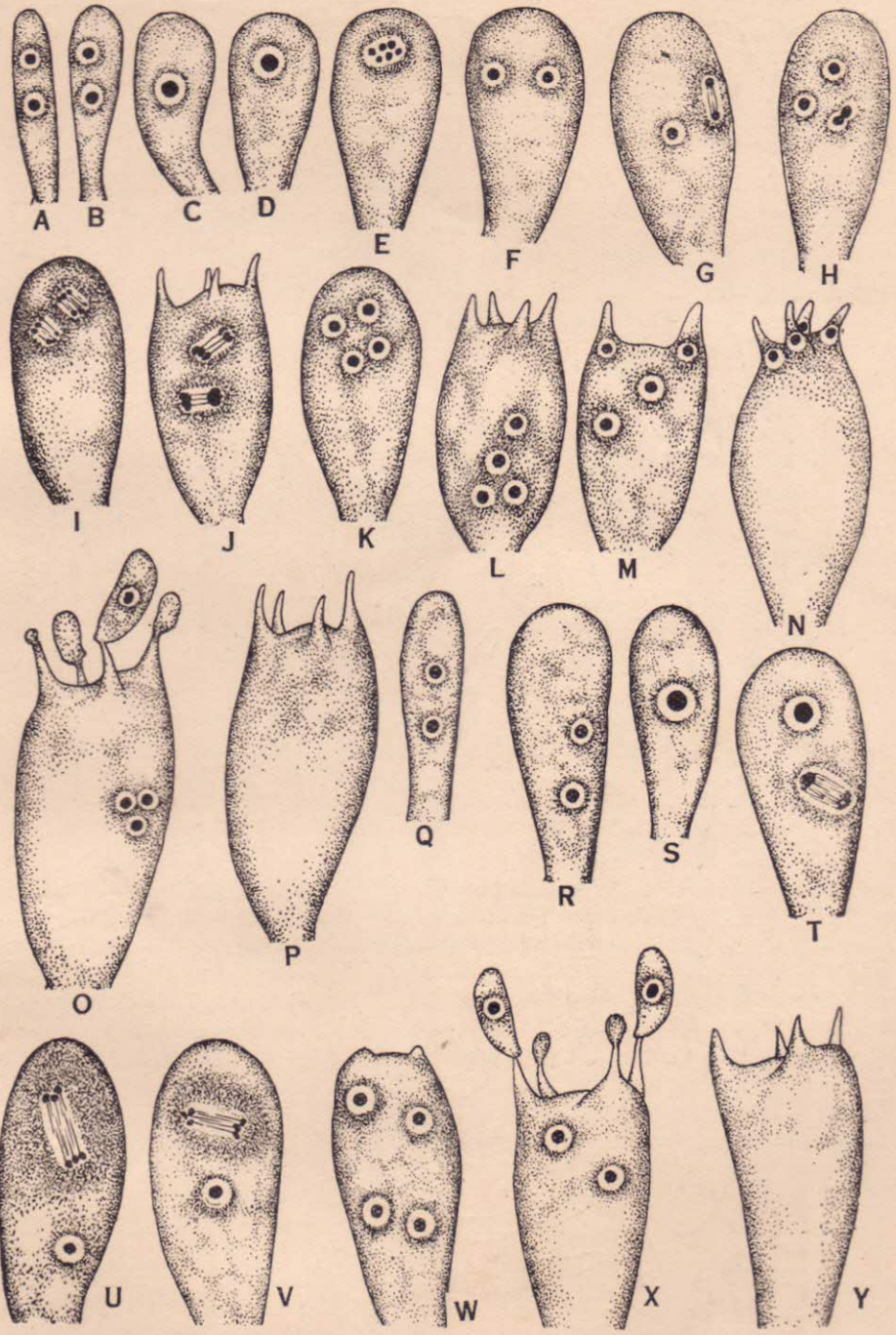
For studying the nuclear conditions in basidia, fresh fruit-bodies of both the fungi were fixed in several fixing fluids at different intervals of time between 1 to 3 a.m. The fixing fluids used were 'Flemming' (weak and strong), 'La Cour (2BD)', 'Bouin-Allen', 'Sass', 'Chromo-Acetic acid', 'Formal-Acetic-Alcohol (weak and strong), and 'Navaschin (A and B)'. Initial fixation was done under reduced atmospheric pressure to facilitate rapid penetration of the fixing fluids. After fixation washing, dehydration and embedding were done following the standard alcohol-chloroform schedule. Microtome sections, 8μ in thickness, were found to be suitable for observations. Several stains, such as Heidenhein's Iron-Alum-Haematoxylin, Crystal Violet (1% aq. soln.) and aqueous Basic Fuchsine, were tried. The first one yielded very satisfactory result when used after fixation with 'Flemming (Strong)' or 'La Cour (2BD)'.

In order to study the karyological conditions in the spores, germinating spores and primary and secondary mycelia, Kniep's (1913) agar-film technique and that modified by Banerjee (1958) were employed. Spore-deposits were directly taken on thin films of cleared 2% *malt-agar* on sterile slides. Preparations for spore-cytology were immediately fixed in all the fixatives mentioned above, while those for germinating stages were incubated at room temperature (28–30°C.) and fixed when the desired stages of germination were obtained. Total preparations of primary and secondary mycelia were also prepared. The nuclei of the spores and germinating spores were most satisfactorily fixed with 'Chromo-Acetic acid' and 'Sass'. 'Flemming (weak)' and Chromo-Acetic acid', on the other hand, gave best results for primary and secondary mycelia. In all these preparations Iron-Alum-Haematoxylin proved to be the most satisfactory one.

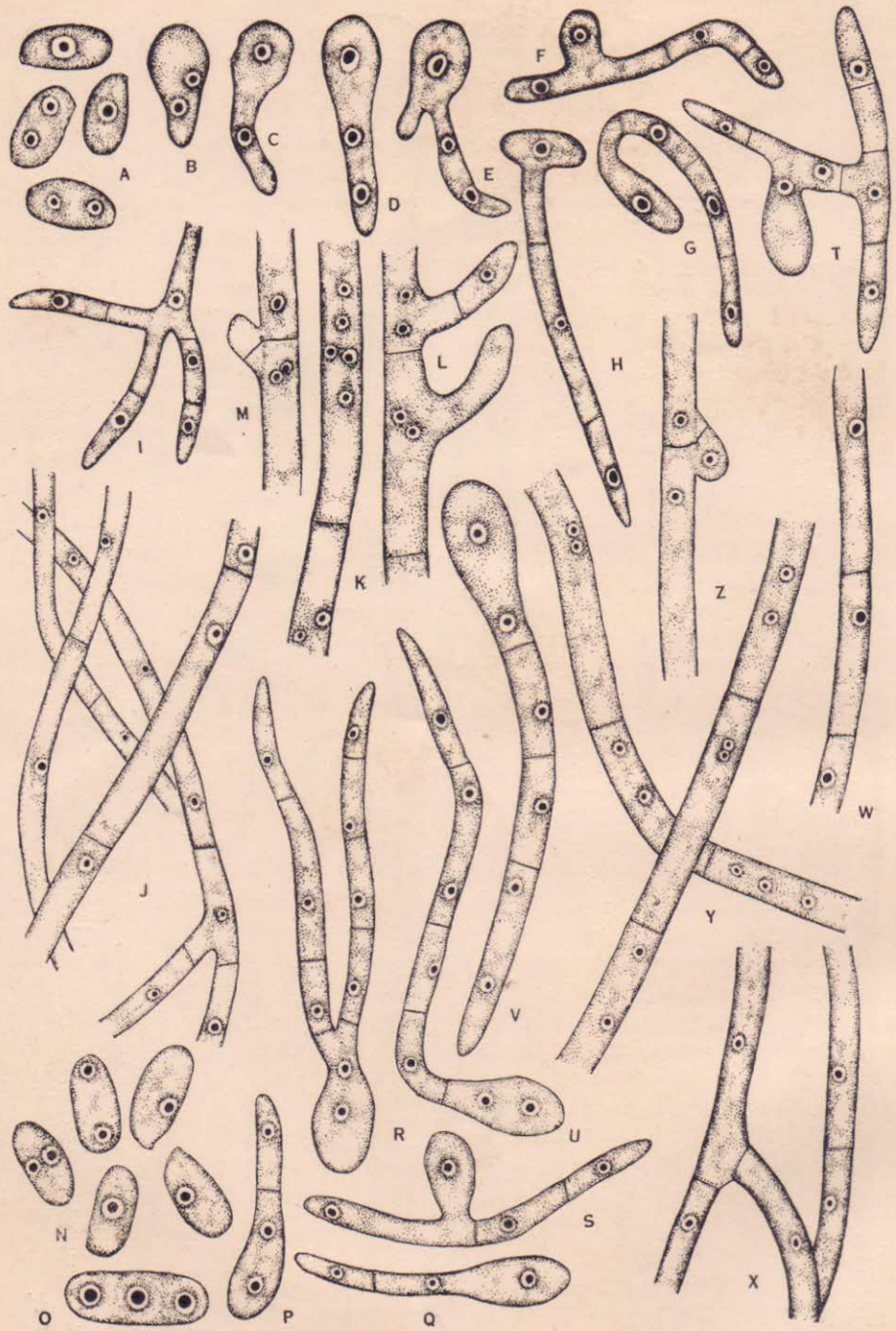
Observations

Lentinus subnudus Berk.

At maturity, each basidium is broadly clavate, slightly wider than the terminal hyphal cells, contains two nuclei about half-way up the basidium and lying one above the other in the general mass of cytoplasm (Text-fig. 1, A & B). This conjugate condition soon comes to an end and karyogamy occurs to produce a synkaryon which gradually moves towards the upper part of the basidium (Text-fig. 1, C & D). The synkaryon at this stage is conspicuously large, compact, homogenous and well-defined. Soon meiosis occurs but the details of early prophase have not been clearly observed in the preparations. The chromosomes, however, have been clearly demonstrated in first division metaphase and can be counted with sufficient accuracy ($2n = 6$: Text-fig. 1, E). The first division spindles are intranuclear in origin and of chiastobasidial type in orientation. After the first division the two nuclei (Text-fig. 1, F) divide either simultaneously (Text-fig. 1, I & J) or in succession (Text-fig. 1, G & H). The spindles are usually obliquely placed and are often parallel to each other (Text-fig. 1, I & J). In some cases they are longitudinal (Text-fig. 1, G) or even transverse (Text-fig. 1, J). No centrosomes



TEXT-FIG. 1. Nuclear phenomena in the basidia of *L. subnudus* (A—P) and *L. praerigidus* (Q—Y). × 3000. (For explanation vide text).



TEXT-FIG. 2. Nuclear phenomena in the basidiospores, germinating basidiospores and mycelia (primary and secondary) of *L. subnudus* (A—M) and *L. praerigidus* (N—Z). × 1500. (For explanations vide text).

have been observed in any stage of meiosis. After the completion of meiosis four nuclei are formed, which are all alike in size and staining properties. Their orientation within the basidium is extremely variable (TEXT-FIG. 1, K–M) and this is a striking feature of the basidial nuclei in this species. Before or after the completion of meiosis four conical sterigmata begin to develop on each basidium and a spore is eventually formed at the apex of each sterigma. As the sterigmata and the spores develop, the nuclei move upwards (TEXT-FIG. 1, M & N) within the basidium and finally migrate through the sterigmata into the spores (TEXT-FIG. 1, N). Each spore thus receives a single nucleus. The spores are formed in succession (TEXT-FIG. 1, O) and the migration of the nuclei towards the top of the basidium is either simultaneous (TEXT-FIG. 1, N) or in succession (TEXT-FIG. 1, O). After the formation and discharge of the four spores the basidium becomes empty (TEXT-FIG. 1, P).

Mature spores, although in great majority of cases uninucleate, occasionally contain two nuclei (TEXT-FIG. 2, A). This is probably due to division of the pre-existing nucleus. Germination of the spores takes place at this binucleate stage by the formation of a germ-tube from any point of the spore-wall into which one of the two nuclei migrates (TEXT-FIG. 2, B & C). Frequently two germ-tubes may develop from the same place on the spore-wall but one of them soon takes the lead (TEXT-FIG. 2, E & F). The first migrated nucleus divides again to produce two nuclei (TEXT-FIG. 2, D & E) and these are eventually separated from the spore by the formation of a transverse septum (TEXT-FIG. 2, F–H) or the septum formation may be delayed (TEXT-FIG. 2, D & E, I). Further elongation of the terminal cell of the germ-tube is followed by nuclear division and wall-formation in succession, so that, after branching a mycelium of uninucleate cells is eventually formed (TEXT-FIG. 2, G–J). This is the condition of the primary mycelium in the advanced stage and the clamp-connexions are entirely absent (TEXT-FIG. 2, J). Cells of the secondary mycelium, on the other hand, are typically binucleate although three to five nuclei with plane septa are not uncommon (TEXT-FIG. 2, L & K). The occurrence of clamp connexions in the secondary mycelium is not regular. The same hypha may bear plane septa as well as clamp-connexions (TEXT-FIG. 2, M).

Lentinus praerigidus Berk.

Young basidia are distinctly binucleate. The two nuclei lie half-way up the basidium and a little distance apart from each other (TEXT-FIG. 1, Q & R). Karyogamy follows and a Synkaryon is formed. The Synkaryon is conspicuously large and occupies the middle position of the basidium (TEXT-FIG. 1, S). First division is stigobasidial as can be assumed from the position of the two resulting daughter nuclei (TEXT-FIG. 1, T). Second division of meiosis takes place in succession and the spindles appear to be somewhat obliquely placed (TEXT-FIG. 1, T & U). The number of chromosomes at each pole of the spindle of the anaphase during second division has been found to be $n = 3$ (TEXT-FIG. 1, U & V). Spindles are intranuclear in origin and no centrosomes have been found in any of the preparations. After the formation of 4 daughter nuclei (TEXT-FIG. 1, W), four sterigmata begin to develop at the apex of each basidium and four

spores are ultimately formed at the apices of the sterigmata. The development of the spores is not simultaneous but they are produced in succession (TEXT-FIG. 1, X). Each spore receives a single nucleus from the basidium and after the discharge of the spores the basidium becomes empty (TEXT-FIG. 1, Y).

Mature spores are mostly uninucleate although occasionally 2 to 3 nuclei in each spore have been observed (TEXT-FIG. 2, N & O). Trinucleate condition is rare, and when it appears the spore becomes somewhat enlarged. When the germination starts the spore-nucleus divides into two daughter nuclei. A germ-tube is formed at either end of the spore into which one of the daughter nuclei migrates, completes another mitosis and a transverse septum is formed in between the two in such a way that a terminal uninucleate cell is formed in the germ-tube while the other two nuclei remain within original spore-case or one towards the base of the germ-tube and the other within the spore-case (TEXT-FIG. 2, P & Q). Sometimes two germ-tubes develop from the same point of origin on the spore-wall and as they develop they grow side by side more or less parallel to each other (TEXT-FIG. 2, R) or in opposite directions (TEXT-FIG. 2, S & T). The terminal cell of the germ-tube then elongates, its nucleus divides, septum-formation follows and this process is repeated so that eventually a mycelium consisting of uninucleate cells is formed (TEXT-FIG. 2, T–X). The cells of the primary mycelium is decidedly uninucleate and lack clamp-connexions (TEXT-FIG. 2, W & X) while the cells of the secondary mycelium is typically binucleate although three to four nuclei with plane septa are not uncommon (TEXT-FIG. 2, Y). The occurrence of clamp-connexions in the secondary mycelium is, as in the case of *Lentinus subnudus*, not regular and are somewhat infrequent (TEXT-FIG. 2, Z).

DISCUSSION

Nuclear phenomenon in the life-cycle of the two fungi can be considered as corroborative evidences to their heterothallic nature. On the basis of karyological conditions, the distribution of sex-factors and the consequent sex-reactions in these two fungi can be explained. In both the fungi the basidiospores are at first uninucleate but the nucleus of the spore always divides into 2 or 3 nuclei at the time of germination and gives rise to a primary haploid mycelium of uninucleate cells. When primary mycelia of opposite sexes meet they form secondary mycelium with clamp-connexions. The number of dicaryons in each hyphal cell is usually one but three or more nuclei may be present in each cell. The occurrence of clamp-connexions is not regular and the same mycelium may have both clamp-connexions and plane septa. This shows that the conjugate pair can divide independently. This binucleate condition comes to an end with the formation of basidia in each of which the two nuclei fuse to form a synkaryon. The synkaryon divides reductionally to form four haploid nuclei. Since both the species are 'bipolar' seggregation takes place during the first division of the synkaryon. The cytological data again suggest the way in which the tetracyte nuclei of the basidium are distributed to form four haploid basidiospores. The nuclei are all of 'homogenous' type

of Pinto-Lopes (1949) in which the chromatin and nucleolus being contracted to an intensely stainable body and the major part of nucleoplasm is free from chromatin. Since the chromosomes are too small, the details of their karyotype is out of the scope. Chromosome number, however, have been demonstrated with sufficient clearness and has been found to be $2n = 6$ in both the cases. Maire (1902), Levine (1913) and others have shown centrosomes and astral rays in meiosis but no such centrosomes or astral rays are visible in any of the preparations of the writers. The variable distribution of the tetracyte nuclei of the basidium is an interesting phenomenon. Further, the orientation of the spindle in the first division of the fusion nucleus is transverse in *L. subnudus* and probably longitudinal in *L. praerigidus* while that of the spindles during second division is extremely variable. In any case the spindles are intranuclear in origin.

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