

CONTRIBUTIONS TO THE CYTOLOGY OF HYMENOMYCETES

I. CYTOLOGICAL STUDIES IN *MARASMIUS CAMPANELLA* HOLTERM.

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INTRODUCTION

Cytological investigations on Hymenomycetes in India has so far been a minor feature. It was Bose (1937) who first made an attempt in this direction only with eleven species of polypores and showed a great deal of consistency in karyogamy being subsequently followed by meiotic divisions in the basidial development. So far as the authors are aware, no further work has been done in India either on other members of Polyporaceae or those of allied families which will be evident from Olive's (1953) excellent review on the subject. In order to throw additional light on nuclear phenomena in other members of Hymenomycetes in India, investigations are now in progress of which the present paper forms the first contribution to the series.

While studying the biology of *Marasmius campanella* Holterm., it was found by the writers (1954a) that the fungus is homo-heterothallic. In order to determine what cytological basis underlies this phenomenon and what light it throws on the life-cycle of the fungus, the present investigation was carried out.

MATERIALS AND METHODS

For the study of the hymenium, pilei of fresh fruit-bodies of *M. campanella*, at all stages of development were collected from Calcutta, cut into small pieces and fixed in several killing and fixing fluids. Of these, Bouin-Allen, Flemming's fluid (weak) and acetic-formalin-alcohol were found to be satisfactory. The materials were fixed at different intervals of time, viz., at 12 noon, and at 3 and 6 p. m. After fixation, washing, dehydration and imbedding were done in the usual way. Sections were cut and those 8–10 μ in thickness were found to be most satisfactory. Several stains, such as Heidenhein's iron-haematoxylin, aqueous basic fuchsin, i.e., Feulgen's method (1924) and aqueous crystal violet were tried, of which Heidenhein's iron-haematoxylin yielded the most satisfactory result. It is to be noted that for proper staining with iron-haematoxylin both mordanting and staining for 25–30 minutes were found quite suitable. For studying the nuclear conditions in spores, spore-deposit from fresh fruit-bodies were taken on clean grease-free sterilized slides. These were then fixed directly for 24-hours in Flemming's fluid and finally stained with iron-haematoxylin. Knip's (1913) agar-film technique and that modified by Sass (1929) was tried for making total preparations in order to study nuclear phenomena of spore-germination and in the mycelium. For studying spore-germination, spores from fresh fruit-bodies were allowed to drop on the agar-film on the slide and extreme aseptic precautions were taken to avoid contamination. The cultures at the desired stage of development were fixed and stained by the

methods already mentioned. Prior to fixation the slides were, however, transferred to dry Petri-dishes and the thin films of the medium were allowed to dry down only at the margin in order to avoid washing out of the films from the slides during the long process of washing and staining. In the agar-film technique, the film of the medium although stained deeply could be destained readily. The degree of destaining of the agar-film afforded a convenient guide to progress of differentiation.

OBSERVATION

The nuclear condition in this species may well begin with the study of the hymenium. The basidia are more or less clavate, disterigmatic, bisporous and each bears a clamp-connection at its narrowed base (Fig. 1, *r-s*). When young they are distinctly binucleate (Fig. 1, *a-c*). This is best demonstrated in certain cases when the two nuclei have been found about half-way up the basidium lying side by side in the general mass of cytoplasm (Fig. 1, *c*). Karyogamy occurs after this stage (Fig. 1, *d-e*) and the fusion nucleus moves towards the upper part of the basidium. The fusion nucleus is conspicuously large, homogeneous and well defined. Its nucleolus is brightly stained and the chromatin-reticulum is not in evidence. The chromosomes have, however, been demonstrated in the meiotic stages (Fig. 1, *f-h*) along with other stained granules in the cytoplasm and the number is $10 (= 2n)$. The presence of synkaryons following karyogamy clearly indicates that the basidial nuclei are in the diploid condition. Two successive nuclear divisions of the fusion nucleus take place in the basidium and four daughter nuclei have been found which seem to be alike in size, structure and staining properties (Fig. 1, *f-s*).

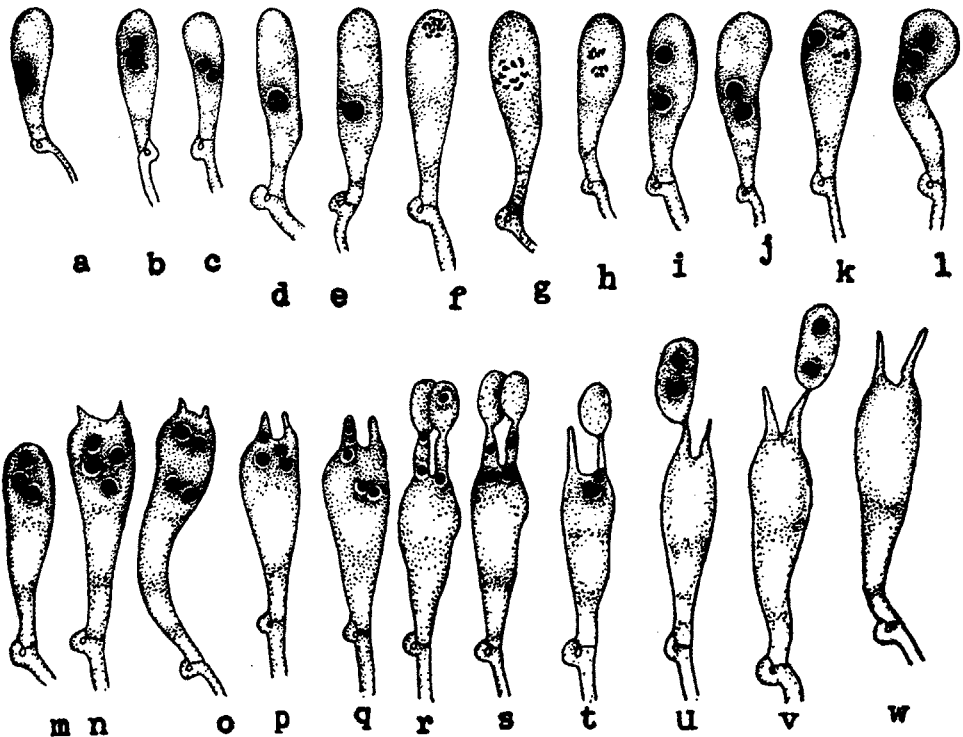


FIG. 1. Successive stages in the development of basidia and the associated nuclear phenomena. ($\times 2240$).

Migration of the daughter nuclei into the spores has been clearly demonstrated but the details of the procedure could not be observed (Fig. 1, *r-v*). Prior to the formation of sterigmata, the four nuclei within the basidium do not show any definite orientation. As the sterigmata and the spores develop, the nuclei, no doubt, move upward within the basidium, but at no time do they become attached to its upper wall (Fig. 1, *n-p*). Nuclei in pairs then prepare to undergo migration, one nucleus of each pair moving into the broad base of each sterigma (Fig. 1, *p*). In some cases, a pair of nuclei, one following the other, have been observed at the base of a sterigma and the other two nuclei are to be found at some distance away from it (Fig. 1, *q*). Subsequently, one nucleus has been found to complete its migration into a spore, while a second nucleus is found at the base of the same sterigma (Fig. 1, *r*). Usually, when all the daughter nuclei migrate into the spores, the basidium becomes empty

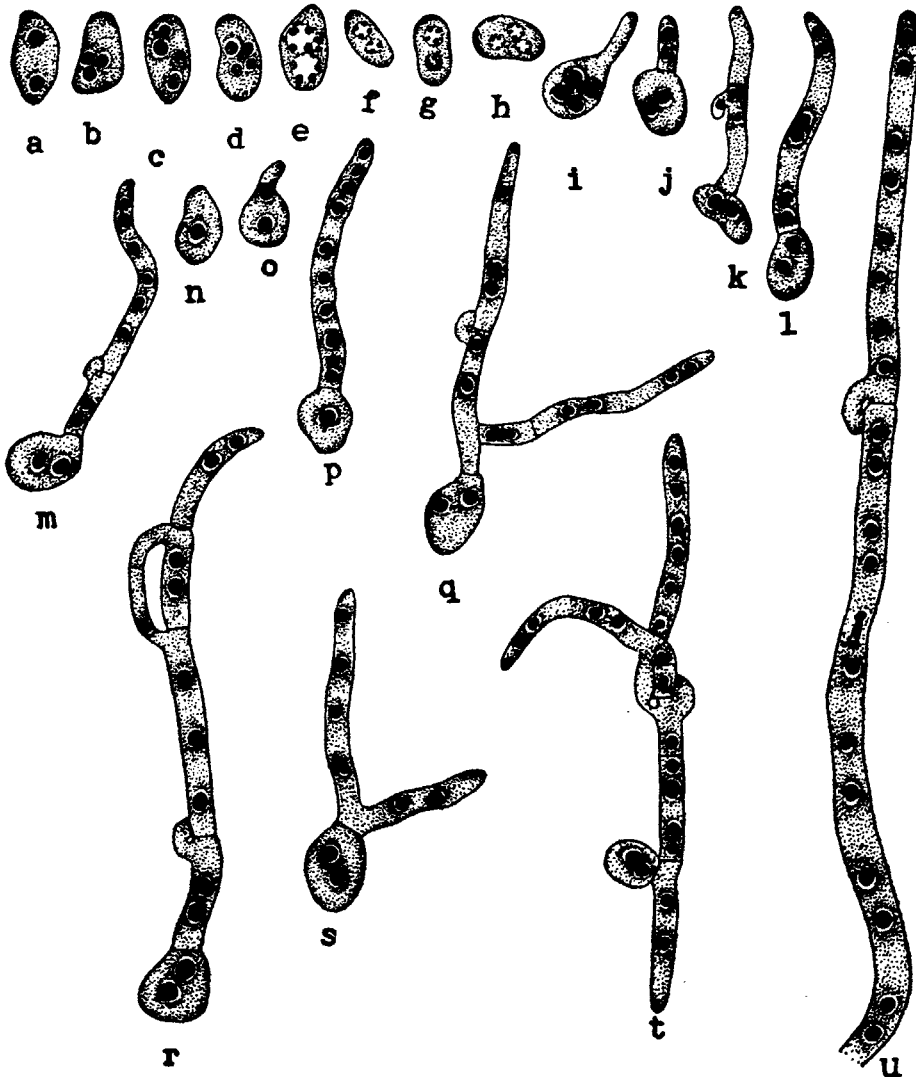


FIG. 2. Nuclear phenomena of spore-germination and in the mycelia. ($\times 2240$).

(Fig. 1, *u-w*). Each spore, when young, contains two nuclei in great majority of cases (Figs. 1 and 2, *u-v*; *a*), although a few uninucleate spores have been found also in a total preparation after the spores are discharged from the sterigmata (Fig. 2, *n*). In a properly fixed and stained preparation it has been found that the mature spores occasionally contain three or four nuclei (Fig. 2, *b-d*). Following Pinto-Lopes (1949) the nuclei of the spores can be grouped into two main types, viz., (i) homogeneous (Fig. 2, *a-d*) and (ii) disperse-expanded (Fig. 2, *e-h*). In most cases mycelia developing from single spore show clamp-connection, which appears early, usually, at the very first septum of the germ-tube (Fig. 2, *k, m, q-r, t-u*). Frequently, two germ-tubes may develop from any point of the spore-wall, but only one of them soon takes the lead (Fig. 2, *s-t*). In early stages of germination, when the germ-tube is first formed, the two pre-existing nuclei of the spore divide once forming four daughter nuclei (Fig. 2, *i*). Of these, two nuclei migrate into the germ-tube, while other two remain within the original spore-case (Fig. 2, *j*). The two nuclei of the germ-tube are eventually separated from the spore by the formation of a transverse septum at the junction of the germ-tube and the spore-wall. The formation of the first septum may be delayed for some time till the germ-tube attains a considerable length. As the germ-tube elongates, its two nuclei either divide by conjugate division forming clamp-connections (Fig. 2, *k*) and this process is repeated to form regular binucleate cells or the nuclei simply increase in number by irregular divisions and without the formation of any clamp-connection (Fig. 2, *l, m, q, u*). In the latter case, the largest number of nuclei in the cell of the germ-tube may be up to ten. Each nucleus occupies almost the entire width of the hypha, and its nucleolus stains intensely with iron-haematoxylin. Each uninucleate spore, on the other hand, at the time of germination produces the germ-tube in the form of a protrusion and the pre-existing nucleus divides once (Fig. 2, *o*). One of the nuclei then migrates into the germ-tube and the other remains within the spore-case. The germ-tube with one nucleus now elongates and soon a transverse septum is formed at its base near the junction of the spore-wall. Eventually, a primary mycelium is formed. Sometimes, wall-formation following nuclear divisions appears to be somewhat delayed (Fig. 2, *p*).

DISCUSSION

Since *Marasmius campanella* is homo-heterothallic, it will be of interest to discuss salient cytological features of this agaric. The spores are usually in the binucleate condition, but occasionally they may be uninucleate or contain more than two nuclei. The uninucleate condition may be interpreted as due to migration of a single nucleus from the basidium to the spore while trinucleate or quadrinucleate conditions appear to have originated due to subsequent divisions of the pre-existing nuclei within the spore prior to germination. In case of the spores that receive one nucleus only, the fate of the remaining nuclei in the basidium could not be followed clearly. The clamp-bearing mycelium is undoubtedly in the secondary condition and as the mycelium is of monosporous origin, the species is considered to be homothallic. However, the cells of the secondary mycelium are typically dicaryotic and with clamp-connections although three to seven nucleate hyphal cells with plain septa are not uncommon. This shows that the members of the conjugate nuclei can divide independently even when there is no question of clamp-connection. In the monocaryotic mycelium arising from uninucleate spores, the hyphal cells are not always associated with the formation of septa.

The basidia have always been found to be disterigmatic and bisporous. As usual, they are binucleate when young but the two nuclei soon fuse to form a synkaryon in preparation for the inception of meiosis. After meiotic divisions four daughter nuclei are formed of which usually two nuclei enter into each spore. In some cases, each of the two spores of a basidium receives a single nucleus. The

distribution of sex-factors and the consequent sex-reactions in this agaric can be explained on the basis of nuclear phenomena. It has been observed that a binucleate spore when germinates always gives rise to a typical dicaryotic mycelium with clamp-connections and from this mycelium normal fructifications are formed as reported by the authors (1954b). Occasionally, when a single nucleus enters into a basidiospore, it or its descendants possibly carry one sex-factor and this type of spore gives rise to exceptional primary mycelium. In such cases, secondary condition arises by hyphal fusions among primary mycelia of opposite-sex, and once the secondary condition is established, the nuclear cycle appears to be the same in all cases. It may be assumed that each binucleate spore, on the other hand, receives a compatible pair of nuclei from the basidium and is evidently in a dikaryophasic condition and must carry a complete set of opposite sex-factors. Increase in the number of nuclei in such a spore is purely vegetative.

SUMMARY

1. *Marasmius campanella* is an agaric with disterigmatic two-spored basidia and there is an alternation of diplophasic and haplophasic nuclei, the latter condition being represented by the haploid nuclei of the basidia following meiosis and those in the cells of dicaryotic mycelia. Karyogamy occurs in the basidium to form a fusion nucleus and the diploid condition is restored. This nucleus divides soon meiotically to form four haploid nuclei.

2. Each basidiospore normally receives two nuclei which may or may not divide again. Occasional spores are found to receive one nucleus. The uninucleate spores give rise to haploid mycelia with uninucleate or multinucleate hyphal cells which in order to produce secondary mycelium, fuse with one another in a limited number of combinations.

3. Early stages in the development of secondary mycelium show the presence of clamp-connections even at the first septum of the germ-tube or they are formed very soon thereafter. The clamp-connections are, however, not present at every septum, the same hypha bears both plain septa and septa with clamp-connections. Further, the cells of the secondary mycelium are not strictly dikaryotic since cells containing more than two nuclei are not uncommon.

4. Chromosomes have been found in pre-meiotic, meiotic and post-meiotic stages, the diploid number being ten and this represents the largest number discovered so far in Hymenomycetes.

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