

CONTRIBUTION TO THE CYTOLOGY OF HYMENOMYCETES: IX. KARYOLOGICAL STUDIES IN *POLYPORUS SANGUINEUS* KLOTZSCH

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(With 3 Text-Figures)

Karyological study of *Polyporus sanguineus* has been undertaken to investigate the nuclear phenomenon in its life-cycle.

The young basidium is the terminal cell of a hypha and is distinctly binucleate. The two nuclei fuse to form the synkaryon. The synkaryon represents the end of dikaryophase and is the only diplophasic stage in the life-history of the fungus. It then divides reductionally. Chromatin reticulum appears in all cases during the early prophase. The reticulum then resolves into typical chromosomes and the diploid number ($2n$) for the fungus is 8. The orientation of the spindle during first division of meiosis is always transverse while that of the second division is irregular. After completion of meiosis, the four daughter nuclei divide again forming eight nucleate basidia. This third division is either simultaneous or in succession. Ultimately, four nuclei migrate at random into four basidiospores, one in each, while the remaining four gradually degenerate within the collapsing basidium.

The basidiospores, when first formed, are always uninucleate and represent the starting point of monokaryophase in the life-cycle of each fungus.

During germination of the basidiospore, usually a single germ-tube is produced and the nucleus directly migrates into it. A transverse wall then develops between the germ-tube and the spore-case. Subsequent nuclear division and wall formation finally forms a septate primary mycelium of uninucleate cells without clamp-connexion.

The secondary mycelium obtained by pairing compatible primary mycelia represents the dikaryophase and is characterised by regular binucleate hyphal cells having simple clamp-connexion almost at every septum.

INTRODUCTION

The critical reviews of Olive (1953) and Boidin (1954) show that considerable work on the karyological phenomena of Basidiomycetes have already been done in other parts of the world. The present investigation on *Polyporus sanguineus* Klotzsch, a member of the family Polyporaceae, is a further addition to the list of Indian species worked out by Banerjee and his co-workers (1955, '56, '57, '60, '61, '62, '66a and '66b).

MATERIAL AND METHODS

Fresh fructifications of *P. sanguineus* were collected after a shower from the timber-yards while growing luxuriantly on logs of *Shorea robusta* Gaertn f. The fructifications were cut into small rectangular pieces (5 mm. $\times$ 5 mm.) and fixed for specified periods in "Bouin-Allen", "Sass", and "Carnoy". After fixation, the materials were washed, dehydrated following Ehrlich and McDonough (1949) and finally embedded in paraffin.

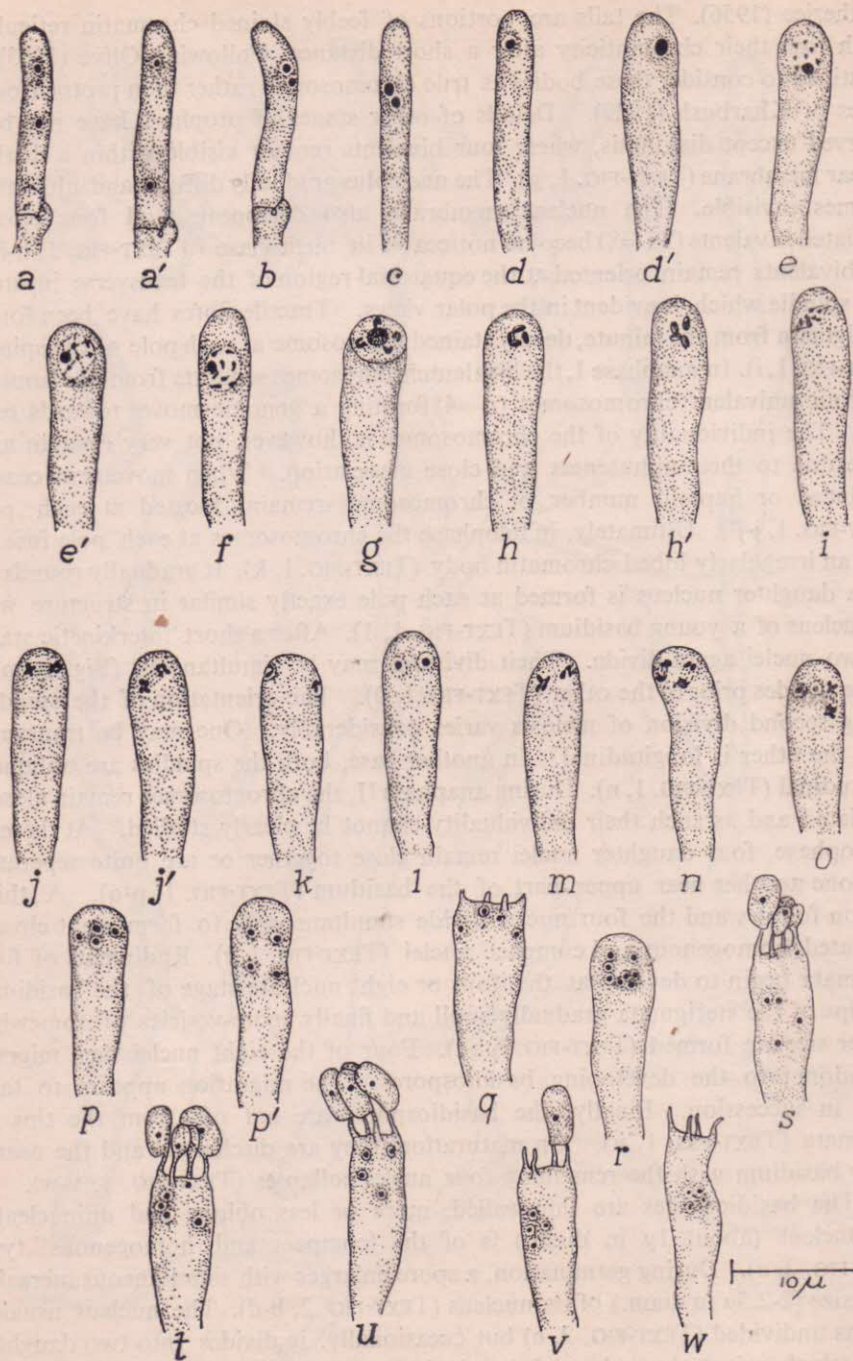
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Microtome sections, 7μ thick, were stained in Heidenhein's Haematoxylin, Crystal violet (1% aq. soln.), and Pyronin- Methyl green. Following Gwynne-Vaughan and Barnes (1937), best preparation has been obtained by mordanting the sections for 1 hr. in 4% aqueous Iron-alum, staining in Haematoxylin for 2 hrs. and finally making differentiation in saturated aqueous solution of Ironalum.

Karyological phenomena in spores, spore-germination, primary and secondary mycelia have been studied by using Banerjee and Mukherjee's (1955) modification of agar-film technique of Kniep (1913) on glass slide. A few slides with ungerminated spores were fixed in "Bouin-Allen" or "Sass" to study the karyological conditions in the spores. Germinating spores at various stages of development were also similarly fixed. For studying the nuclear behavior in the primary and secondary mycelia, agar-films on slides were separately inoculated with the respective types of mycelia and after considerable amount of growth those were fixed as before. Both "Bouin-Allen" and "Sass" yielded satisfactory results when stained with Haematoxylin.

OBSERVATIONS

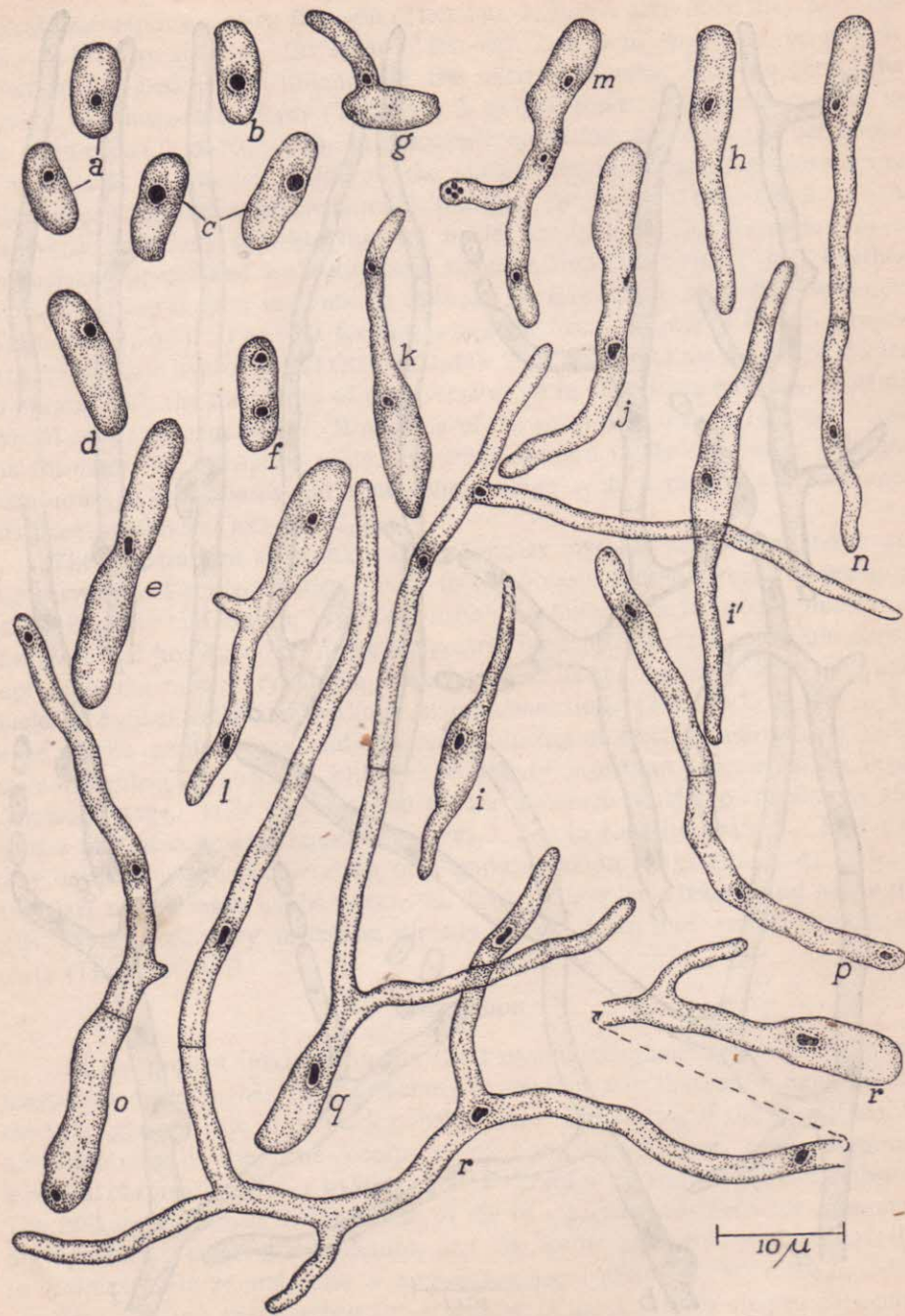
The hymenium of *P. sanguineus* is composed of a compact layer of basidia showing different stages of development. A young basidium is but very little swollen than the terminal hyphal cell and is provided with a clamp-connexion at its base (TEXT-FIG. 1, a-a¹). Its cytoplasm is granulated and there are two conspicuously small (about 1μ in diam.) 'condensed and homogenous' type of nuclei (Pinto-Lopes, 1949). The nuclei are either widely separated or a little distance apart from each other. In a stained preparation, nuclear details at this stage are not clear as the chromatin matter is condensed into a central deeply stained mass. This is surrounded by a hyaline karyolymph and an outermost indistinct nuclear membrane. The two nuclei gradually approach each other and lie close together at the upper part of the basidium (TEXT-FIG. 1, b). This is followed by the fusion of hyaline zones and coalescence of the chromatin masses (TEXT-FIG. 1, c-d¹). The coalescing chromatin masses lose their identity eventually rounds up and a synkaryon is formed. The synkaryons are identical in structure and staining properties with the nuclei of the young basidium but are much larger in size (TEXT-FIG. 1, d¹). With the formation of synkaryon (diplophase), the dikaryophasic stage in the life-cycle of the fungus comes to an end. This stage probably lasts for sometime as is evident from its higher frequency of occurrence in some of the preparations. The synkaryon then enters the first stage or 'interphase' of the meiotic division and enlarges considerably. One nucleolus and 7-8 chromocenters are the only definable structures present at this stage (TEXT-FIG. 1, e). With the onset of prophase a delicate net work of threads, beaded at places with coarser granules, become visible within the nucleus (TEXT-FIG. 1, e¹). These coarser granules are the heterochromatin regions of chromosomes that stain darker than the net work. In later stages, certain regions of the reticulum undergo thickening and condensation. Ultimately, the reticulum breaks down into eight chromatin bodies provided with 'chromatin tails' (TEXT-FIG. 1, f) as has been observed by Banerjee and



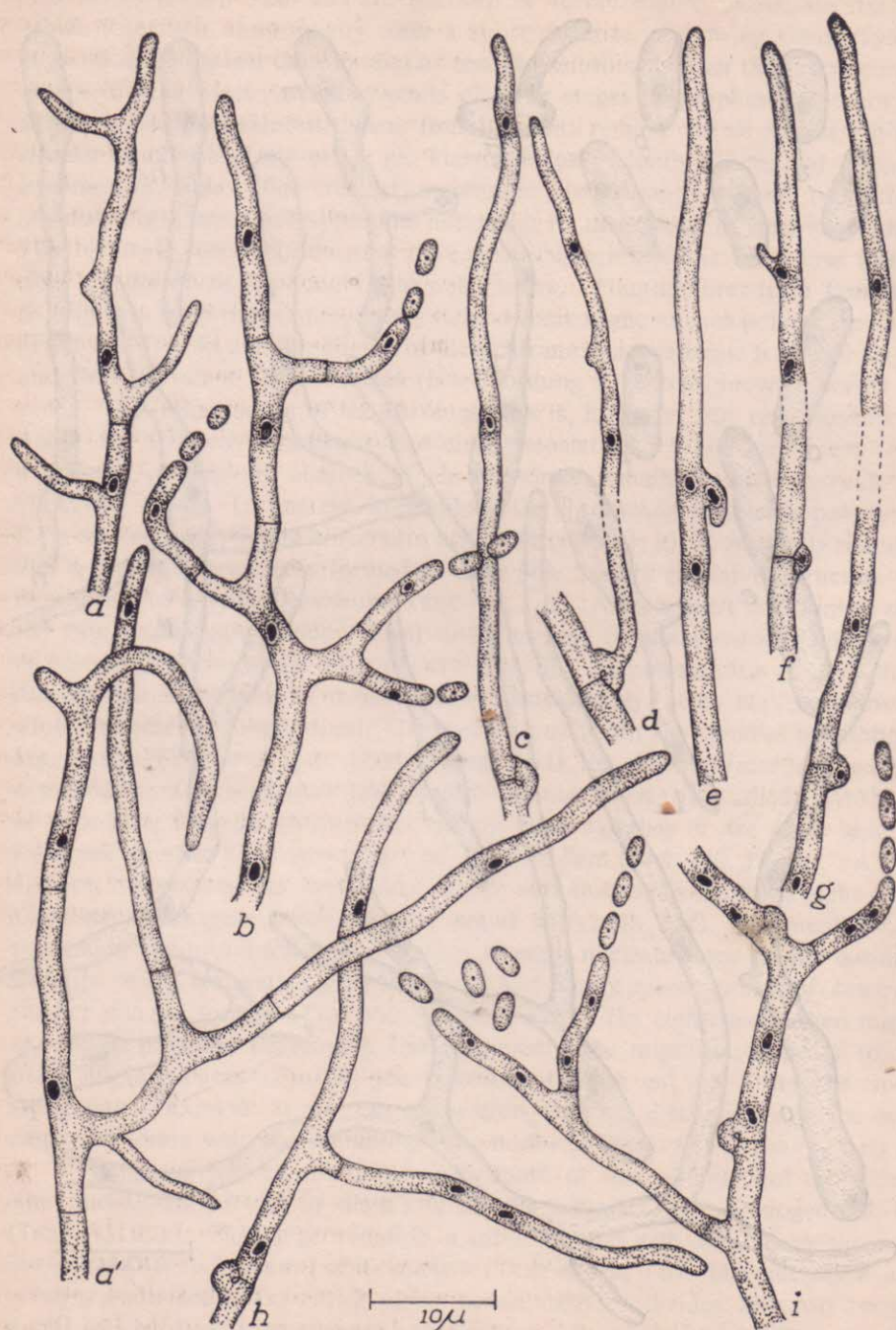
TEXT-FIG. 1. Nuclear phenomena in the basidia of *Polyporus sanguineus* Klotzsch (vide TEXT).

Mukherjee (1956). The tails are portions of feebly stained chromatin reticulum which lose their chromaticity after a short distance. Following Olive (1953), it is justified to consider these bodies as true chromosomes rather than protochromosomes of Kharbush (1929). Details of other stages of prophase have not been observed except diakinesis, where four bivalents remain visible within a distinct nuclear membrane (TEXT-FIG. 1, g). The nucleolus gradually diffuses and ultimately becomes invisible. The nuclear membrane also disappears and four closely associated bivalents ($2n=8$) become noticeable in metaphase I (TEXT-FIG. 1, h-h¹). The bivalents remain oriented at the equatorial region of the transverse intranuclear spindle which is evident in the polar views. Tractile fibres have been found to originate from the minute, deeply stained centrosome at each pole of the spindle (TEXT-FIG. 1, i). In anaphase I, the bivalent chromosomes separate from one another and four univalent chromosomes ($n=4$) forming a genome moves towards each pole. The individuality of the chromosomes is, however, not very clear in anaphase due to their minuteness and close association. When movement ceases, a reduced or haploid number of chromosomes remains located at each pole (TEXT-FIG. 1, j-j¹). Ultimately, in telophase the chromosomes at each pole fuse to form an irregularly lobed chromatin body (TEXT-FIG. 1, k). It gradually rounds up and a daughter nucleus is formed at each pole exactly similar in structure with the nucleus of a young basidium (TEXT-FIG. 1, l). After a short 'interkinetic stage' the two nuclei again divide. Their divisions may be simultaneous (Fig. 1, m-n) or one divides prior to the other (TEXT-FIG. 1, o). The orientation of the spindles during second division of meiosis varies considerably. One may be transverse while the other is longitudinal. In another case, both the spindles are obliquely longitudinal (TEXT-FIG. 1, n). During anaphase II, the chromosomes remain closely associated and as such their individuality cannot be clearly studied. At the end of telophase, four daughter nuclei remain close together or are quite separated from one another near upper part of the basidium (TEXT-FIG. 1, p-q). A third division follows and the four nuclei divide simultaneously to form eight closely associated 'homogenous and compact' nuclei (TEXT-FIG. 1, r). Rudiments of four sterigmata begin to develop at the four or eight nucleate stage of the basidium. The tips of the sterigmata gradually swell and finally spore-vesicles of somewhat smaller size are formed (TEXT-FIG. 1, s-t). Four of the eight nuclei then migrate at random into the developing basidiospores. The migration appears to take place in succession. Finally, the basidiospores are cut off from the tips of sterigmata (TEXT-FIG. 1, u). On maturation, they are discharged and the nearly empty basidium with the remaining four nuclei collapses (TEXT-FIG. 1, v-w).

The basidiospores are thin-walled, more or less oblong and uninucleate. The nucleus (about 1μ in diam.) is of the 'compact and homogenous' type (TEXT-FIG. 2, a). During germination, a spore enlarges with simultaneous increase in the size ($2-2.5\mu$ in diam.) of its nucleus (TEXT-FIG. 2, b-d). The nucleus usually remains undivided (TEXT-FIG. 2, e) but occasionally, it divides into two daughter nuclei which orient themselves along the long axis of the spore (TEXT-FIG. 2, f). A germ tube then develops from any part of the spore wall (TEXT-FIG. 2, g-h). Rarely two germ-tubes appear from the two ends of the spore (TEXT-FIG. 2, i-i¹).



TEXT-FIG. 2. Nuclear phenomena in the basidiospores and germinating basidiospores of *Polyporus sanguineus* Klotzsch (Vide TEXT).



TEXT-FIG. 3. Nuclear phenomena in the primary and secondary mycelia of *Polyporus sanguineus* Klotzsch (Vide TEXT).

Apical emergence are most frequent (TEXT-FIG. 2, j). A germ-tube may have the same width with that of the spore (TEXT-FIG. 2, e & p) or it may be narrow (TEXT-FIG. 2, g-o). When uninucleate, the nucleus migrates into the germ-tube leaving the spore-case empty (TEXT-FIG. 2, g) or it sends a daughter nucleus in the germ-tube (TEXT-FIG. 2, k). In binucleate condition, however, the behaviour of the nuclei is different. One of the nucleus migrates into the developing germ-tube while the other remains within the spore-case (TEXT-FIG. 2, l). A transverse wall may separate the two nuclei resulting in the formation of a uninucleate tip-cell and an uninucleate basal portion (TEXT-FIG. 2, n). Further nuclear division may or may not be followed by immediate cross-wall formation (TEXT-FIG. 2, o-r). The wall formation is sometimes delayed to form a temporary 2-3 nucleate hyphal cell (TEXT-FIG. 2, m). This multinucleate condition comes to an end with the formation of transverse septa in succession and the resulting hyphal cells are uninucleate. Branching of the germ-tube may start even before the formation of transverse septa. Nuclear division in the vegetative cells has been noted in a few cases and a metaphase stage with 4 distinct chromosomes has been observed (TEXT-FIG. 2, m).

The preparations of primary and secondary mycelia, when examined reveal the karyological characteristics similar to the observations noted in the previous cases (Banerjee and Nandi, 1962, '66a '66b). In both the mycelia, the nucleus is 'compact and homogenous'. The irregularly branched primary mycelium which represents the monokaryophasic stage has been found to be divided into uninucleate hyphal cells and without clamp-connexions (TEXT-FIG. 3, a-a'). In some of the preparations, oidiophores producing numerous uninucleate oidia are noticeable (TEXT-FIG. 3, b). The secondary mycelium represents the dikaryophasic stage. It is characterized by the presence of clamp-connexions and with a pair of conjugate nuclei (TEXT-FIG. 3, c-g) in each hyphal cell. Branches may develop by the proliferation of clamp-connexion (TEXT-FIG. 3, d) or from any part of the main hypha (TEXT-FIG. 3, h). Cases have been found where the cells of the secondary mycelium directly break down into small uninucleate oidia (TEXT-FIG. 3, i).

DISCUSSION

In the present investigation on the karyological phenomenon of *Polyporus sanguineus* evidence has been presented to demonstrate that the fungus follows the same general rule in its nuclear behaviour. The nuclei of the young basidia are essentially of the same 'compact and homogenous' type, each containing a central chromatin mass, a hyaline zone of karyolymph and a nuclear membrane. The synkaryon formed by the fusion of the two nuclei then enters the interphase during which it enlarges considerably and this according to Swanson (1961) is due to synthesis of large molecules of nucleic acids and proteins. With the initiation of meiosis, one or two nucleoli and a number of small, deeply stained chromatin bodies, become visible within the nucleus. This may be due to the fact that the nucleic acids of the chromosomes remain either too diffused to absorb much dye or as so hydrated that the dye is not accumulated in sufficient amount to stain

them intensely. Stages of prophase including typical bivalent formation at diakinesis have been observed with sufficient accuracy. The intranuclear spindle with polar centrosomes observed probably indicate their primitive character, since these structures are absent in higher plants. After the completion of meiosis, four haploid daughter nuclei are formed within each basidium. It is interesting to note that following meiosis a third nuclear division takes place resulting into eight nucleate basidia at maturity. Four of the eight nuclei in each basidium are, however, utilized at random in the formation of four uninucleate basidiospores while the remaining four nuclei gradually degenerate within the collapsing basidium. This additional division of the tetracyte nuclei has also been observed in a number of previous observations (Banerjee *et al*, 1956, '60, '61, '62, '66a, '66b) but its significance is yet to be determined.

Like all other heterothallic species of Basidiomycetes, *P. sanguineus* shows three nuclear phases in its life-cycle. The uninucleate basidiospores germinate to form always the primary mycelia of uninucleate cells which represent the monokaryophasic stage. Fusion of two compatible primary mycelia give rise to the secondary mycelium with clamp-connexions. This type of mycelium represents the dikaryophasic stage of the life-cycle, in which each hyphal cell contains a dikaryon. This dikaryophasic stage comes to an end with the formation of the basidium in which the two nuclei fuse to form a synkaryon, the only diplophasic stage.

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