Contribution to the Cytology of Hymenomycetes. VII. Karyological Studies in *Fomes Lividus* Kalchbr

Sachindranath Banerjee & Balen Nandi

Published online: 30 Jan 2014.

Submit your article to this journal ↗

View related articles ↗

CONTRIBUTION TO THE CYTOLOGY
OF HYMENOMYCETES.
VII. KARYOLOGICAL STUDIES IN *FOMES LIVIDUS* KALCHBR.

SACHINDRANATH BANERJEE and BALEN NANDI

Botany Department, University of Calcutta, Calcutta, India.

INTRODUCTION

A knowledge of the karyological phenomena in the life-cycle of a Hymenomycetes is undoubtedly essential in understanding its biology properly. A critical review on the subject has already been made by OLIVE (1953) where it will be evident that a major part of the work has been done on Agaricaceae. Recently BOIDIN (1954) has further supplemented our knowledge on this line of research in some Hydnaceae and Thelephoraceae. In India, it was BOSE (1937) who first takled the problem and worked on the basidial cytology of eleven species of Polyporaceae. Following his lead, BANERJEE and his coworkers (1955, 1956, 1957, 1960, 1961, 1962) have added informations on the karyological phenomena in some species of Polyporaceae, Thelephoraceae and Agaricaceae during recent years. With a view to add further contribution along this line, the authors have selected *Fomes lividus* Kalchbr., a resupinate member of the family Polyporaceae of which any information as to its nuclear behaviour is lacking.

MATERIAL AND METHODS

For the study of nuclear conditions in the basidia, rectangular blocks (5 mm. × 5 mm.) were made from fresh fructifications of *F. lividus*, collected from the timberyards of Calcutta while growing luxuriantly on logs of *Shorea robusta* Gaertn. f. in the months of July to October. It was found that before fixation pre-soaking of the fruit-bodies by rain or water was essential for obtaining divisional stages of the nuclei. Different fixatives, such as, « Bouin-Allen », « Sass », « Carnoy », « Nawaschin (A×B) » and « Formol-Acetic-Alcohol » were used of which « Bouin-Allen » proved to be the best although « Sass » yielded satisfactory

results. Following SASS (1929), the air from the pore-tubes was pumped out to obtain rapid penetration of the fixative. After fixation the materials were washed, where necessary, dehydrated following EHRLICH and McDONOUGH (1949) to avoid difficulties in sectioning, and finally embedded in paraffin.

Microtome scetions 7 μ were cut and the sections were stained separately in Heidenhein's iron-alum haematoxylin, crystal violet (1% aqueous solution), pyronin-methyl green, leuco-basic fuchsine (FEULGEN and ROSSENBECK 1924) and Giemsa staining. Of these, iron-alum-haematoxylin gave best results when used after fixation with « Bouin-Allen » or « Sass ». Following the method suggested by GWYNNE-VAUGHAN and BARNES (1937), it has been found that best preparation could be obtained only after mordanting the sections for 1 hr. in 4% aqueous ironalum followed by staining in haematoxylin for 2 hrs. Subsequently, differentiation was done in saturated aqueous solution of iron-alum.

KNIEP's (1913) agar-film technique, as modified by SASS (1929) was used for studying haryological phenomena in spores, spore germination, primary and secondary mycelia. Spore-deposits were directly taken on thin film of cleared 3% malt-agar on sterile slides. Some of the slides were immediately fixed while others were incubated at 30° C for germination of the spores. The germinating spores were fixed at different stages of development. Preparations of primary and secondary mycelia were made separately on agar films and fixed in the same way. Both « Sass » and « Bouin-Allen » yielded satisfactory results in case of spores, germinating spores and the two types of mycelia.

<center>OBSERVATIONS</center>

The nuclear cycle in this species may well begin with the study of hymenium. The hymenium, consisting of basidia, is a compact laer. The mature basidia are typically clavate, tetrasterigmatic and quadrisporous. The young basidia are elongated, slightly swollen than the terminal hyphal cells and always binucleate. They contain densely granular cytoplasm and without conspicuous vacuoles. In each basidium, the two nuclei remain a little distance apart and are arranged one above the other along the long axis about half way up the basidium (Fig. 1, a-a'). Each nucleus is conspicuously small (1-1.5 μ across) and although not distinctly visible, is bounded on the outside by a nuclear membrane. The main mass of the nucleus, in a stained preparation, consists of deeply stained, small chromatin body separated from

Fig. 1. — Nuclear phenomena in the basidia of *Fomes lividus* Kalchbr.: *a-a"*, young binucleate basidia; *b-c'*, stages of karyogamy; *d-f*. stages of prophase during the first division of meiosis showing reticulate structure of the nucleus with condensed heterochromatin bodies; *f'-b'*, subsequent stages of the division; *i-j*, binucleate basidia formed after first division; *k-p*, stages of the second division of meiosis of two daughter nuclei; *q*, tetranucleate basidium; *q'r*, formation of sterigmata and a 8-nucleate basidium; *s-t*, migration of four nuclei in the four basidiospore and the other four remain within the basidium; *u-y*, gradual disintegration of the four nuclei in the collapsing basidia; *y*, binucleate basidiospores.
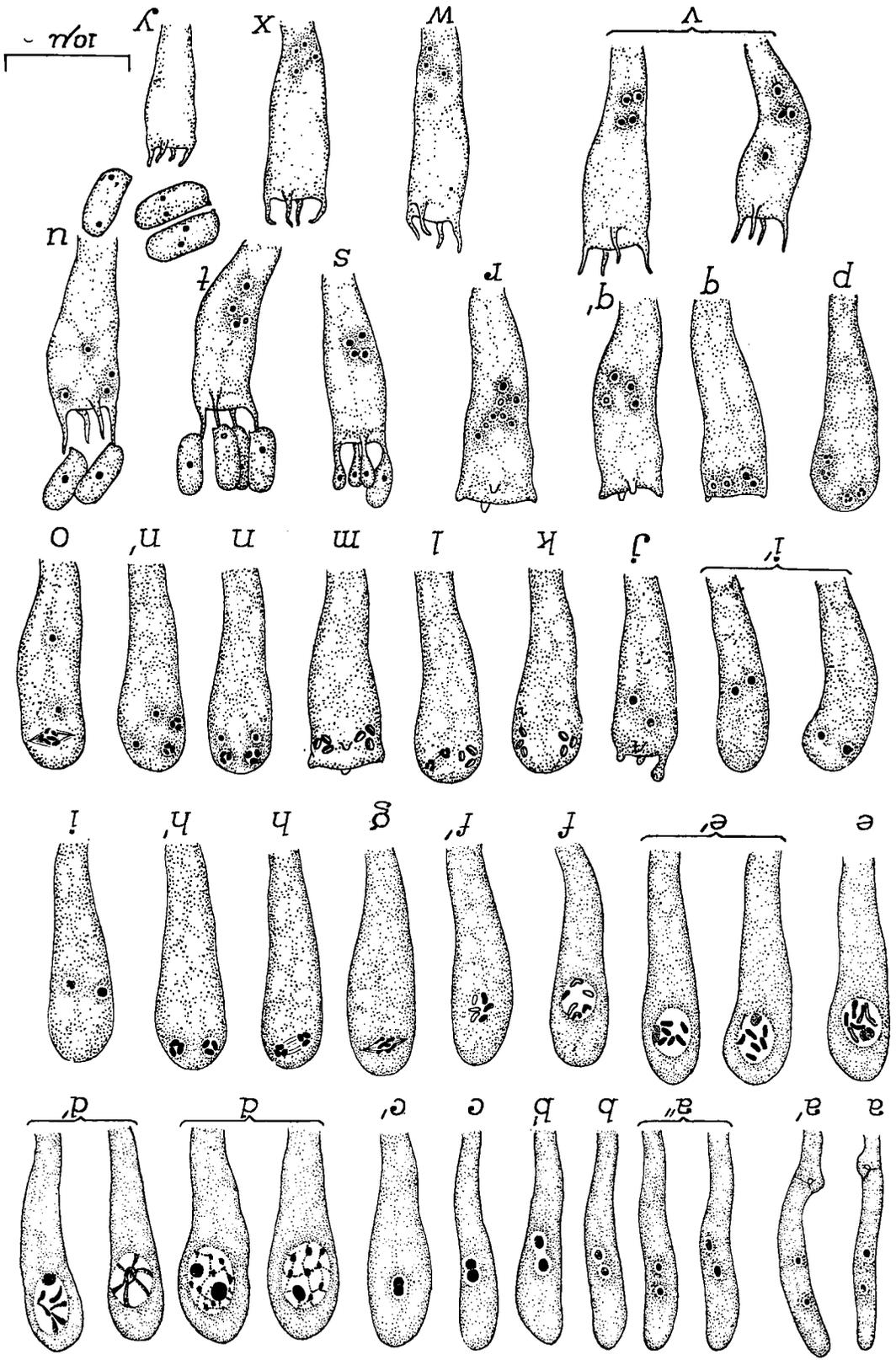
the nuclear membrane by a clear, hyaline zone of karyolymph, about 0.5 μ wide (Fig. 1, a-a'). The two nuclei gradually approach each other (Fig. 1, a") and ultimately fuse to form a synkaryon. During early stages of fusion, the two nuclei come close together and their hyaline zone appear first to coalesce (Fig. 1, b-b'). At this stage, the two chromatin bodies lie within a common, hyaline, somewhat dumb-bell shaped area. Fusion of the chromatin bodies then takes place (Fig. 1, c-c'). The fusion nucleus, at his stage, is similar in structure to those in the young basidium but somewhat conspicuous and larger (3.5-4.5 μ) in size. This type of nucleus where the nuclear matter and chromatin portions are contracted into an intensely stainable, homogenous body free from the majer part of karyolymph is considered as ' compact and homogenous' by PINTO-LOPES (1949). Simultaneously, the basidium also enlarges and gradually attains its maximum size. With karyogamy and formation of synkaryon, the dikaryophasic stage in the life-cycle of the fungus comes to an end. The synkaryon, the only diplophasic stage in the life-cycle, at first occupies approximately the central region of the basidium but subsequently migrates to its upper part. From this it can be assumed that this stage lasts longer than other stages and the fusion nuclei probably undergo a period of rest in preparation for the inception of meiosis. With the onset of meiosis, the synkaryon enters the first stage or ' interphase ' of the division-cycle and enlarges considerably and in the following prophase almost attains the width of the basidium (Fig. 1, d). Delicate reticulum, beaded at places due to deeply stained heterochromatic bodies, and one or two nucleoli make their appearance particularly in preparations stained with haematoxylin and crystal violet (Fig. 1, d). These heretochromatic bodies represent the chromo-centers of chromosomes. In some higher plants, it is often possible to esti-mate the number of chromosomes in a metabolic nucleus by counting such heterochromatic masses (SHARP 1943). In this case, however, the number of chromosomes cannot be calculated accurately from these heterochromatin bodies since their number varies in nuclei of different basidia. During late prophase, chromosomal condensation starts and the nucleolus gradually disappears by losing its chromaticity (Fig. 1, d-e'). Ultimately, the nucleolus disappears completely and the chromosomes become visible within a distinct nuclear membrane (Fig. 1, f). Six distinct chromosomes ($2n = 6$) have been observed (Fig. 1, f'). Typical bivalent chromosomes, however, have not

Fig. 2. — Nuclear phenomena in the basidiospores and germinating basidiospores of *Fomes lividus* Kalchbr: *a,* uninucleate basidiospore; *b-d,* binucleate basidiospores, *c-k,* production of germ-tubes from any point on the spore-wall and subsequent migration of the nucleus or nuclei; *h,* one nucleus migrating into the germ-tube; *j,* migration of both the nuclei into the germ-tube; *l-p,* subsequent nuclear divisions form a multinucleate condition; *q-t,* septum-formation results in the production of terminal uninucleate hyphal cell.

a   *b*   *c*   *d*   *e*   *f*   *g*   *h*   *i*   *j*   *k*   *l*   *m*   *n*   *p*   *q*   *r*   *s*   *t*

10 μ
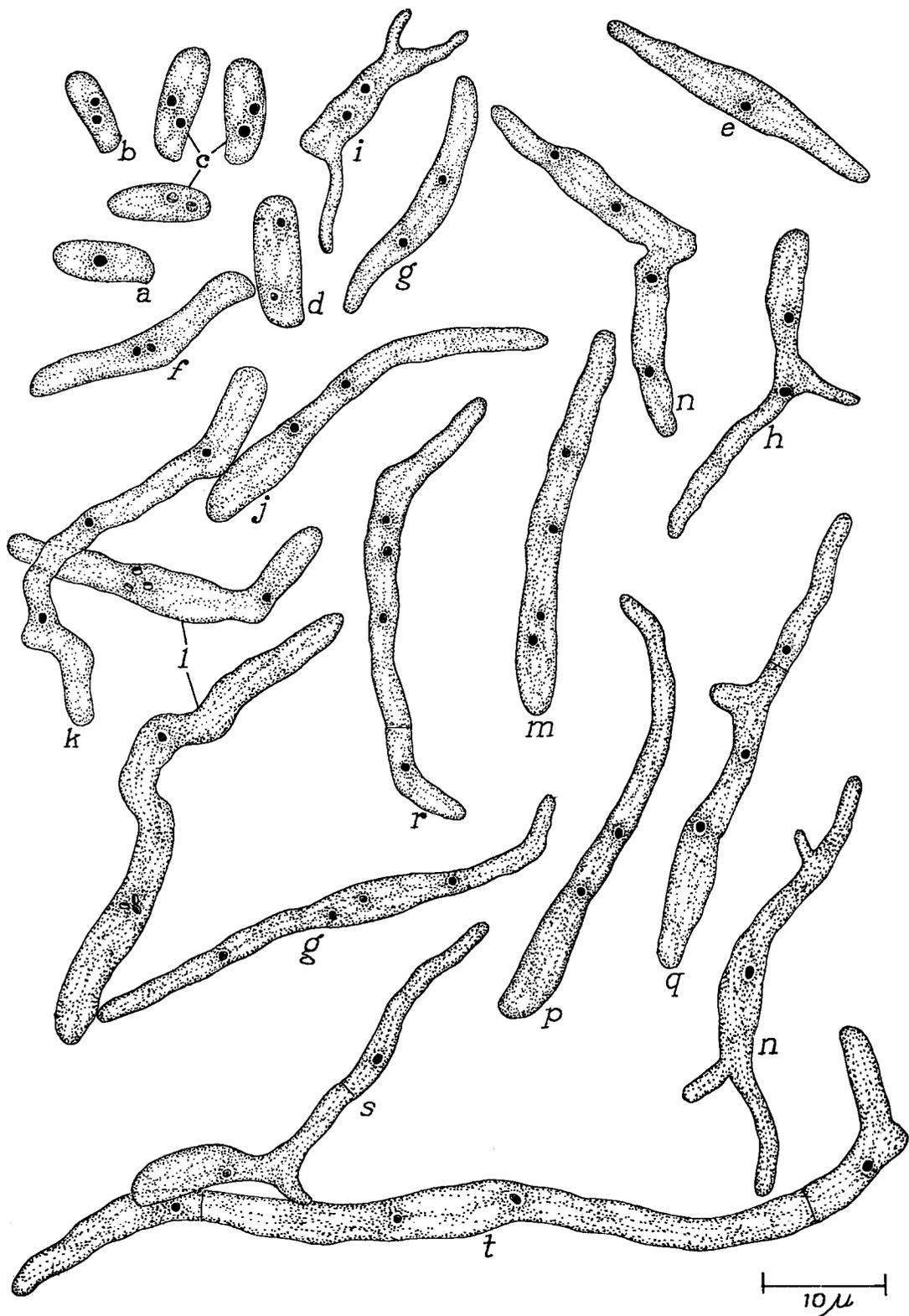
been observed. The nuclear membrane disappears with the formation of a transverse spindle (Fig. 1, f'-g). Closely associated chromosomes are clearly visible near its equatorial region (Fig. 1, g). Subsequently, this stage is followed by anaphasic separation and one member of each of three pairs of chromosomes ($n = 3$) constituting a genome moves towards each pole (Fig. 1, h). The individuality of the chromosomes during late anaphase and early telophase is distinct (Fig. 1, h-h') while the fibres of the spindle disappear. Ultimately, the chromosomes at each pole lose their identity and form a daughter nucleus consisting of a rounded chromatin mass, a surrounding hyaline zone, the nuclear membrane and exactly similar in structure with the nuclei of the young basidium. The two daughter nuclei formed due to first meiotic division lie opposite to each other and at right angles or obliquely so to the long axis of the basidium (Fig. 1, i-j). At this stage basidia enlarge considerably and are typically clavate in form. Their swollen apices projected a little beyond the young basidia. From the preponderance of binucleate condition it can be stated that a short ' interkinetic ' stage probably exists between the first and second divisions of meiosis. The two nuclei then divide in succession (Fig. 1, 1, n-o) but sometimes their divisions are simultaneous (Fig. 1, k, m). The detailed chromosomal changes similar to prophase I have, however, not been observed. Stages have been found where one nucleus is in the metaphase (Fig. 1, l) or anaphase stage (Fig. 1, o) while the other has already completed its division. The orientation of the spindles during the second division shows much variation. In some cases both the spindles are transverse (Fig. 1, k-n) while in others one is transverse and the other longitudinal or obliquely longitudinal (Fig. 1, n'-p). The four nuclei resulting from the second meiotic division, are alike in size, structure and staining properties but their orientation in the basidium is extremely variable. They do not show any definite arrangement within the basidium and either lie closely associated together at the centre (Fig. 1, q') or in the upper part of the basidium (Fig. 1, q). Rudiments of sterigmata, four in number, begin to develop at the apex of the basidium during the later stages of meiosis (Fig. 1, m). Rarely, however, sterigmata also develop at the binucleate stage (Fig. 1, j) of the basidium. The position of the sterigmata does not bear any relation to the position of the four nuclei in the basidium. At this stage, the four daughter nuclei undergo a third homotypc division

Fig. 3. — Nuclear phenomena in the primary and secondary mycelia of *Fomes lividus* Kalchbr.: *a-b*, primary mycelium with uninucleate hyphal cells; *c-e*, secondary mycelium with binucleate hyphal cells having characteristic clamp-connexions at the septa, *f-j*, stages in the formation of a clamp-connexions; *k*, formation of a branch and migration of two « conjugate nuclei » into it.

a

b

c

d

e

f

g

h

i

j

k

10μ
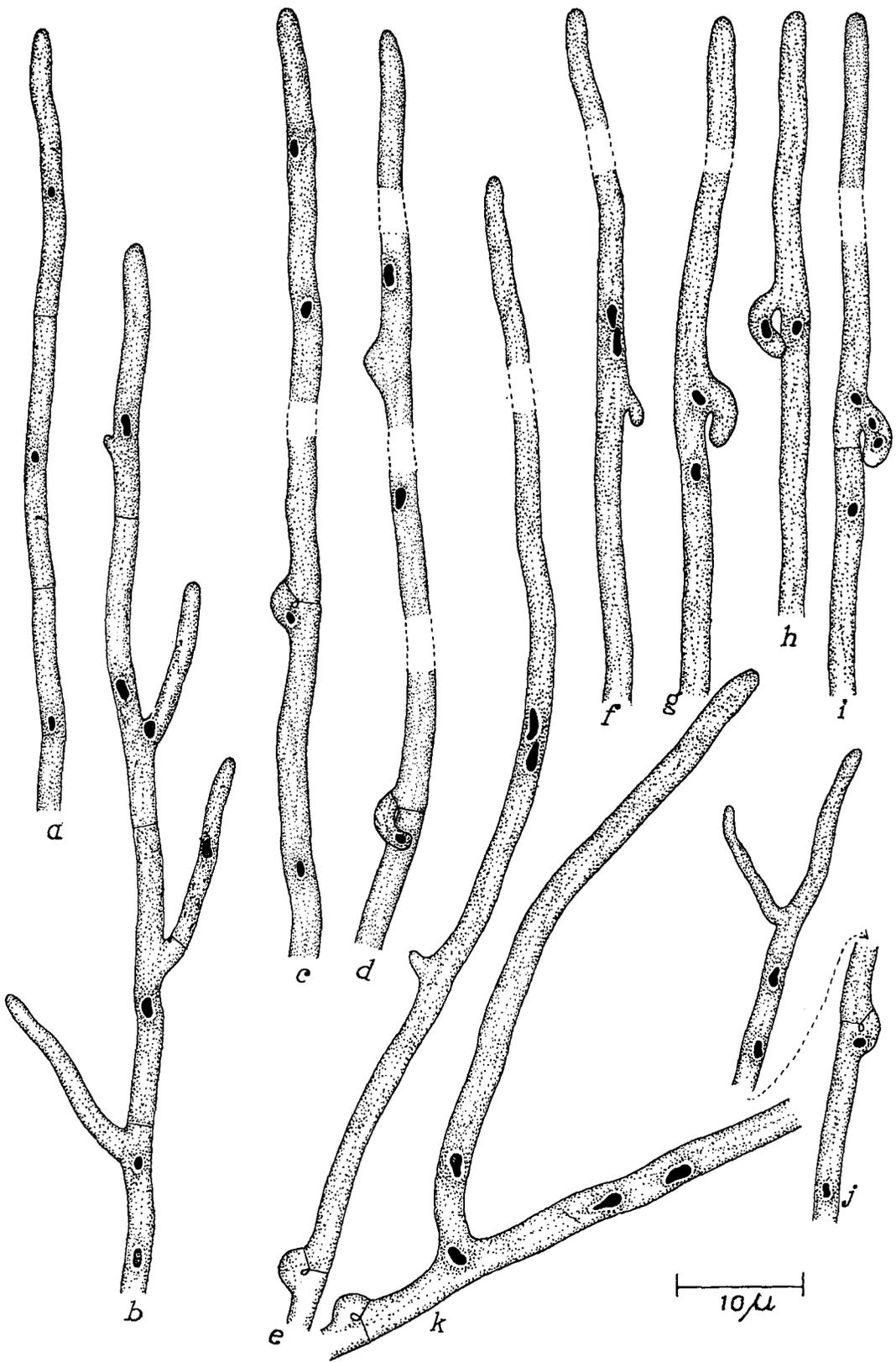
forming eight closely associated nuclei (Fig. 1, r). Divisional stages and spindle-orientation of the third division have not been observed. This stage is followed by the swelling of the tips of the sterigmata and migration of the eight daughter nuclei at random into the four developing spores (Fig. 1, s). Stages of migration of nuclei from basidium into the spores have not been observed. The spores are uninucleate and are separated by walls from the sterigmata. The nucleus in each basidiospore usually occupies a central position (Fig. 1, t-u). Occasionally, the nucleus in each spore undergoes further homotypic division forming two daughter nuclei which lie side by side in a plane at right angle or parallel to its long axis (Fig. 1, y). After the formation of four basidiospores, the remaining four nuclei within each basidium gradually degenerate (Fig. 1, v-w). The sterigmata lose their identity, shorten and the basidium ultimately collapses (Fig. 1, x-y).

The basidiospores are thin-walled, hyaline, somewhat oblong with a distinct apiculus and in a stained preparation reveal the presence of one or two 'compact and homogenous' type of nuclei that are exactly similar in structure with the nuclei of the young basidia (Fig. 2, a). In an uninucleate spore the nucleus occupies the central position (Fig. 2, a). When binucleate, the nuclei remain close together usually in the middle (Fig. 2, b) or quite separated from each other along the long axis of the spore (Fig. 2, d). During germination, the spores somewhat enlarge and one to three germ-tubes emerge from any point on the spore-wall (Fig. 2, e-i), single or opposite germ-tubes being more frequent (Fig. 2, e-g). The germ-tubes are either quite narrow (Fig. 2, i) or of the same width as that of the spores (Fig. 2, n). Branching of the germ-tubes may start immediately after their emergence (Fig. 2, h) or at a late stage (Fig. 2, t). In case of the binucleate spores one or both the nuclei migrate into the germ-tubes (Fig. 2, h, j). When the spore is uninucleate, the nucleus remains within the spore-case but supplies one daughter nucleus in the germ-tube (Fig. 2, k) after division. Occasionally, the nucleus in the germ-tube is immediately separated from the remaining portion by a transverse wall (Fig. 2, s). But more frequently the nucleus in the germ-tube repeatedly divides to make it multinucleate without laying down of transverse septa immediately (Fig. 2, m-n). During this mitotic division, three metaphasic chromosomes ($n = 3$) have been observed. Eventually wall-formation starts from the tips of the elongating germ-tube or from the tips of its branches (Fig. 2, q-t, t) and a primary mycelium of uninucleate cells is produced.

The branched primary mycelium with uninucleate cells represents the monokaryophasic stage. The nuclei are of 'compact and homogenous' type comparable to those of the young basidium and basidiospores. The central chromatin mass of the nucleus is spherical, oval or irregular in outline

(Fig. 3, a-b). The secondary mycelium, on the otherhand, typically dika-ryophasic and is made up of binucleate cells having a simple clamp-connexion almost at every septum (Fig. 3, c-k). The nuclei are exactly similar in structure and staining behavior to those present in the primary mycelium.

DISCUSSION

The basidiospores of *F. lividus* are phenotypically similar. They are uni- or binucleate and germinate to produce primary mycelia of uninucleate cells. The nuclei of basidiospores and those of primary mycelia are haploid. Secondary mycelium consists of binucleate cells and shows simple clamp-connexion. This binucleate condition of the secondary mycelium continues until two haploid nuclei fuse in the young basidium forming a synkaryon. Then the interphasic enlargement of synkaryon follows. With the initation of meiosis, one or two nucleoli and a number of small, deeply stained chromatin bodies become visible within the nucleus. SWANSON (1961) considers that « the nucleic acid of the chromosomes are either too diffused to absorb much dye or as so hydrated that the dye is not accumulated in sufficient quantity to stain them intensely ». Such chromatin bodies have also been previously reported by WAKAYAMA (1930, 1932), BANERJEE *et al.* (1956, 1960, 1962) and others. As the division proceeds chromosomes appear and the diploid and haploid chromosome numbers have been found to be 6 and 3 respectively. The four nuclei formed after meiosis, undergo a third equational division giving rise to eight daughter nuclei. Of these, four nuclei migrate into four developing basidiospores, one in each, and are finally cut off at the tips of four sterigmata.

REFERENCES

BANERJEE S. N. and BANERJEE N. C., 1961. — Contribution to the cytology of Hymenomycetes. V. Karyological observations in Trametes lactinea Berk. Osterr. Bot. Zeitschr., 18: 184-193.

BANERJEE S. N. and MUKHERJEE A. K., 1956. — Contribution to the cytology of Hymenomycetes. II. Karyological observations in Stereum fuscum (Sch.) Quel. Indian Journ. Mycol. Res., 2: 41-50.

BANERJEE S. N. and MUKHERJEE N., 1955. — Contribution to the cytology of Hymenomycetes. I. Cytological studies in Marasmius campanella Holterm. Proc. Nat. Inst. Sci. India, 21 (3): 115-119.

BANERJEE S. N. and NANDI B., 1962. — Contribution to the cytology of Hymenomycetes. VI. Karyological studies in Flammula dilepis B. & Br. Caryologia, 15 (2): 257-366.

BANERJEE S. N. and SAMADDER K. R., 1960. — Contribution to the cytology of Hymenomycetes. III. Karyological studies in Daedalea flavida Lev. La Cellule, 60: 321-330.

BANERJEE S. N. and SANYAL S., 1957. — Contribution to the cytology of Hymenomycetes. IV. Cytological Studies in Lentinus subnudus Berk. and L. praerigidus Berk. Indian Journ. Mycol., Res., 3: 29-35.

BOIDIN J., 1954. — *Essai biotaxonomique sur les Hydnes resupines et les Corticies. Etude speciale du comportement nucleaire et des myceliums*. Thesis.

BOSE S. R., 1937. — *Cytology study of basidia of Polyporaceae*. Journ. Indian Bot. Soc., **16**: 119-128.

EHRLICH H. G. and McDONOUGH E. S., 1949. — *The nuclear history in the basidia and basidiospores of Schizophyllum comune Fr*. Amer. Journ. Bot., **36**: 360-363.

FEULGEN R. and ROSSENBECK H., 1924. — *Mikroskopish-chemischer Nachweis einer Nuclein saure von Typus der Thymonuclein saure und die darauf beruhende elektive Farbung vom Zellkernen in mikroskopischen Praparate*. Zts. Phys. Chem., **135**: 203-248.

GWYNNE-VAUGHAN H. C. and BARNES B., 1937. — *The structure and development of the fungi*.

KNIEP H., 1913. — *Beitrage zur Kenntris der Hymenomyceten I, II*. Zeitschr. Bot., **5**: 593-637

OLIVE L. S., 1953. — *The structure and behavior of fungus nuclei*. Bot. Rev., **19**: 439-586.

PINTO-LOPES J., 1949. — *Contribution to the study of structure in fungi. I*. Portug Acta Biol. Ser. A.. **2**: 191-210.

SASS J. E., 1929. — *The cytological basis for homothallism and heterothallism in the Agaricaceae*. Amer. Journ. Bot., **16**: 663-701.

SHARP L. W., 1943. — *Fundamentals of cytology. London*.

SWANSON C. P., 1962. — *The Cell*. Prentice-Hall, London.

WAKAYAMA K., 1930. — *Contribution to the cytology of fungi I. Chromosome number in Agaricaceae*. Cytologia, **1**: 369-388.

—, 1932. — *Contribution to the cytology of fungi. IV. Chromosome number in Autobasidiomycetes*. Cytologia, **3**: 260-284.

SUMMARY

1. The karyological phenomena in the life-cycle of *Fomes lividus* Kalchbr., a resupinate member of the family Polyporaceae has been studied in detail.

2. The young basidium is distinctly binucleate. This dikaryotic condition comes to an end with their fusion to form a synkaryon. The synkaryon first undergoes an interphasic enlargement followed by the appearance of chromatic materials as reticulate structure. Subsequently, from the reticulum typical chromosomes ($2n = 6$) are formed. A haploid chromosome set constituting a genome ($n = 3$) forms a daughter nucleus at each pole. The orientation of the spindle of the first division of meiosis (heterotypic) is more or less transverse while in the second division (homotypic) it shows irregularities. A third nuclear division occurs forming the eight nucleate condition of the basidium. Four of these eight nuclei migrate into four developing basidiospores while the other four degenerate in the collapsing basidium. The nucleus within the basidiospore often divides before the liberation from the sterigma.

3. The mature uni- or binucleate basidiospore on germination produces one to three germ-tubes. The germ-tubes are either quite narrow or of the same width as that of the spores. As the germ-tubes enlongate, their nuclei repeatedly divide, formation of septa follows and eventually a septate primary mycelium of uninucleate cells without clamp-connexion is formed.

4. The secondary mycelium, with characteristic clamp-connexion and binucleate cells, is formed only when two compatible primary mycelia unite.