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VARIATION IN *POLYSTICTUS XANTHOPUS* FR.

By

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(With Plates I to IV and 4 Text-figures)

THE present study has been undertaken to determine the degree of variation and the differences that exist between the variants in *Polystictus xanthopus* Fr. So far, three forms of the fructifications of the fungus have been recognised based only on the nature of the hymenial surface and they have been reported mainly from the hills of Assam. They usually grow on logs of *Shorea robusta* under identical environmental conditions.

It has been found that all the three forms of the fungus differ not only in the characters of the hymenial surface but also in the external morphology and microscopic anatomy of the sporophores, in growth characteristics on artificial and natural media, in oxidase reactions, in the rate of decaying sapwood of *Shorea robusta* and in the reaction to a toxic substance. Some of these characteristics have been found to be overlapping. The differences between the three forms in growth characteristics are both morphological, such as colour, topography and texture of the superficial mats, and physiological, as exemplified by the existence of antagonism between the uniting pairs of mycelia on artificial media, effects of variation in *p*H of a medium on growth, reactions to zinc chloride and the rate of growth. No hyphal fusions between the polysporous mycelia of the three forms have been observed.

INTRODUCTION

There are records (Stakman, 1929) of many species of fungi which exhibit physiologic forms. These forms differ greatly in their parasitic activity, growth characteristics under cultural conditions and also to some extent in morphological characters. Among Hymenomycetes, wide variation of this type within the same species has been noted only in a few species by Schmitz (1925) and Verrall (1934). In any comparative work it is, therefore, essential to find out the range of variation existing within the same species in the characters being studied. Considerable cultural work has already been done with wood-rotting fungi from India by Bose (1929, 1929a, 1930), Banerjee and Bakshi (1945), Bagchee and Bakshi (1950) and Bakshi and Bagchee (1950), but no work has been done on form-variation on any species in India so far. It is, therefore, desirable to study on a comparative basis the range of variation exhibited by a given species.

The present study has been undertaken to determine the degree of variation existing in *Polystictus xanthopus* Fr. This fungus has been selected, because Bose (1936) has reported form-variation within this species mainly on the characters of the hymenial surface. According to him "the hymenial surface of specimens of *Polystictus xanthopus* Fr.

at high altitude shows three different kinds of porous area, viz., some with typical very small pores, some with much bigger pores and some with hydnoid pores''. He also concludes that they are variations of one and the same species. Such variations, however, are hardly found in the specimens collected from the plains. Bose's report has been based on materials from different localities of Cherrapunji (8000 ft.) and Lokra hills (8000-10,000 ft.) Assam, collected in 1929 and 1934 respectively.

So far as the authors are aware, very little work has been done on this fungus up to the present time. Although first named and described by Fries (Saccardo, 1888), it was subsequently re-described by various workers like Lloyd (1910), Bose (1918, 1919, 1936), Corner (1932) and others based on collections from different localities. Their descriptions were mainly based on external morphology of the fruit-bodies. In 1932, Corner not only described the characteristic form of *Polystictus xanthopus*, but also extended his researches on the hyphal anatomy of the fruit-body of the fungus. Further, its sexuality has been recently studied by Banerjee and Samadder (1957). Nothing more in particular, is known about the fungus on other aspects.

The scope of the present investigation is to determine how much this species varies with regard to external form and anatomy of the fruit-bodies together with general cultural characteristics on artificial media including the rate of growth, habit of growth, colour-variation and hyphal characteristics of the mycelium. Attempts have also been made to find out the nature of antagonism and compatibility that exist between the mycelia of different forms, their rate of decaying wood and also their response to a toxic substance of different concentrations.

Geographical Distribution

Polystictus xanthopus Fr., a common saprophyte, has been known for more than a century in the tropics of the old world. In India, it is known to occur on logs and fallen branches of *Shorea robusta* Gaertn.f. and on *Cedrela toona* Roxb. (Banerjee 1947; *List of common names of Indian Plant Diseases, I. C. A. R.*, 1950) causing white spongy rot. Lloyd (1910) reports that the home of this plant is Africa and probably it was first published by Ehrenberg as *Polyporous katui* which was subsequently re-named as *Polystictus xanthopus* by Fries. The African collections run to the mesopodial types mainly. In tropical America they are very rare. There are records of this species from America in Saccardo (1888), but according to Lloyd (1921), they are erroneous, as in his wide experience he did not receive a single specimen from this country. More recently, however, Killermann (1928) has again stated it to be pantropical. Petch (1916), mentions that it generally occurs in low countries. According to Corner (1932), besides these, there are collections in Singapore herbarium from many of the low lying parts of Malaya and specimens from high altitudes, such as from Camerons highlands in Pahang (6000 ft.), have also been found. In Fraser's hill, Pahang, (4000 ft.) luxuriant growth of this fungus has been reported. It has also been reported from Negri Sembilan

on Gumong Angsi (2700 ft.). Therefore, it can be stated that *Polystictus xanthopus* seems to be generally distributed throughout the Peninsula and is independent of altitudinal variations. Saccardo (1888) reports it to be commonly found in the tropics, Sierra Liona (Africa), India, Java, Guyana, Brazil, Peru, Pegu, Sumatra, Cocincina (S. America), Australia, North America and Islands of the Pacific Ocean. It has also been reported from various places in India by Bose (1919, 1936) and others (Butler and Bisby, 1931).

Materials

The fructifications used for the study were collected from Cherrapunji, Assam, in 1953, in the month of September. The situation was rather cool, very damp, somewhat shady and provided a favourable condition for the growth of the fungus. They were found to grow luxuriantly on posts and logs of *Shorea robusta*. Several collections of the fungus from this locality were made from which three morphologically different types were carefully selected. The materials when brought into the laboratory, were revived by placing the fructifications with their upper surface downwards on moist cotton wool in several Petri-dishes and from these spores were collected in sterile agar plates. From these spores initial polysporous cultures were made by transferring loopfulls of spores aseptically to 2.5% *malt-agar* slants. These were then incubated at 30°C. Within a week good mycelial growth was obtained in all the tubes and these were finally kept as stock-cultures for subsequent study and comparison. Microscopic examination of all the cultures showed the presence of much branched, hyaline, septate hyphae with abundant clamp-connections.

Morphology of the Sporophores

A. *External features*

It has been previously stated that there exists considerable variation in external morhpology of the fruit-bodies of *Polystictus xanthopus* which first attracted the attention of Bose in the year 1936. He recognises three different types of fructifications based mainly on the character of the hymenial surface. After careful study of the external features of all the materials three distinct types of fruit-bodies have been seggregated as follows :

Type–I. Fructifications with long and narrow excentric stipe, very thin, more or less obliquely infundibuliform and with very minute, regular pore-mouths ;

Type–II. Fructifications small, with somewhat stout and thick excentric stipe, more or less obliquely saucer-shaped, with a central depression and with minute, regular and slightly larger pore-mouths ;

Type–III. Fructifications thin, always sessile, bracket-shaped and with angular to 'Irpecoid' or 'Hydnoid' pore-mouths.

Forms with intergrading characters have also been noticed in many specimens, but these cannot be strictly placed in either of the above-mentioned three distinct categories. The external features of the types recognized by the writers have been fully described below. In all cases the colour of the fruit-bodies has been expressed according to Ridgway (1912).

Type–I (Plate I, figs. 1 and 2)

Fructifications : Stipitate. *Stipe :* Usually excentric, sometimes central but never lateral, more or less cylindrical, arising from a small disc-like concolorous base, gradually expanding upwards into the pileus but sharply delimited from it, smooth, Ochraceous-Buff to Ochraceous-Orange in colour, often with Ferruginous spots, somewhat stiff on drying, dimension about 10–25×1–3 mm. *Pileus :* Infundibuliform, usually obliquely so; coriaceous, becoming stiff on drying; sometimes laterally confluent; usually about 7–9 cm. or more in diameter; margin very thin, entire, even, becoming lobed in larger, specimens, involute on drying; white but becoming Cinnamon-Buff in dry specimens. *Upper surface :* Glabrous, shining; concentrically zoned; somewhat stiff; rugulose due to longitudinal wrinkles; colour showing various shades of Cinnamon-Buff, Orange-Cinnamon, Vinaceous Tawny; Garnet Brown and Maroon in regular concentric bands of variable thickness, Cinnamon-Buff colour being predominant in some specimens. *Context :* Very thin, less than 0.5 mm. in thickness; white; coriaceous. *Hymenial surface :* Smooth, somewhat longitudinally wrinkled in dry specimens; dull white in colour but becoming Pinkish-Buff when old; pore-mouths minute, regular, more or less circular, ending abruptly near the margin forming a sterile zone, about 1 mm. wide; pore-tubes very short, forming a distinct layer, not separable from the substance of the context.

Type–II (Plate I, figs. 3 and 4)

Fructifications : Stipitate. *Stipe :* Always excentric, never central or lateral; usually short cylindrical, sometimes elongated, dilating upwards into the pileus but sharply delimited from it, arising from a more or less concolourous discoid base; Ochraceous-Orange or Antimony Yellow in colour, sometimes with Ferruginous spots; smooth and glabrous, sometimes rugulose; somewhat stiff and corky on drying; dimension about 5–7 (12)×5–8 mm. *Pileus :* Always shallow, saucer-shaped but oblique; coriceous, becoming stiff on drying; sometimes laterally confluent; about 3.5–7 cm. wide and 2.5–4 cm. deep. Margin thin, usually entire, even, sometimes slightly wavy but never lobed, not involute on drying, at first white, but turning Pinkish-Buff with age. *Upper surface :* Glabrous; shining with numerous narrow to broad concentric zones; somewhat rugulose due to longitudinal wrinkles; colour showing various shades of Garnet Brown, Maroon and Claret Brown throughout, a broad band of Maroon colour invariably present just behind a narrow Pinkish-Buff margin; a prominent ride around the central depression almost invariably present. *Context :* Somewhat thick, less than 1 mm. in thickness; white; coriaceous. *Hymenial surface :* Usually smooth, sometimes with wide longitudinal

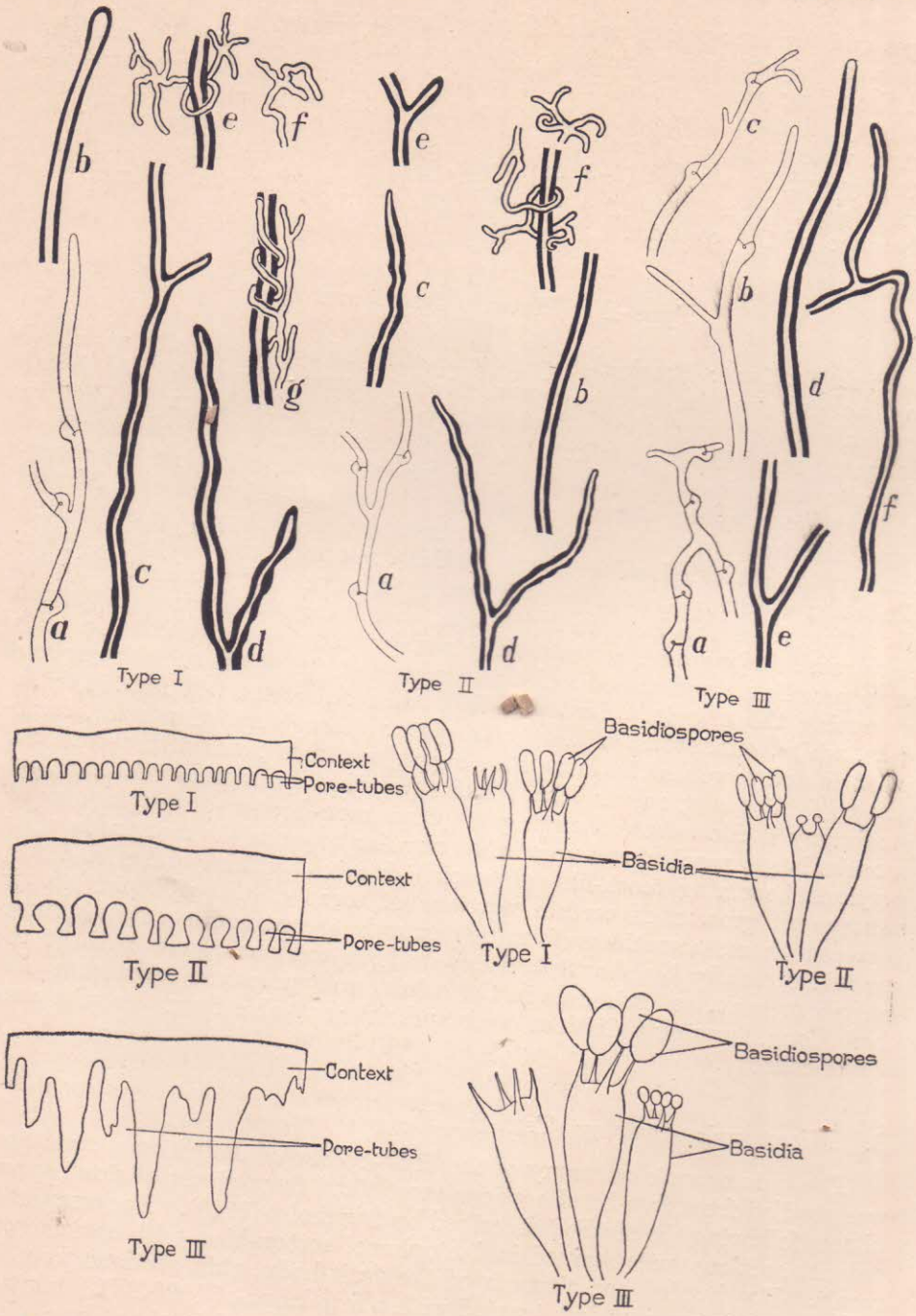
ridges and furrows; colour not uniform, a broad central light Wood Brown zone with Light Buff to Pinkish-Buff coloured areas towards the margin and the stipe; pore-mouths minute, regular, more or less circular throughout but delimited towards the periphery by a distinct narrow zone, about 1 mm. wide and consisting of somewhat angular pores; this narrow zone abruptly ends in a broad (2–3 mm. wide) sterile edge; pore-tubes comparatively large; tubes forming a distinct layer and not separable from the substance of the context.

Type–III (Plate I, figs. 5 and 6)

Fructifications : Bracket-shaped (reflexed), and sessile; usually attached to the substratum by a comparatively narrow base or the basal portion near the point of attachment with the substratum is slightly elongated resembling a stalk; thin; flabelliform, in the form of an arc of a circle; sometimes imbricate; coriaceous when fresh, becoming somewhat stiff on drying; about 3–7 cm. across and 2.5–4 cm. deep. Margin very thin, uneven, slightly lobed, not involute on drying; whitish at first but turning Pinkish-Buff to Warm Buff in dry specimens. *Upper surface:* Glabrous; somewhat shining; stiff; with numerous longitudinal wrinkles diverging from the base outwards and rendering the surface somewhat rugulose; faintly zoned, zones usually narrow but one or two zones usually very prominent and raised; colour Maroon and Garnet Brown throughout excepting the margin. *Context :* Very thin, less than 0.4 mm. in thickness, white, coriaceous. *Hymenial surface :* Rough and 'Irpecoid', somewhat folded towards the margin in bigger specimens, sometimes with one or two faint zones; colour Warm Buff towards the margin, other portions Antimony Yellow but changing to Cinnamon-Brown on drying; pore-mouths large, regular, and somewhat angular towards the margin, in older portions becoming teeth-like (Irpecoid) due to breaking down and also subsequent growth of certain portions of some of the dissepiments in between them, sometimes forming somewhat continuous cavities interrupted internally by short partition walls; a uniform sterile zone at the margin present, not raised as in the other two types; pore-tubes comparatively long, forming a distinct layer and not separable from the substance of the context.

B. *Microscopic Anatomy* (Text-fig. 1)

The microscopic anatomy of the fruit-bodies of *Polystictus xanthopus* was studied from free-hand sections mostly cut from pieces of dried sporophores following the method described by Overholts (1929). For temporary mounts sections were stained in lactophenol-cotton-blue with satisfactory result. For hyphal constituents of the fruit-body, thin sections from its different parts were at first treated with a few drops of 10% KOH solution on a slide and washed with water. Small groups of hyphae were then teased from these sections in warm lactophenol and examined without staining. Staining of the hyphae was considered unnecessary, because when the substage diaphragm and the condenser of the microscope were



Text-fig. 1. Anatomical features of the three types of fructications of *Polystictus xanthopus*. (Hyphal systems and basidia & basidiospores. × 700)
(For explanation, vide text)

slightly regulated, visibility of the hyphae was decidedly increased. These teased materials were also mounted in Erythrosine-glycerine-agar jelly for making permanent preparations. Measurements regarding the width of the hyphae and other elements or structures of any given specimen were based entirely, according to the suggestion given by Bisby (1945). Ten to twenty routine examinations and measurements of carefully selected elements to include the range of sizes were found to be sufficient for this purpose. The nomenclature adopted by Corner (1932) in describing different types of hyphae of the fruit-bodies have been followed.

(i) *Thickness of the context.* The thickness of the context varies greatly in the three types, maximum being in Type–II and minimum in Type–III. The thickness of the context in Type–I is intermediate between the two and varies from 300–420μ, that of Type–II and Type–III ranging from 600–870μ and 120–330μ respectively.

(ii) *Length of the pore-tubes.* The pore-tubes, with regard to length, vary greatly and do not form a well distributed series. The pore-tubes in Type–II are almost double in length than those in Type–I, but between Type–II and Type–III there is a wide gap. The length of the pore-tubes in Type–I varies from 80–140μ and that in Type–II 170–230μ. Maximum length of the pore-tubes has been attained in Type–III which varies from 660–1950μ.

(iii) *Thickness of the dissepiments.* As regards the thickness of the dissepiments it is also different in the three types. In Type–I it is narrow, more or less uniformly thick from base to apex of the pore-tubes and about 4–8μ in thickness. In Type–II the dissepiments appear as biconcave columns in longitudinal section, dilating towards the pore-mouths and the bases of the pore-tubes, and finally merging into the context. Its thickness varies between 8–12μ in the middle and about 16–20μ at either ends. In Type–III, the dissepiments appear as more or less conical structures in longitudinal section being widened towards the context and more or less tapering towards the pore-mouths. Its thickness varies from 12–14μ towards the apex and 44–48μ near the base.

(iv) *Diameter of the pore-tubes.* The diameter about the middle of the pore-tubes does not vary much in Type–I and Type II, being 60–110μ in the former and 60–120μ in the latter, but in Type–III it is very much widened and ranges from 150–300μ.

(v) *Length and width of basidia and basidiospores.* The shape and size of the basidia and basidiospores in Types I and II agree more or less closely with each other. The basidia in Type-III appear to be larger although the basidiospores do not vary much with those in other two types so far as their measurements are concerned. Table 1 will furnish the relevant data.

Table 1. *Basidia and basidiospores of* Polystictus xanthopus

	Type I	Type II	Type III
Basidia	Broadly clavate, tetra-sterigmatic and quadri-sporous; dimension about 12.2–13.5×3μ.	Broadly clavate, mostly di-sterigmatic and bi-sporous, some tetra-sterigmatic and quadri-sporous; dimension about 12—13×3—3.5μ.	Clavate, elongated, tetra-sterigmatic with long sterigmata and quadri-sporous; dimension about 18—19×5.5—6μ.
Basidiospores	Oval to cylindrical, sometimes slightly curved; hyaline, thin-walled, smooth; dimension about 5–5.5×2–2.2μ.	Shape and structure like those in Type–I; dimension about 5.4×1.5–2μ.	Mostly oval to sub-cylindric; hyaline, thin-walled, smooth; dimension about 6.4–7.5×3μ.

(vi) *Hyphal characteristics.* In hyphal composition of the fruit-bodies, there appear to exist two distinct groups. Fructifications of Types I and II together form one group in which all the four types of hyphae, viz., *generative*, *skeletal*, *mediate* and *binding*, as defined by Corner (1932), are present. Fructification of Type–III alone forms the other group in which there is a tendency towards the elimination of binding hyphae. The hyphal composition of the three types of fruit-bodies has been summarized in Table 2.

Table 2. *Hyphal system in the three types of fruit-bodies of* Polystictus xanthopus

Generative hyphae	Frequent; usually thin-walled, sometimes the walls become slightly thick but with uniform linear lumen; branched extensively or sparingly; longitudinal or interwoven; hyaline; about 1.5–3 (4) μ wide; usually nodose septate and having a simple clamp-connexion at each septum; with abundant protoplasmic contents, in the older portions contents becoming scanty. (Text-fig. 1, a)	Frequent; characters same as those in Type–I; about 2–6μ in diameter. (Text-fig. 1, a)	Very abundant; profusely branched and interwoven; width varying from 2–3 (4) μ; full of granular contents; nodose septate; branches often becoming narrow. coralloid, nodulose or spiculiferous; general characteristics like those in Type–I. (Text-fig. 1, a–c)

	Type I	Type II	Type III
Skeletal hyphae	Most abundant; thick-walled; straight or slightly flexuous; unbranched; more or less longitudinal; without clamp-connexions; hyaline to pale yellow in colour; about 2–6μ wide; wall thickness very variable, in some cases lumen almost obliterated in mature parts, sometimes uniformly thick throughout; apices mostly thin-walled, usually aseptate, but in a few occasional septa may be present. (Text-fig. 1, b)	Most abundant; characteristics more or less similar to those in Type-I; width of the hyphae 4–6μ. (Text-fig. 1, b–c)	Very abundant ; mostly flexuous but sometimes straight; usually interwoven due to flexuous nature; hyaline to yellowish brown in mass; about 5–6μ wide; other characters similar to those in Type–I. (Text-fig. 1, d)
Mediate hyphae	Frequent, more or less uniformly thick-walled, with lumen linear; usually aseptate, a few occasional septa may be present; pale yellow in colour about 1.5–3.5μ wide; straight or slightly flexuous; somewhat inter woven; scanty granular contents may be present; apices thin-walled with granular contents. (Text-fig. 1, c–d)	Present but less frequent; characters similar to those in Type–I ; about 2–4.5μ wide. (Text-fig. 1, d–e)	Present but comparatively very few; about 2–4μ wide; characters similar to those in Type–I. (Text-fig. 1, e–f)
Binding hyphae	Frequent; thick-walled; much branched; often inter-twining or twisted, usually coiling around skeletal and mediate-hyphae, sometimes coralloid, nodulose or spiculiferous; without clamp-connexions; mostly hyaline to pale yellow coloured; about 1–2.5 (3)μ wide; wall thickness variable with lumen linear or obliterated in older parts; apices thin-walled with few granular contents. (Text-fig. 1, e–g)	Frequent; characters more or less similar to those in Type-I; width varies between 1.5–2.5(3)μ. (Text-fig. 1, f)	Not in evidence ; structures appearing as binding hyphae are in reality much branched generative hyphae.

Fungus in Culture

A. *Cultural Characteristics*

(i) On agar medium

So far as the authors are aware, nothing is known about the cultural characteristics of *Polystictus xanthopus*. For studying the cultural characteristics of the three forms recognized by the writers, polysporous mycelia of all the types were grown separately on *potato-dextrose-agar* in Petri-dishes (90 mm. in diameter). The medium was prepared, according to the recipe of Fritz (1923). The cultures were grown in complete darkness under ordinary conditions of temperature (28°–30°C.) of the laboratory. In order to maintain the size of the inocula as constant as possible, small discs (5 mm. in diameter) of agar with the polysporous mycelium of each type were cut out from the periphery of the advancing mycelium by means of a sterile cork-borer from 7-days-old culture. Each disc was then placed with upside downwards upon the medium at the centre of the Petri-dish containing approximately about 30 c.cm. of the medium. The *p*H value of the medium after sterilization was found to be 5.2. Observations were based on an average of four Petri-dish cultures of each type. The terms used to describe the texture of the mat are those proposed by Nobles (1948) and the colour exhibited by the mycelium has been expressed, according to Ridgway (1912). The cultural characteristics of the three types have been described below separately.

Type–I (Plate II, fig. 7)

(i) *Habit of growth.* In all cases, growth started with much rapidity and within 24 hours after inoculation, formed a thin, cottony mycelium over the surface of the inoculum. Within the next 24 to 36 hours, growth appeared over the agar medium forming an appressed mycelium which later tended to become somewhat raised and cottony towards the periphery, while the mycelium over the inoculum turned somewhat felty. As the growth advanced, the superficial mat gradually condensed and in about six days after inoculation it was differentiated into an outer raised felty zone surrounding a central appressed area. On the tenth day the central zone was somewhat lacunose and the peripheral zone became slightly raised and felty to farinaceous. No further change in the habit of growth was, however, noticed even in cultures 15 to 20-days-old, excepting the mycelium appeared to be more compact. No change in colour of the medium was noticed in any case.

(ii) *Colour.* The colour of the mycelium was white throughout during the early period of growth, but from the eighth day pigmentation of the mycelium started. At first patches of Light Vinaceous appeared only in the central lacunose zone. A gradual change of colour was, however, noted from the ninth day onwards with the age of the culture and the range of colour included Vinaceous to Drab towards the centre and

Pinkish-Buff with Apricot Buff spots here and there towards the periphery.

(iii) *Rate of growth.* The rate of growth was more or less uniform from the very beginning and increased about 5 mm. daily in radial direction.

Type–II (Plate II, fig. 8)

(i) *Habit of growth.* Growth started with moderate rapidity forming thin mycelium over the inoculum within 24 hours after inoculation. On the following day it turned somewhat patchy felty and became surrounded by a thin, narrow zone of appressed mycelium over the agar medium. On the fourth day the major portion of the superficial mat became uniformly smooth but tended to become somewhat raised and felty towards the margin and with a narrow appressed zone of advance. Within a couple of days a somewhat rugulose area appeared in the central region of the mat which soon became separated from the inoculum by a slightly raised circular felty area. Towards the periphery of the mat, the texture remained uniform. Between the periphery and the rugulose region, the mat became slightly depressed and assumed Chamoise texture in culture, about 10-days-old. No change in colour of the agar medium was, however, noticed.

(ii) *Colour.* At the onset, the mycelium was pure white throughout but tinting of the mat started on the sixth day after inoculation. The central area of the mat at first became coloured and the range included Pinkish Cinnamon and Pinkish-Buff. Further change in colour was, however, not noticed excepting in that certain portions of the coloured area became more intense.

(iii) *Rate of growth.* To start with the growth was rather slow but within three to four days after inoculation it became more rapid and remained more or less uniform throughout in all cases. The average daily increment of growth was 5.5 mm. in radial direction.

Type–III (Plate II, fig. 9)

(i) *Habit of growth.* At first the growth was rather slow to start with and within 24 hours after inoculation practically no growth was observed over the inoculum. After 32 to 34 hours, thin, white sub-felty growth could be recognized only over the inoculum which within the next 12 to 16 hours descended over the medium forming a narrow circular zone of appressed and sodden mycelium. As the growth advanced, the superficial mycelium gradually became raised, smooth and felty forming a zone towards the periphery. On the tenth day the superficial mat became smooth and felty throughout, but with two to three raised concentric zones about the middle portion of the mat. No further change in texture was, however, noticed during later stages of development of the mycelium excepting in that it became more or less tightly interwoven. There was no discolouration of the medium during this period.

(ii) *Colour.* At the beginning the colour of the superficial mat was white throughout. On the sixth day of inoculation slight tinting of the

mycelium started at the periphery in the form of a circular zone of Pinkish-Buff while other portion of the mat remained white. With age, pigmentation gradually extended towards the centre showing lighter shades on the tenth day. Finally, the colouration of the mat deepened and showed gradual shades of Saccardo's Umber, Twany-Olive and Pinkish-Buff from the periphery to the centre.

(iii) *Rate of growth.* Although the growth started slowly, the mycelium soon became active and subsequently grew more or less at uniform rate. The daily increment of growth was found to be 4.5 mm. in radial direction.

In Table 3 a comparative account of the cultural characteristics of the mycelia of the three types of *Polystictus xanthopus* on the tenth day on *potato-dextrose-agar* has been summarized.

Table 3. *Comparative cultural characteristics of the mycelia of the three types of* Polystictus xanthopus *on the tenth day of inoculation on potato-dextrose-agar.*

	Type–I	Type–II	Type–III
Habit of Growth	Growth slightly raised, felty to farinaceous towards the periphery, surrounding a central lacunose zone.	Peripheral region somewhat raised and uniformly felty; behind it a slightly depressed Chamoise zone surrounding a central somewhat rugulose area.	Smooth and felty throughout but with two to three raised concentric zones about the middle portion of the mat.
Colour	Light Vinaceous towards the centre and a few Pinkish-Buff spots here and there towards the white peripheral mat.	Central portion of the mat showing shades of Pinksih Cinnamon and Pale Pinkish-Buff; other portions white.	Pinkish-Buff peripheral zone with lighter shades extending from the periphery towards the white central region.
Daily increment of growth	Uniform; 5 mm. in radial direction.	Uniform; 5.5 mm. in radial direction.	Uniform; 4.5 mm. in radial direction.

(ii) On wood-blocks (Plate II, figs. 10-12)

Growth characteristics of the superficial mycelia on sapwood blocks of Sāl were also studied (Plate II, figs. 10–12). For this purpose, several Kolle flasks, each containing sterile 2.5% *malt-agar* slants, were inoculated with polysporous mycelia of the three types of *Polystictus xanthopus* separately and after 10-days-growth, several water-soaked sapwood blocks were exposed to them. These flasks were then kept in complete darkness and room temperature (25°–30°C) of the laboratory.

After three to four days, almost all the blocks were attacked and partly covered by the mycelia, but the rate of growth in case of Type-III appeared to be rather slow. The mycelium in each case was white throughout at the beginning. On the seventh day after exposure of the wood-blocks, very luxurient growth of the mycelium of Type–II was observed and the texture was Chamoise like in appearance. In case of Type–I, the free faces of the wood-blocks were covered with densely felty to cottony mycelium, which later became more active and uniformly compact. The growth of the mycelium in case of Type–III was somewhat thin and cottony which later became compact to form patchy felty areas while the other portions remained as before. Gradually, shades of colour appeared in all cases. In Type–I, the mycelium at first turned Vinaccous which later deepened to Drab colour in about a month. In Type–II, the mycelium became Pale Pinkish-Cinnamon and remained in that condition during subsequent stages of development. In Type–III, the mycelium turned Pinkish-Buff at places and no further change of colour was observed in this case even in cultures 1-month-old.

B. *Oxidase Tests* (Plate II, figs. 13–18)

Oxidase tests, as originally recommended by Bavendamm (1928) to distinguish a "white rot' fungus from the 'brown rot' type, were also performed. These tests were made in the laboratory by growing polysporous mycelia of the three types of *Polystictus xanthopus* on 2.5% malt-agar containing 0.5% gallic or tannic acid in Petri-dishes. Dark brown rings due to the presence of oxidase appeared in all cases within 36–48 hours indicating that all the three types of mycelia are capable of decomposing lignin constituents of the wood and can be considered positive reactors. Therefore, they are all of "white rot' type. In general, the intensity of reaction was greater on gallic acid medium than that on the medium containing tannic acid due to both types I and II while the reverse condition prevailed in case of Type–III. The variation in the intensity of reaction and diameters of the diffusing zones after 48 hours have been shown in a comparative way in Table 4. The terms used in describing the intensity of reaction are those of Nobles (1948).

Table 4. *Comparative account of the oxidase activities of the polysporous mycelia of the three types of* Polystictus xanthopus.

		Type–I	Type–II	Type–III
Tannic acid	Diameter	20 mm.	17 mm.	22 mm.
	Intensity	Strong	Moderately Strong	Very Strong
Gallic acid	Diameter	17 mm.	20 mm.	18 mm.
	Intensity	Strong	Strong	Strong

C. *Effects of acidity and alkalinity* (Plate IV, figs. 31–42)

In order to find out whether there was any variation in the rate of growth of the polysporous mycelia of the three types of *Polystictus xanthopus* in a medium having different *p*H, an experiment was set up. The medium taken was 2.5% *malt-agar* and N./10 HCl and N./50 NaOH solutions were used as acid and alkali to prepare four different *p*H grades, viz., 4, 6, 8, and 10, two on the acid side and two on the alkali. A small disc of inoculum was put at the centre of each plate with the mycelial side downwards. The inoculated plates were then kept in diffused light and at room-temperature (28°-32°C.) of the laboratory.

In all cases, except in Type–III, growth started after 24 hours while in Type–III it was evident in about 36 hours. On the fifth day of inoculation the variation in the rate of growth of the three types of mycelia were noted as shown in Table 5.

Table 5. *Comparative data showing the rate of growth in diameter of the three types of mycelia of* Polystictus xanthopus *on the fifth day after inoculation on malt-agar having different p*H.

*p*H (range)	Type–I	Type–II	Type–III
4	5.5 cm.	6.6 cm.	5 cm.
6	6 cm.	5.5 cm.	5.7 cm.
8	4.2 cm.	3.5 cm.	3.5 cm.
10	2 cm.	1.5 cm.	1.7 cm.

From the table it is evident that there is no marked variation in the rate of growth of the three types of mycelia on a medium having different *p*H. There is, however, a decline in the rate of growth in Types I and III on both sides of *p*H 6. In Type–II, on the other hand, this decline in the rate of growth becomes evident even from *p*H 6. That is to say, the growth in diameter of the colonies is maximum in *p*H 6 in Types I and III, while in Type–II it is maximum in *p*H 4. In *p*H 10, the growth of mycelia of all the types is considerably checked.

D. *Hyphal Characteristics*

In all cases preparations for microscopic examination were made of mycelium mounted directly in water and examined with the aid of a Phase-contrast microscope. For making permanent preparations, hyphae from different regions of the mat were mounted in Erythrosine-glycerine-agar jelly. In order to note size, form and gradual differentiation of the actively growing hyphae, observations were made from different regions of 7, 10 and 14-days-old cultures beginning from the periphery to the centre of the mat before it attained tough consistency.

In describing the different types of hyphae, the descriptive terms as proposed by Corner (1932) have been used throughout. In order to avoid unnecessary repetition a full description of the hyphal types and their

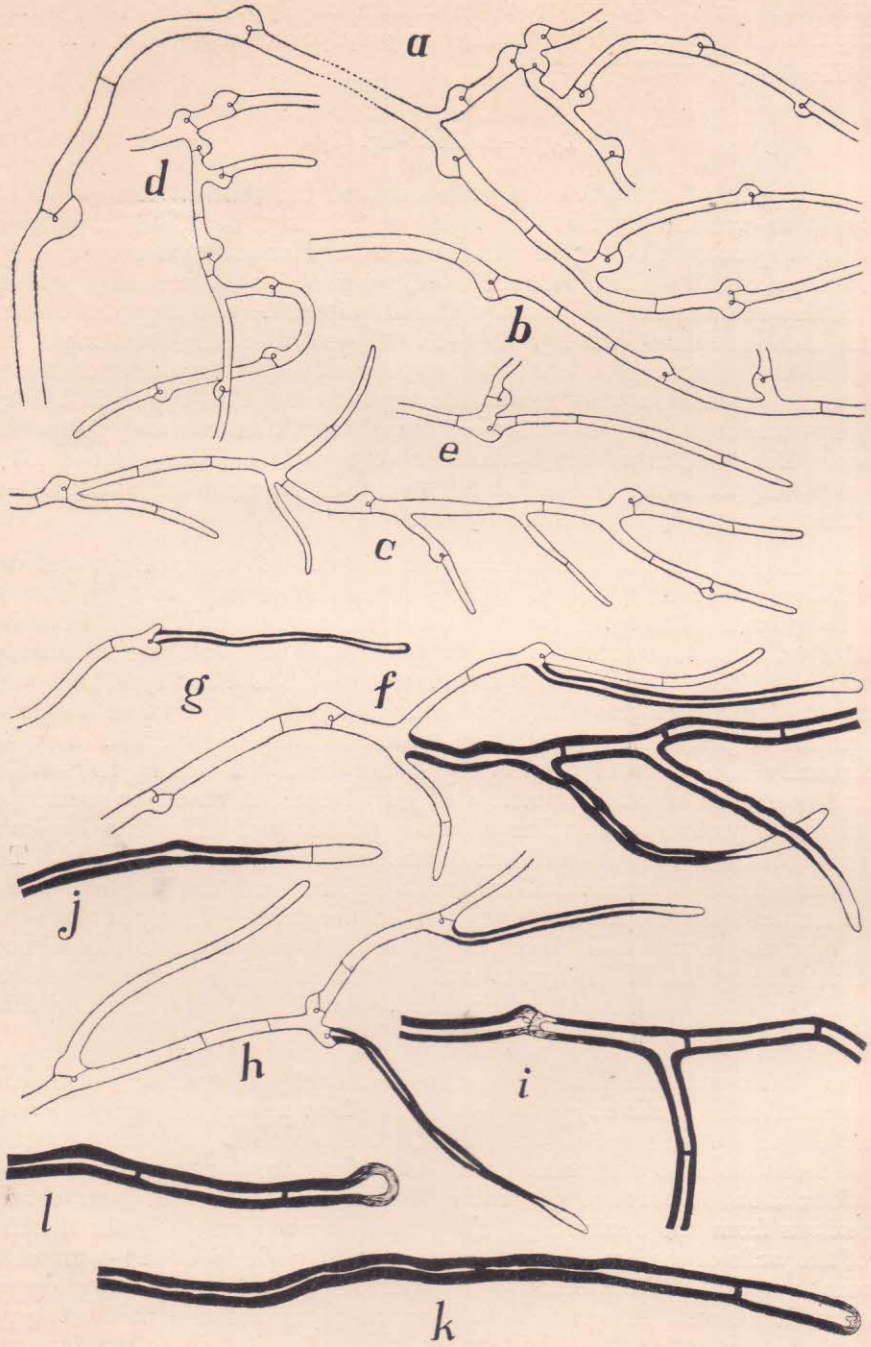
interrelations in the polysporous mycelium of Type–I and the salient descriptive features of the hyphae in the polysporous mycelia of the other two types are given separately.

Type–I (Text-fig. 2)

The hyphae of the advancing zone are of the *generative type* being always thin-walled, hyaline, straight or slightly flexuous, closely or distantly septate, full of granular contents and usually bearing a single clamp-connexion at each septum. Many plain septa are also present. The clamp-connexions are usually found alternately on opposite sides of the same hypha (Text-fig. 2, a–e). Opposite clamp-connexions are rarely found (Text-fig. 2, a). The hyphae are sparingly branched and each branch either originates from the opposite side of a hypha just below a clamp-connexion or the clamp-connexion proliferates and forms the branch. The width of the hyphae is very variable, being 1.5–4 (5) μ. The wider hyphae have been found to contain less granular contents and as such they appear to be more hyaline.

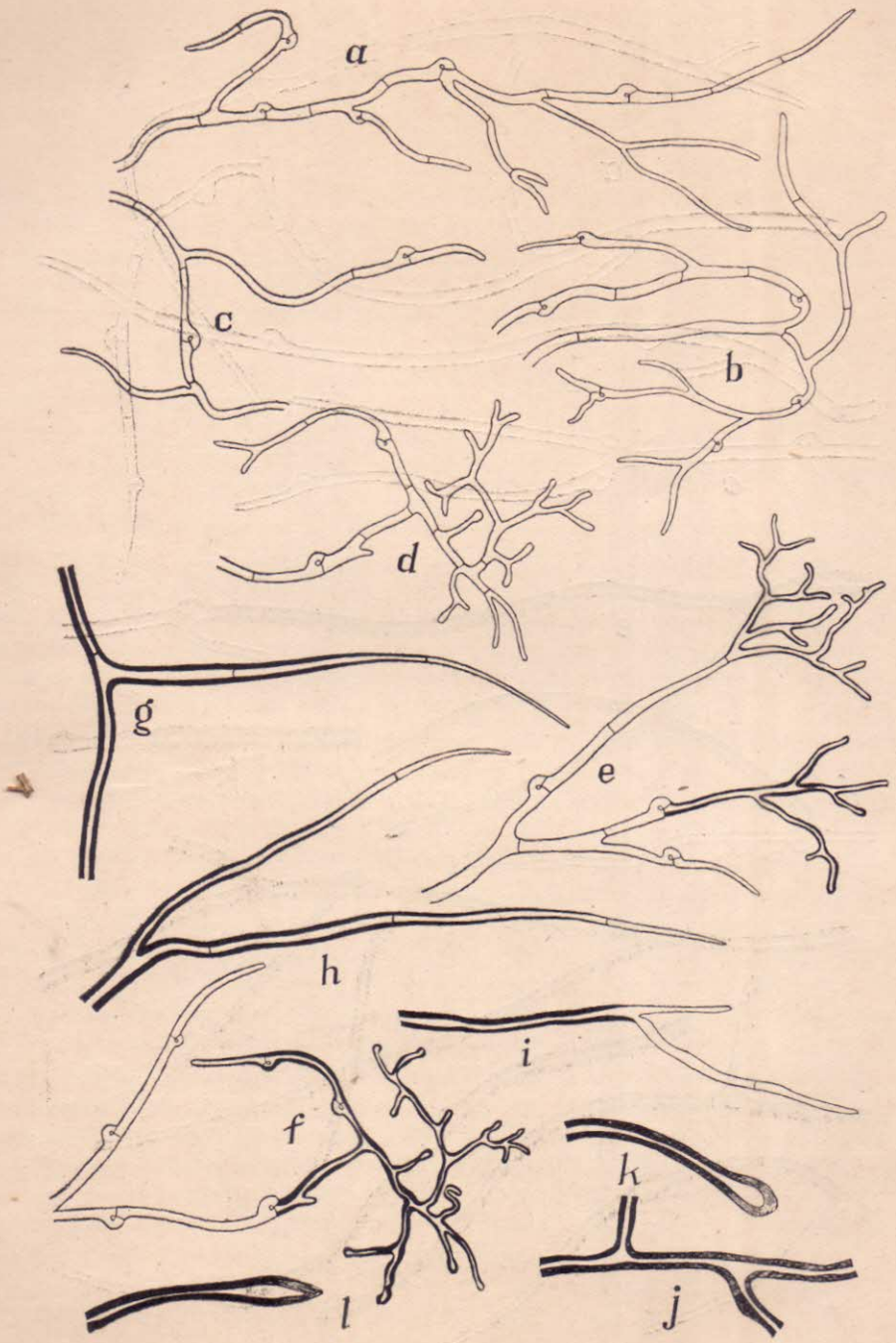
Just in the region a little behind the zone of advance, origin and differentiation of other types of hyphae from the generative type can be well-recognized. The latter type becomes less conspicuous, often branches profusely forming a complicated network and with infrequent clamp-connections. Thick-walled hyphae, mostly of the *skeletal type*, are very abundant at this stage. They are mostly hyaline, always unbranched, straight or slightly flexuous, always without clamp-connexions, aseptate to distantly septate and originating either from the main thin-walled generative hypha on the opposite and just below a clamp-connexion or by its direct proliferation. The thickness of the wall of such a hypha is very variable. It may be uniform or unequeal in the same hypha. The lumen sometimes becomes obliterated at places so that its continuity is broken and the hypha appears to be aseptate. The granular contents become fewer and fewer and sometimes appear to be almost absent. The width of the hypha varies between 2–6 μ. The tip of the skeletal hypha is attenuated or rounded, may be thick-walled or thin-walled and sometimes slightly inflated (Text-fig. 2, g-h, j-l).

Some thick-walled hyphae appear to be distantly or sparingly branched, and consitute the *mediate type* (2.5–3.5 μ). They are undoubtedly derived from the generative hyphae like the skeletal type but they are branched and without clamp-connexions. Sometimes they are formed due to direct transformation of parts of generative hyphae which do not bear clamp-connexions from the beginning. They may also be formed by the thickening of the walls of the branched generative hyphae whose clamp-connexions become totally obliterated due to thickening and stretching or by their proliferation. All gradations between generatve and mediate types of hyphae are noticeable (Text-fig. 2, f, i). In the older portions and towards the centre of the mat, all the three types of hyphae, namely, generative, skeletal and mediate, can be recognized but of these, the generative type predominates. Some of the generative hyphae are distinctly



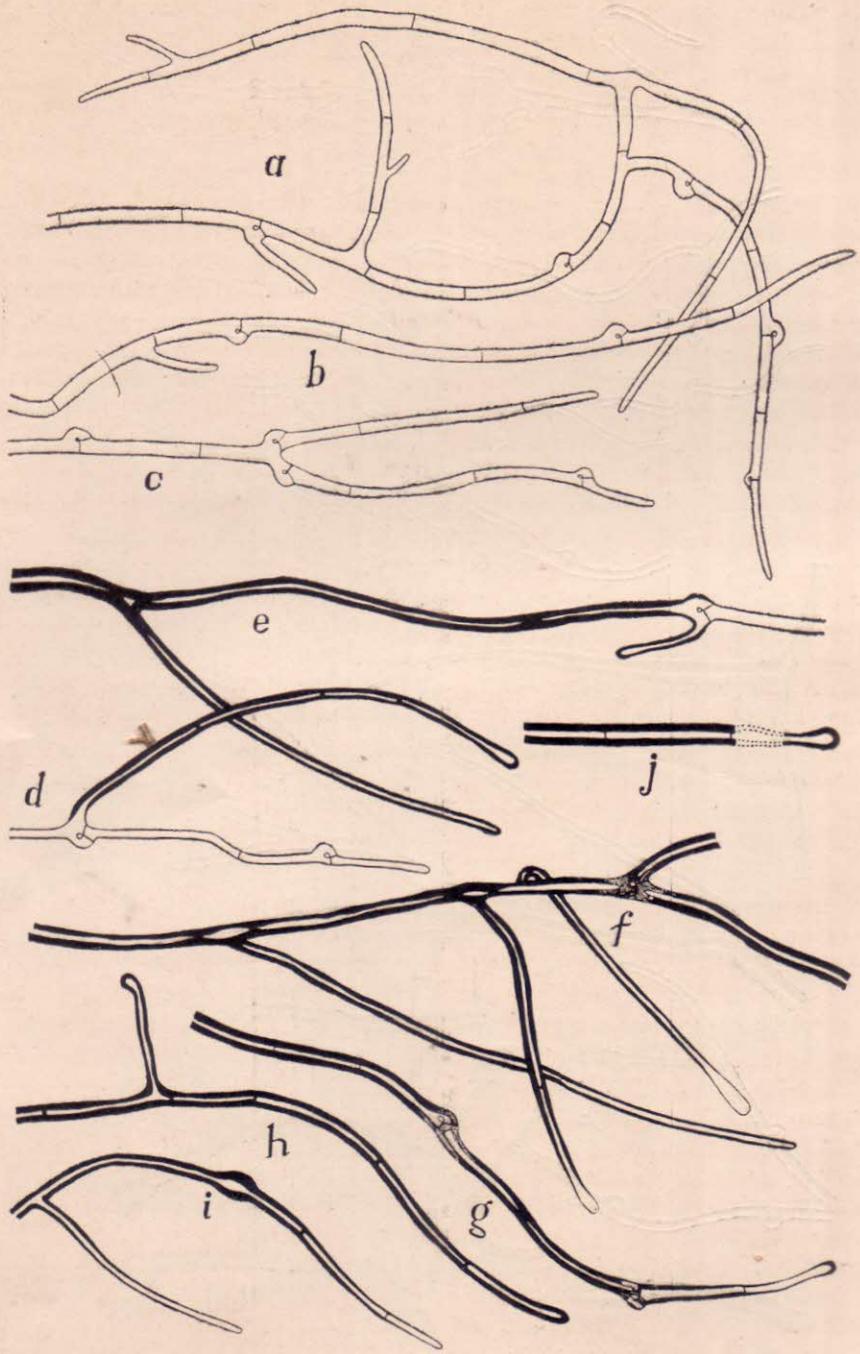
Text-fig. 2. Hyphal system in the polysporous mycelium of *Polystictus xanthopus* (Type–I). × 1400.

(For explanation, vide text)



Text-fig. 3. Hyphal system in the polysporous mycelium of *Polystictus xanthopus* (Type–II). × 1400.

(For explanation, vide text)



Text-fig. 4. Hyphal system in the polysporous mycelium of *Polystictus xanthopus* (Type–III). × 1400.

(For explanation, vide text)

thick-walled and show plenty of proliferating clamp-connexions. The *binding hyphae*, so characteristic of the fructifications of this type, are not in evidence even in old cultures.

Type–II (Text-fig. 3)

As in Type–I, the advancing zone is full of *generative hyphae* (1–3.5 μ) with abundant simple clamp-connexions and also with plain septa (Text fig. 3 a–d). Partial or entire transformation of some of the generative hyphae into thick-walled branched hyphae with or without clamp-connexions can be clearly seen. This may be designated as much branched *mediate hyphae* and their width varies between 1–2 (4) μ (Text-fig. 3, e, f). Some of the generative hyphae appear to be entirely empty, closely septate and without clamp-connexions. No skeletal hyphae have been differentiated in this region.

In the regions behind the advancing zone and towards the centre, three types of hyphae, viz., generative, mediate and skeletal, become gradually differentiated. The *skeletal hyphae* [1.7–2.8 (4) μ] in the older region of the mat become most abundant. The *generative hyphae* are fewer and there are transitional stages between these and the *mediate type* (Text-fig. 3, e, f). The hyphal characteristics are more or less similar to those in Type–I, but the apices of the skeletal and those of the branches of mediate hyphae (1–2.5 μ) upto a considerable distance from the tip, remain thin-walled and become gradually attenuated showing nuclei and sparse granular contents within (Text-fig. 3, g, i). The skeletal and mediate hyphae (Text-fig. 3, i-l) are usually uniformly thickened showing more or less linear lumina. Partially twisted or coiled binding hyphae which are present in the fructification of this type cannot be observed.

Type–III (Text-fig. 4)

The same three types of hyphae, viz., *generative*, *skeletal* and *mediate*, enter into the composition of the mycelium of this type. The advancing zone is similar in having only the characteristic generative hyphae (Text-fig. 4, a-c). The width of the generative hyphae vary between 1.5–3.5 μ. The clamp-connexions are comparatively few and the plain septa are frequent. Just behind the zone of advance, only the skeletal hyphae become differentiated from the generative type and they become more and more abundant towards the centre (Text-fig. 4, d, g, i, j). Their characterististics are similar to those found in Type–I. In the central portion of the mat, all the three types of hyphae, viz., generative, skeletal and mediate, are present of which the skeletal type (2–5 μ) predominates. The mediate hyphae (2–5 μ) are springly branched (Text-fig. 4, e-f, h) and agree closely with those in the other two types.

Antagonism and Compatibility

The nature of antagonism between the polysporous mycelia of the three types of *Polystictus xanthopus* have been ascertained. They were tested on 2.5% *malt-agar* slants in special culture-tubes ($10'' \times 1\frac{1}{2}''$). The