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SIGNIFICANCE OF THE CLAMP-CONNECTION IN THE BASIDIOMYCETES

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(With Plates 6–9)

The cytogenetic phenomena affecting clamp-connection formation and the interpretation of this peculiar type of septum in the classification of the higher Basidiomycetes were reviewed from the literature and discussed. The cytogenetic data available are restricted to a small number of basidiomycetous species, but the formation of clamp-connection is included among the major phenomena whose genetic control is generalized for the Basidiomycetes.

Clamp-connection formation in heterothallic species is controlled by the factors affecting sexuality. Simple septa appear in the hyphae of clamp-connection bearing species when (1) the simple septa result from independent nuclear division of the dikaryon, with or without subsequent hyphal growth of the newly recovered monokaryon, (2) when there is production and further development of apomictic (asexual) spores containing only one of the nuclear components of the dikaryon, or (3) when there appears any kind of monokaryotic hyphal growth caused by the splitting of the dikaryon. The monokaryotic hyphae are invariably simple-septate. Genetic experiments also show that simple septa appear in successful crosses between monokaryons of tetrapolar heterothallic species carrying homoallelic *A* or *B*, or even both at the same time, incompatibility factors. Therefore, the clamped and simple-septate hyphae of the Basidiomycetes are genetically and cytologically distinct. In homothallic species the control of clamp-connection formation is not well known, but whenever clamp-connections are formed there are nuclear pairings. In pseudohomothallic species the formation of clamp-connections follows the pattern of the heterothallic species, but masked by the dikaryotic nature of the basidiospores.

The taxonomic interpretation of the clamp-connection is somewhat divergent. In many cases the authors did not investigate the cytogenetic condition of the hyphae to formulate their hypothesis. This shows the necessity of the proper evaluation of the pattern of septation of the hyphae before any hypothesis is formulated.

Introduction

The clamp-connection is a character of questioned taxonomic significance because of its inconspicuousness and irregular pattern of occurrence in many Basidiomycetes, especially the Polyporales and Agaricales. In contrast to this erratic occurrence, this peculiar type of septum is both prominent and abundant in mycelia derived from culture of basidiocarp tissues of many species in which the septation is not evident in nature.

The careful study of the septation in the Basidiomycetes has been stressed only recently by a few mycologists, although the cytologic studies involving the formation of clamp-connections are advanced. This manuscript is essentially a review paper. The cytologic preparations used to illustrate this paper and many of the taxonomic studies on polypores are original. The cytogenetic data were all taken from the literature. I hope to bridge the gap between taxonomy and modern genetics of the Basidiomycetes, emphasizing the necessity to define the septation of the species properly.

Material and methods

Cytologic preparations for nuclear demonstration were made by the HCl-Giemsa technique (Ward & Ciuryzek, *1962*) with the tropical wood-rotting polypore, *Polyporus pseudoboletus* Speg. Hyphal studies of fresh and dried specimens (of several species of polypores) were made by Teixeira's technique (*1956*, *1962a*) of sampling and immediate mounting. Semipermanent mounts of unstained material were made in lactophenol-cotton blue medium (Alexopoulos & Beneke, *1962*). Permanent mounts of stained and unstained preparations were made with the use of the water-soluble plastic "Abopon" (Hrushovetz & Harder, *1962*). Additional technique used for herbarium specimens included staining with 0.5 percent aqueous solution of toluidine blue, washing with distilled water and mounting in either distilled water or "Abopon".

Photographies were made with 35 mm black-and-white Adox KB-14 film under bright-field, dark-field, and phase-contrast illuminations. Prints were made on the high contrast Kodabromide F4 and Agfa 6 papers.

Nomenclature of the mycelium and its components

Homokaryotic and monokaryotic are genetic terms for the haploid mycelium of the Basidiomycetes; the corresponding taxonomic term is primary mycelium. The hyphal segments of the primary mycelium are uninucleate or multinucleate (Olive, *1953*) and invariably simple-septate (Figs. 1–5). Two contradictions to this generalization are (1) the presence of true clamp-connections in the primary mycelium of *Stereum hirsutum* (Willd.) ex Fries and *Coprinus narcoticus* (Batsch) ex Fries, respectively claimed by Kniep and Brunswik (Gäumann & Dodge, *1928*; Raper, *1953*), and (2) the presence of incomplete clamp-connections in the primary mycelium of *Itersonilia perplexans* Derx reported by Olive (*1952*). Both cases will be discussed later on.

Heterokaryotic and dikaryotic are genetic terms applied to the diploid-equivalent phase of the life-cycle of the Basidiomycetes. The corresponding taxonomic term is secondary mycelium. The hyphae of the secondary mycelium are usually binucleate, but also multinucleate (Olive, *1953*), and either simple-septate or bearing clamp-connections (Figs. 6–7). In species with both types of septa (either in the mycelia from culture of basidiocarp tissues, or in the mycelium of the basidiocarp in nature), the simple-septa never appear in hyphal segments of dikaryotic constitution.

Context, dissepiment, trama, hymenium, and so on, are terms used currently in classification. A new terminology has been introduced with the addition of the microstructural criteria in classification. The hyphae that can divide and form new structures such as basidia, cystidia, setae, and any other modified hyphal types are termed generative hyphae (Corner, *1932a*, *1932b*). By its original definition, any dividing hypha could be called generative. Teixeira (*1962a*, *1962b*) limited the definition of the generative hyphae to the totipotent dikaryotic elements of the species. In many Basidiomycetes the generative hyphae are the only constituent of the basidiocarp context. In others, the generative elements differentiate into morphologically and functionally distinct structures, particularly the skeletal and binding hyphae (Corner, *1932a*, *1932b*, *1953*; Cunningham, *1954*, *1963*; Teixeira *1956*, *1962a*, *1962b*). In contrast to the totipotent nature of the generative hyphae, the true skeletal and binding hyphae lose their capacity for cell division and are characterized by having a limited growth. Therefore, the generative hyphae are the structures in which the pattern of septation of the species should be surveyed.

Sexuality and clamp-connection formation

Contemporary research has demonstrated a strict relationship between the genetic factors controlling sexuality and clamp-connection formation in the Basidiomycetes. The sexual processes in this class of fungi (Raper, *1960*) are characterized by (1) a generally haplo-dikaryotic life-cycle; (2) a (a) homothallic, (b) bipolar and tetrapolar heterothallic, or (c) pseudohomothallic or secondary heterothallic patterns of sexuality; and (3) a mechanism of somatic copulation by hyphal fusion followed by nuclear migration.

The first survey of the distribution of these patterns of sexuality among the Basidiomycetes (Whitehouse, *1949a*) showed that, in the sample analysed, only 10 percent of the species was homothallic. Of the remaining 90 percent, 35 percent was bipolar heterothallic and 55 percent tetrapolar heterothallic. This is a biased sample since species with clamp-connections were and still are selected for study. It does indicate, however, the predominance of heterothallism and, within this, of tetrapolarity, a particular attribute of the Basidiomycetes.

In homothallic species, a single, haploid basidiospore completes the entire life-cycle, including karyogamy and meiosis. In the clamped, homothallic species studied, the initial mycelial growth is usually characterized by hyphae possessing simple septa. Clamp-connections appear later on and the hyphae now show paired nuclei (Buller, *1958*; Boidin, *1958*; Berthier, *1963*). The use of the term dikaryon for the secondary mycelium of the homothallic species is misleading because, although association of genetically diverse nuclei (except the mating system, because there is no such a thing in homothallic species) can be established (hybridization), all the nuclei can perform the entire life-cycle "*per se*". Olive (*1953*) proposed the distinguishing term "homodikaryon" for the secondary mycelium of the homothallic Basidiomycetes.

Bipolar heterothallism in the Basidiomycetes follows the analogous *A*, *a* system of the heterothallic Mucorales and Ascomycetes. Tetrapolar heterothallism is interpreted as the consequence of the addition of a new factor, *B*, located on a different chromosome.

To the classical interpretation of tetrapolarity (Quintanilha, *1933*, *1935*; Buller, *1941*, *1958*; Whitehouse, *1949a*, *1949b*; Papazian, *1950*, *1958*; Raper, *1953*) the concept of compound loci for incompatibility (Papazian, *1951*) has been added. Tetrapolarity is now interpreted as follows; (1) both the *A* and *B* incompatibility factors are formed of at least two subunits; (2) each subunit is composed of a multiple allelomorphic series; (3) the total expression of each factor, either *A* or *B*, results from the individual composition of each subunit; (4) any allelic change at one of the subunits leads to an entire change of expression of the factor affected; and (5) the subunits of both *A* and *B* factors form new combinations by crossing-over and by spontaneous and induced mutations (Raper, Baxter & Middleton, *1958*; Day, *1960*; Raper, Baxter & Ellingboe, *1960*; Parag & Raper, *1960*; Takemaru, *1961*; Parag, *1962*; Finchan & Day, *1963*; Raper, *1963*; Raper & Esser, *1964*).

According to the allelic constitution of the incompatibility loci, four types of heterokaryons can be recognized: (1) heteroallelic *AB* heterokaryon, (2) heteroallelic *A*, homoallelic *B* heterokaryon (common-*B*), (3) homoallelic *A*, heteroallelic *B* heterokaryon (common-*A*), and (4) homoallelic *AB* heterokaryon (common-*AB*). Geneticists usually refer to the first type of heterokaryon as the dikaryon, and to the others simply as heterokaryons.

When primary (homokaryotic) mycelia of clamped, tetrapolar species are paired, true clamp-connections are formed only if the homokaryons carry different alleles at both *A* and *B* loci. If the mates are homoallelic at one or at both incompatibility loci, the heterokaryon eventually formed is limited and unstable, often resolving into its homokaryotic components. In rare instances, however, the heterokaryons may fruit like the dikaryon (Raper, *1963*; Raper & Raper, *1964*). Genetic investigations indicate that the *B* factor controlls extensive nuclear migration leading to heterokaryosis. In some cases the common-*B* heterokaryon has simple septa (unclamped) whereas in others the clamp-connections are replaced by incomplete, false or pseudoclamp-connections (Quintanilha, *1935*; Fulton, *1950*; Papazian, *1950*, *1958*; Raper, *1953*, *1963*; Raper & San Antonio, *1954*; Parag & Raper, *1960*; Swiezynski & Day, *1960a*; Takemaru, *1961*; Parag, *1962*, *1965*; Raper & Esser, *1964*; Raper & Raper, *1964*). In the pseudoclamp-connections the hook may grow insufficiently to reach the eventual penultimate cell, may touch the subterminal cell without fusing with it, or may grow independently as a hyphal branch. The hook of the pseudoclamp-connection fails in transfering the complementary nucleus to the penultimate cell. Therefore, the eventual heterokaryon is restricted to some terminal cells of the hyphae. The *A* locus has a specific control on clamp-connection formation: in common-*A* heterokaryons neither clamp-connections nor pseudoclamp-connections are ever formed. Only when the *A* factor is heteroallelic are true clamp-connections or pseudoclamp-connections formed (Finchan & Day, *1963*).

A cytoplasmic influence upon clamp-connection formation was claimed by Harder (Papazian, *1958*). He destroyed the terminal cell and its hook before fusion with the subterminal element and reported that true clamp-connections were formed during considerable growth of the newly-formed subterminal, homokaryotic, dicytoplasmic cell. Harder's claims were not confirmed by Aschan (*1952*) and Fries & Aschan (*1952*) who reported neither clamp-connections nor pseudoclamp-connections in the 'neohaplonts', i.e., the homokaryons obtained from the dikaryotic dividing hyphae. The problem of the dicytoplasmic influence on clamp-connection formation was reconsidered recently by Raper & Raper (*1964*).

The specificity of the genetic control of clamp-connection formation is also revealed in homokaryons carrying one or more mutations that disrupt the mechanism of the control of incompatibility (Raper, *1963*). Under these circumstances, the mutant-*B* homokaryon mimics the common-*A* heterokaryon; the mutant-*A* or the modified-*A* homokaryon mimics the common-*B* heterokaryon; and the mutant-*B* modified-*A* homokaryon mimics the dikaryon. The latter mutant-type forms pseudoclamp-connections and eventually fruits, but true clamp-connections are not formed because there is only one nucleus per cell. The necessary participation of two genetically distinct nuclei for formation of true clamp-connections in heterothallic species raises doubts concerning Kniep's and Brunswik's claims of clamped primary mycelia (Gäumann & Dodge, *1928*; Raper, *1953*). The natural occurrence of homokaryons with pseudoclamp-connections (Olive, *1952*) might be tentatively assumed as the consequence of mutations that disrupt the mechanism of incompatibility control in nature. The dependence of septation on proper allelic constitution of the *A* and *B* factors in *Schizophyllum commune* Fries is shown in Table 1 (provisional, tentative and unpublished data kindly given by Dr. John R. Raper, Harvard University, used here with his permission).

Although the reports of clamp-connections in the hyphae of the primary mycelium of heterothallic species are questioned, the special phenomenon termed spontaneous dikaryotization of the homokaryon still remains to be explained. Raper (*1953*) mentioned the possibility of contamination of the homokaryon by spores carrying the opposite mating type, but Papazian (*1951*) claimed unquestionable cases of spontaneous dikaryotization. Papazian (*1958*) stated, however, that the normally behaving homokaryon "might be carrying extra *A* and extra *B* factors which later segregate out into a separate nucleus and produce a dikaryon, but they would have to be carried without their influencing the incompatibility phenotype which is incongruous."

Lange (*1952*) introduced the term 'amphithallism' for the phenomenon of formation of 'homothallic' and 'heterothallic' mycelia from the spores of the same basidiocarp. Lange's reports, as well as the results presented by French authors who

TABLE I

COMPARATIVE SEPTATION AND CLAMP-CONNECTIONS IN NORMAL AND MODIFIED MYCELIA OF SCHIZOPHYLLUM COMMUNE PERCENTAGE

(Provisional, unpublished data obtained by Dr. John R. Raper)

	Homokaryon	Heterokaryons					
	Ax Bc	Common-*A*		Common-*B*		Dikaryon	
		Nor.	Mod.	Nor.	Mod.	Nor.	Mod.
Simple septa	100	97.2	7.3	2.6	20.3	0.6	7.3
True clamp-connections	—	—	—	—	—	98.2	—
Septal pseudoclamps	—						
Non-septate	—	1.1	39.3	11.2	39.5	—	18.4
Non-septate, nucleate	—	0.6	2.5	4.3	0.7	—	1.4
Septate	—	—	21.3	8.6	12.0	—	24.3
Septate, nucleate	—	—	18.0	67.2	8.8	0.6	14.0
Interseptal pseudoclamps							
Non-septate	—	—	1.5	2.6	1.3	0.6	7.3
Non-septate, nucleate	—	—	6.0	3.5	1.3	—	11.0
Septate	—	—	0.7	—	—	—	1.4
Septate, nucleate	—	—	3.3	—	1.3	—	14.7
Sample	149	183	150	116	158	168	136

have accepted the term 'amphithallism' (Kühner, Lamoure & Fichet, *1962*; Lamoure, *1955*, *1957a*, *1957b*, *1957c*, *1959*, *1960*) show that the so-called 'amphithallic' species do form homokaryotic and dikaryotic basidiospores which generate the homokaryotic and dikaryotic mycelia respectively. The production of dikaryotic basidiospores was discussed by Sass (*1929*), Quintanilha, Quintanilha & Vasermanis (*1941*), and Skolko (*1944*), but it was Dodge (*1927*, *1957*) who analysed the consequences of the incorporation of genetically diverse nuclei in the ascospores of *Neurospora tetrasperma* Dodge. The apparent homothallism suggested by the single ascospore cultures of *Neurospora tetrasperma* was named 'pseudohomothallism' by Dodge, and the homologous phenomenon in the Basidiomycetes was called 'secondary heterothallism' by Whitehouse (*1949a*), a terminology that is used in Alexopoulos' (*1962*) textbook of mycology and Raper & Esser's (*1964*) contemporary review of sex and genetics in the fungi. Unless alternatively used for encompassing both typical heterothallism and pseudohomothallism the term 'amphithallism' should be replaced by Dodge's pseudohomothallism.

The fruiting ability in culture as the basis for the interpretation of some species is somewhat complicated by the phenomenon known as the 'haploid fruiting', sometimes erroneously referred to as parthenogenesis. In the haploid fructification the basidiospore progeny is of one mating type, the parental type. Although it may be possible that sister nuclei fuse in the young basidium, it is generally accepted that karyogamy and meiosis do not take place in the haploid fruiting specimen. Before deciding on the validity of clamps as a significant characteristic for the identification of fruiting bodies, the cytogenetic criteria of haploid versus dikaryotic condition must be securely established. This situation appears to have caused no taxonomic problems yet, especially because the extent of the haploid fruiting phenomenon in nature is unknown.

Growth and stability of the mycelium

Both primary and secondary mycelia are distributed commonly in nature (Nobles, *1958b*) and can grow independently of each other. Nuclear divisions in the vegetative hyphae have been interpreted as amitotic, by Bakerspigel (*1959*), as truly mitotic, by Olive (*1953*), Ward & Ciuryzek (*1961*), and Lu (*1964*). Raper & Esser (*1964*) stated that there remains the possibility of two or more basic modes of nuclear divison in general.

The HCl-Giemsa staining technique has been used most often among the cytologic procedures for nuclear demonstration in the fungi. The selection of the proper technique is important in the study of nuclear division. In addition to that, Ward & Ciuryzek (*1961*) indicated that the smallness of the chromosomes is under the resolutional limits of the optical microscope. Regardless of the small size of the nuclei (Fig. 8), nuclear division appears to be very rapid and the majority of the nuclei appears at interphase (Fig. 9). Chromosomal bodies, however, are prominent but difficult to count (Figs. 10–12).

Nuclear migration through the hyphae is a prominent phenomenon in the Basidiomycetes (Figs. 13–14). Despite the small aperture of the septal pores (Moore & McAlear, *1962*), Buller's (*1958*) statement that the entire nucleus migrates through the septal pore of the hyphae has been confirmed genetically by Snider (*1963*). In addition, Bracher & Butler (*1964*) pictured a nucleus of *Rhizoctonia solani* Kühn contracting through a 0.5 μ distended septal pore of a basidiomycetous-type of hyphal septum. No less remarkably is the picture shown by Giesy & Day (*1965*).

The events of conjugate nuclear division and their relationships with clamp-connection formation in the dikaryon were discussed by Bensaude (*1918*), Buller (*1930*, *1958*), Quintanilha (*1935*), Noble (*1937*), Dodge (*1942*), Routien (*1948*), Olive (*1953*) and others. Basically, three phenomena take place during clamp-connection formation: (1) the parent nuclei divide conjugately, (2) a simple septum is formed between each dividing parent nucleus, and (3) the hook derived from the upper cell fuses with the penultimate cell and transfers one nucleus re-establishing the dikaryotic condition. These phenomena may lack a constant time relationship (Noble, *1937*; Routien, *1948*), and the number of nuclei per cell and of clamp-connections formed at the same hyphal height may also vary (Olive, *1953*). Nevertheless, heterokaryosis is preserved even when the nuclei divide with a considerable lapse in time and space (Fig. 9).

Heterokaryosis is not absolute in the dikaryon. Whenever the parent nuclei are separated into different hyphal segments the dikaryon is split and the original homokaryons are recovered. This recovery has been induced experimentally in several ways. Harder (Papazian, *1958*) achieved it by micrurgical technique. Raper & San Antonio (*1954*) macerated the dikaryon in a Waring Blender, plated out on agar medium, and selected the simple-septate growing hyphae. Miles & Raper (*1956*), Da Costa & Kerruish (*1962*), and Kerruish & Da Costa (*1963*) used various toxic chemicals which, in some way, increased the proportion of homokaryons. In many species the spontaneous splitting of the dikaryon is observed commonly in culture by either the sorting out of the nuclei into uninucleate hyphal branches, or into uninucleate apomictic spores produced by the hyphae, with or without a special conidial apparatus.

Homokaryotic hyphal branches result from disturbance of conjugate nuclear division, especially when the parent nuclei of the dikaryon divide independently (Figs. 15–16) or when the spindle of the dividing nuclei are accommodated widely apart (Noble, *1937*).

Apomictic (asexual) spore formation is a major source for recovery of the original homokaryotic components of the secondary mycelium. The apomictic spores have been termed oidia, conidiospores, chlamydospores, ballistospores, secondary spores, gasterospores, etc. To name such spores as asexual spores is not absolutely satisfactory because apomictic basidiospores are produced by the haploid fruiting specimens.

The asexual spores can be produced by both primary and secondary mycelia. In many cases the spores have been used as additional characteristics for recognition of the species, either in nature or in culture (Cartwright, *1929*, *1932*; Chow, *1934*; Nobles, *1943*; Kühner, *1946*, *1947*; Olive, *1946*, *1947*, *1948*; Kühner, Romagnesi & Yen, *1947*; Rogers, *1947*; Jackson, *1948a*; McKeen, *1952*; Bulat, *1953*; Sarkar, *1959b*; Jacquiot, *1960*; Pantidou, *1961*, *1962*; O. Fidalgo, *1963*).

Genetic and cytologic studies involving the asexual spores show that the primary mycelium may form uninucleate or multinucleate homokaryotic asexual spores (Martens & Vandendries, *1933*; Kaufert, *1935*; Nobles, *1935*, *1937*; Brodie, *1936*; Vandendries, *1937*; Bose, *1943*; Olive, *1950*; Maxwell, *1954*; Doguet, *1956*). These spores (Fig. 17) are formed usually in chains and result from a series of nuclear divisions without immediate formation of cross-walls (Figs. 10–11). They may also arise by budding (Figs. 12 and 19) or by a special conidial apparatus (Nobles, *1935*). Usually released singly or in pairs (Fig. 20), the homokaryotic asexual spores may germinate, giving rise to the primary mycelium, or may function as a dikaryotizing agent (Fig. 21).

The secondary mycelium may form: (1) only homokaryotic, asexual spores (Nobles, *1935*; Brodie, *1936*; Kühner & Yen, *1947*; Aschan, *1952*; Sarkar, *1959a*); (2) only dikaryotic, asexual spores (Kaufert, *1935*; Barnett, *1937*; Nobles, *1937*; Bose, *1943*; Doguet, *1956*; McKay, *1959*; Kühner, Lamoure & Fichet, *1962*); or (3) both kinds (Gilmore, *1926*; Vandendries, *1937*; Kühner, *1949*; Olive, *1952*; Lamoure, *1958*). The germ tube arising from an asexual, dikaryotic spore of a clamped species usually bears clamp-connections from the start (Fig. 22).

Asexual spores have also been reported from nature (Patouillard, *1887*; Heim & Malençon, *1928*; Jackson, *1948b*; O. Fidalgo, *1963*). In some species the asexual spores are produced in conjunction with the basidiospores; in others the asexual spores are formed in quantity to characterize the imperfect stage—the so-called *Ptychogaster*-form—of various species of different genera of Basidiomycetes.

Natural occurrence of clamp-connections

Clamp-connections occur more frequently in nature than is actually recognized. Conspicuous clamp-connections are found in many species, especially those with relatively simple hyphal organization such as the Tremellales (Martin, *1945*), Clavariaceae (Corner, *1950*), many Hydnaceae (Maas Geesteranus, *1962*, *1963a*, *1963b*, *1963c*) and various Thelephoraceae (Cunningham, *1963*). In other species the clamp-connections are not detected easily. This may be due to the construction of the basidiocarp, predominantly formed of skeletal and binding hyphae as in the Polyporaceae, or to the irregular pattern of septation, either in those cases in which clamp-connections and simple septa are found mixed, or in the cases in which septa are not seen. The latter situation—the irregular pattern of septation—is observed commonly in the Agaricaceae. In many cases, however, clamp-connections were not reported because the specimens were not studied carefully. Teixeira (*1960*) and Teixeira & Rogers (*1955*) demonstrated that clamp-connections were present in species which had been previously reported as lacking clamps. Teixeira (*1962a, 1962b*) has reported additional errors in descriptions pertaining to the manner of septation in various polypores.

The regular procedure for microscopic examination of the basidiocarp collected in nature is not often satisfactory for species with inconspicuous generative hyphae. Disregarding the errors made by some taxonomists, many mistakes on septal analysis are caused by optical devices of low resolution. Optimum optic conditions can be achieved with phase-contrast illumination and staining with methylene blue or with toluidine blue aqueous solutions. In addition, detection of the proper hyphae for observation of septa usually requires the proper sampling of the basidiocarp (Teixeira, *1956, 1962a*).

In species with inconspicous septa or with an irregular pattern of septation, examination of the septa is made usually at the base of the hymenial structures such as the basidium, cystidium, setae, pseudoparaphyses, and so on. The absence of hymenial structures and the autolysis of the basidia, however, make such a practice useless. This handicap can be compensated for by the presence of modified generative hyphae as well as the presence of special structures of the pileus cover (Furtado, *1965*). The modified generative hyphae usually undergo changes in breadth, thickness of the wall, coloration, and so on, without losing their ability for cell division. Cell modification may affect contiguous or alternating segments of the generative hyphae. Consequently, the clamp-connection is modified wholly or partially (Figs. 23–24). The presence of clamp-connections can be determined even in separated segments of generative hyphae. Separation of two contiguous segments occurs exactly along the two septa of the clamp-connection. Since the hook originally formed by the terminal cell fuses with the eventual penultimate cell, the basal part of the originally terminal cell is recognized by a kind of lateral truncation which results from the more or less inclined septum formed from nuclear division inside the primitive hook. Furthermore, the two original septa of the clamp-connection form a convex angle at the basal portion of the terminal cell (Fig. 7a). The apical termination of the eventual penultimate cell has a bulge formed by the fusion of the primitive hook from the upper cell with the subterminal cell, and the septa now form a concave angle (Figs. 7a, 15, 23 and 25). The same principles of observation indicate the original direction of hyphal growth: the hook is always directed backward and the presence of the lateral bulge indicates the subterminal cell, therefore opposing the direction of growth. This general statement can be sometimes obscured e.g. by branching immediately from the clamp-connection (Fig. 7b) or by detachment of the segment at one end and reversion of growth direction (Figs. 15 and 23), the latter in need of further observation.

Another, but uncommon, source of hyphae for analysis of septation can be seen in species whose hymenial surface is tubular, plicate, folded, or provided with any irregularity. The generative hyphae can have localized growth and occlude the spaces of the hymenial surface. The generative hyphae that occlude those spaces should be distinguished from contaminant fungi, either by the conidial apparatus or by the distinguishing staining reactions.

The appearance of simple septa in mycelium of species bearing clamp-connections results, therefore, from (1) disturbance of conjugate nuclear division in the generative hyphae; (2) formation of septa regardless of nuclear division, either by localized deposition of wall material (pseudoseptum), or by breakage of the cytoplasm followed by immediate restoration due to the properties of surface tension and further deposit of wall material (cleavage septum; Fig. 7c). These types of septum should not be compared with the clamp-connection: the simple septa derived from disturbance of nuclear division characterize the homokaryotic (haploid) stage of the life-cycle of the Basidiomycetes, whereas the septa formed without nuclear division are not true septa.

Taxonomic meaning of the clamp-connection

The systems of classification for the Basidiomycetes have been based traditionally on the general features of the prominent basidiocarps. The emphasis has been shifted gradually with the addition of microstructural criteria in classification. But yet there is general disagreement concerning the delimitation of higher taxa, especially the genera. Teixeira's (*1962a*) remarks on the chaotic state of classification of the Polyporaceae can be applied to other groups of Basidiomycetes as well.

Several attempts at a natural system of classification have been made; various types of modified hyphae were introduced as additional features in classification (Lentz, *1954*); an entire system of classification was based primarily upon the microscopic characteristics (Patouillard, *1900*); and many discussions and proposals have been made for different groups of Basidiomycetes (Ames, *1913*; Donk, *1933*, *1964*; Martin, *1945*; Wakefield, *1946*; Heim, *1946*; Cooke, *1949*; Pinto-Lopes, *1952*; Cunningham, *1954*; Bondartzev, *1953*; Nobles, *1958b*; Kotlaba, *1961*; Singer, *1962*; Teixeira, *1962a*; Lowe, *1963*). Nevertheless, the subject is still open to many questions.

The study of the basidiocarp from nature has been greatly enhanced by the introduction of microstructural criteria in classification proposed by Corner (*1932a*, *1953*) and developed by Cunningham (*1954*, *1963*) and especially by Teixeira (*1956*, *1962a*, *1962b*) and O. Fidalgo (*1964*). Among the microstructures of the basidiocarp, the presence or absence of clamp-connections has a position that is both important and contested. Some mycologists search for the pattern of septation habitually; others simply consider the septa to be of no taxonomic value. Between these extremes, there are mycologists who emphasize the septation only in species with prominent generative hyphae.

The position taken on the taxonomic significance of the presence of absence of clamp-connections is generally governed by the investigator's interpretation of the origin of the clamp-connection and of the species with simple-septate hyphae. The hypotheses on the origin of the Basidiomycetes are simply speculative and devoid of fossil documentation. It is generally agreed, however, that the clamp-connection of the Basidiomycetes is homologous with the crozier of the Ascomycetes (Rogers, *1934*, *1936*; Linder, *1940*; Bessey, *1942*, *1961*; Arnaud, *1951*), although Savile (*1954*, *1955*) and Buller (*1958*) are contrary to the idea.

Various hypotheses tried to explain the origin of the species with simple-septate hyphae. Jackson (*1948b*) proposed the idea of 'homothallic', simple-septate lines derived from 'heterothallic', clamped species. This proposal is somewhat confusing, unless Jackson meant homokaryotic lines derived from clamped heterothallic species. A similar but better formulated hypothesis was presented by Nobles (*1958b*) who suggested that the absence of clamp-connections in the polypores might have arisen through the propagation of homokaryotic generation or through the suppression of formation of clamp-connection in the dikaryotic mycelium. Nobles also suggested that some simple-septate species appear to be homokaryotic counterparts of modern heterothallic species with clamp-connections, or of similar ancestral forms. Singer (*1962*) said that the clamp-connection has been abandoned in agarics in the evolutionary process of losing the clamp-connection as an unnecessary and uneconomical way of cell division, except for a specific organ. Hesler & Smith (*1963*) mentioned that the clamp-connections seem to have dropped out of *Hygrophorus* (Agaricaceae) here and there without regard to relationships of species.

In its pure taxonomic interpretation, the presence or absence of clamp-connections is considered to be significant at the species level by some authors, and at higher taxonomic rank by others. Hesler & Smith (*1963*) de-emphasized the value of the clamp-connections in their treatment of *Hygrophorus* and stressed that the pattern of septation can be used at the species level; Singer (*1962*), however, said that, in the Agaricales, the presence or absence of clamp-connections can be used for larger groups. Nobles (*1958b*) stated that the pattern of septation in the Polyporaceae can be of significant value at the species level, but Pinto-Lopes (*1952*) and Teixeira (*1962a*, *1962b*) mentioned that larger groups can be separated on the basis of the septation of the hyphae. Pinto-Lopes' and Teixeira's points of view have been confirmed in taxonomic studies of K. Fidalgo (*1959–1961*), O. Fidalgo (*1958–1964*), O. & K. Fidalgo (*1962*, *1963*), Teixeira (*1962b*), and Furtado (*1965*).

In the clamped species studied experimentally, clamp-connections are formed only in one specific heterokaryon, the dikaryon. The simple-septate hyphae of the clamped dikaryon are, invariably, homokaryotic counterparts. The coexistence of clamped and clampless hyphae has been reported often in basidiocarps collected in nature. It appears, however, that the only study of the genetical and cytological condition of the simple-septate hyphae found mixed with others with clamp-connections was made by Papazian (*1958*) who cultured the simple-septate, hair-like hyphae from the upper cover of the pileus of a species of *Coprinus* with clamp-connections and obtained homokaryotic mycelia. Otherwise, the mycologists have neglected the study of the cytogenetic condition of the hyphae and simply report the characteristics of the septation. Under these circumstances, the usual 'aberrations' reported are the species in which clamp-connections are found only in special areas of the fructification such as the hymenium, the base of the stipe, the volva of the agarics, and no septa or simple septa found elsewhere. In other cases, clamp-connections are found throughout the context but the basidia are limited by a basal simple septum. These examples can be included under the general condition of 'irregular pattern of septation'.

The genetical studies are confined to a small number of species, but the genetical control of clamp-connection formation is accepted as a major phenomenon under the strict control of the factors affecting sexuality in pseudohomothallic and bipolar and tetrapolar heterothallic species. Furthermore, it is known that the survival of the heterokaryon (the common-*A*, common-*B*, or even common-*AB*) with simple-septum is difficult because the general tendency is the split of the heterokaryon into the homokaryotic components. These findings indicate how complex it would be for the geneticist to offer any tentative explanation for the idea already introduced by taxonomists of the origin of the species (dikaryon)—especially the heterothallic—with simple-septate hyphae from the clamped ones through the suppression of formation of clamp-connections.

In his discussion on the clamp-connection as a character for classification of the Aphyllophorales (Polyporales), Donk (*1964*) mentioned that "the absence of clamps in a fruitbody may be due, theoretically, to one of at least three factors: (i) the fruitbody being formed by a haploid mycelium, (ii) the species lacking clamps altogether, or (iii) the species in 'diploid' condition occurring in two 'forms', one clampless, one clamp-bearing." The first of Donk's assumption is perfectly possible, but not investigated satisfactorily; the second is totally recognized; but the third could be supported with difficulty on the basis of the cytogenetic data available.

The selection of characteristics which would allow the assemblage of the Basidiomycetes into more natural groups will throw some light on the interpretation of the taxonomic significance of the presence of absence of clamp-connections in the septa of the hyphae. Whether one accepts the traditional system of families of the Basidiomycetes, or the recent splitting of the Aphyllophorales proposed by Donk (*1964*), one verifies that some groups are formed of species with only clamp-connections, others with only simple septa, whereas some have species with both clamp-connections and simple septa. The taxonomic studies of large taxa made by mycologists who adopt the microstructural criteria in classification have also shown such a discrepancy. In his recent treatment of the Thelephoraceae, Cunningham (*1963*) segregated various genera in groups distinguishable additionally on the basis of the presence or absence of clamp-connections, but maintained genera that encompass clamped and clampless species. The coexistence of species with different type of septa within the same genera has not been confirmed in the taxonomic studies of the Polyporaceae by Teixeira (*1962b*), K. Fidalgo (*1959–1961*), O. Fidalgo (*1958–1964*), O. & K. Fidalgo (*1962*, *1963*), Furtado (unpublished).

Segregation of taxa of Basidiomycetes on the basis of microstructures genetically controlled and the behavior of the species in culture are modern and efficient tools in taxonomy. It is important, however, that both conservative and modern taxonomists take into consideration the necessity to investigate the cytogenetic condition of the hyphae whenever the pattern of septation is decisive for definition of any taxa or the proposal of any hypothesis.

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EXPLANATION OF PLATES 6—9

PLATE 6

Fig. 1. Simple-septate hyphae that coexist with the clamped ones in culture, × 500.
Figs. 2–5. Uninucleate, monokaryotic condition of the simple-septate hyphae, × 1000.
Fig. 6. Binucleate, dikaryotic nature of the clamped hyphae, × 1000.

PLATE 7

Fig. 7. The dikaryon: (a) the two septa of the clamp-connection, (b) the detachment of the hyphal segment at the level of several clamp-connections, and (c) the cleavage septa, × 1000.

PLATE 8

Fig. 8. Mitosis in a narrow homokaryotic hypha, × 2000.

Fig. 9. Asynchronous clamp-connection formation, × 1200.

Figs. 10–11. Successive mitosis in a homokaryotic hypha preceding the formation of apomictic spores, × 1000 and × 2000 respectively.

Fig. 12. Formation of apomictic spores through the process of budding, × 2000.

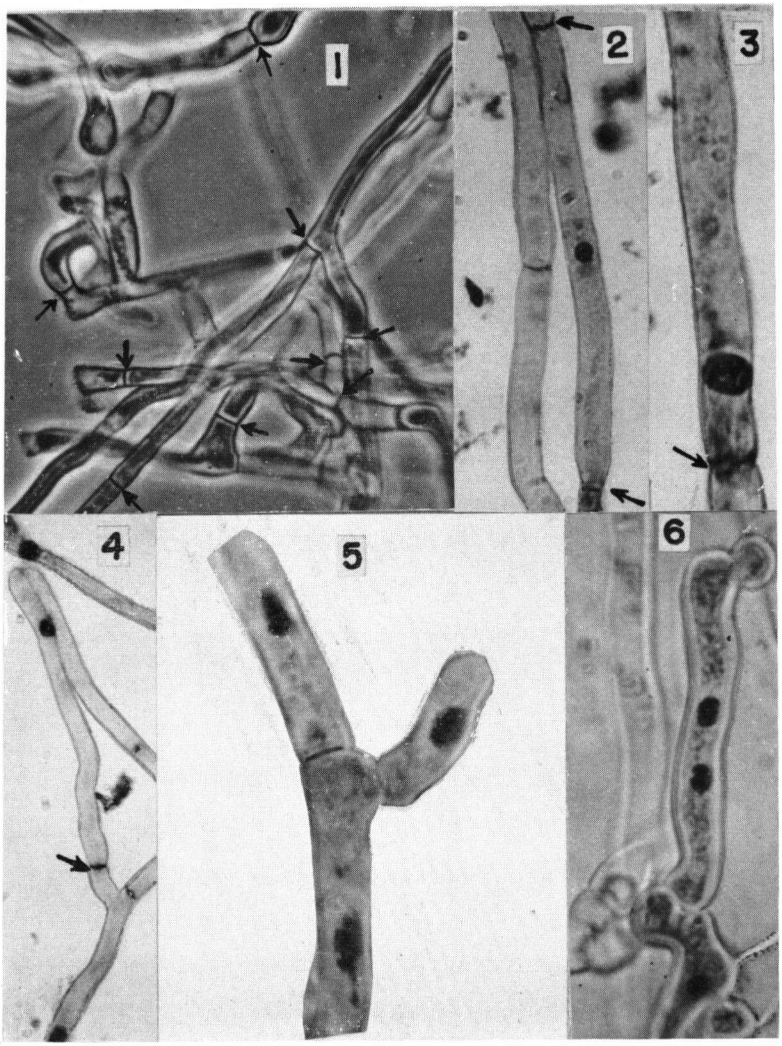
Figs. 13–14. Hyphal fusion and nuclear migration in the homokaryon, × 1000.

Figs. 15–16. Coexistence of simple-septa and clamp-connection in the same hypha. — Fig. 16. Nuclear distribution of the dikaryon that divided independently; both × 1000.

PLATE 9

Figs. 17–22. Apomictic spores. — Fig. 17. Formation in chains, × 400. — Figs. 18–19. Uninucleate, homokaryotic spores, × 1500. — Fig. 20. Bicelled spore, × 1500. — Fig. 21. Dikaryotizing behavior of the apomictic spore, × 1000. — Fig. 22. Clamp-connection formation in a dikaryotic apomictic spore, × 1000.

Figs. 23–25. Clamp-connection in hyphae from basidiocarp collected in nature and preserved in herbarium. — Fig. 23. Detachment of a hyphal segment and inversion of the direction of growth, × 200. — Fig. 24. Generative hypha with clamp-connection, × 1000. — Fig. 25. Modified generative hypha with a broken clamp-connection, × 500.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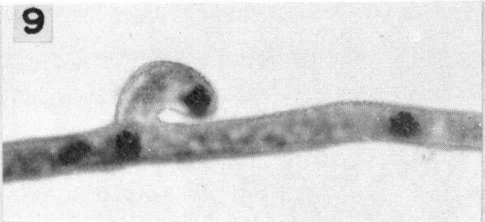
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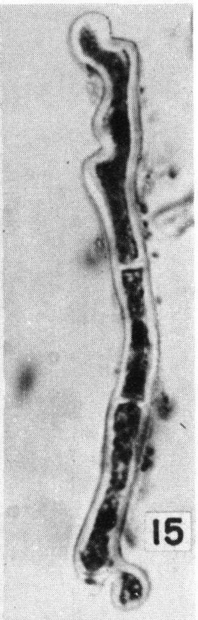
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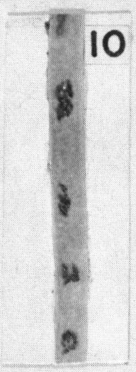
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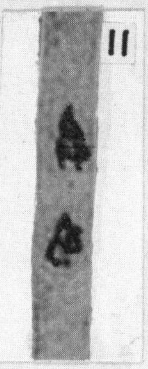
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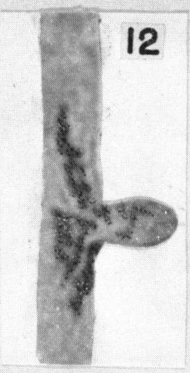
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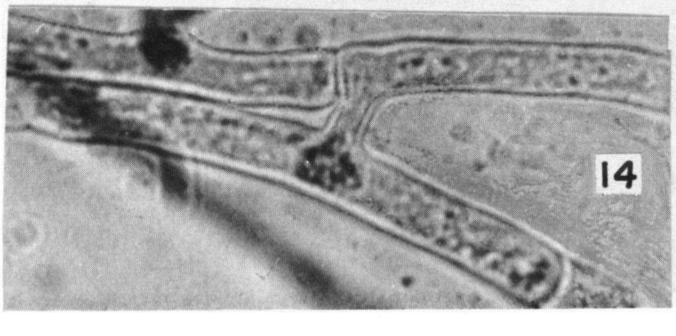
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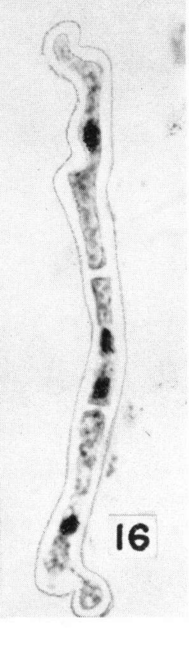
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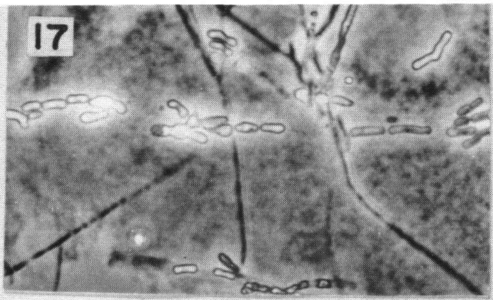
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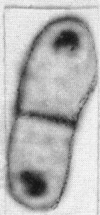
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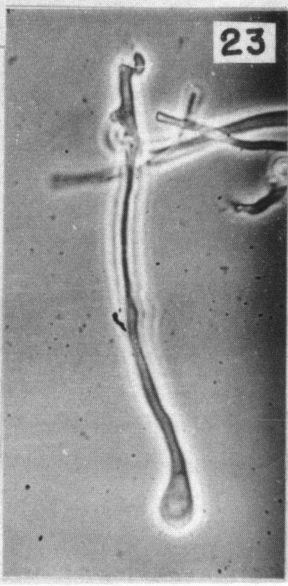
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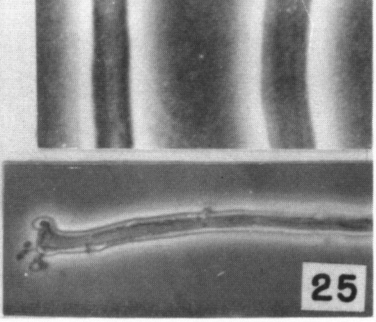
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