

CHAPTER 2

LITERATURE REVIEW

2.1 Taxonomic history and circumscription of *Marasmius*

The mushroom-forming genus *Marasmius*, a member of the family Marasmiaceae in the order Agaricales, was first described by Fries in 1835, but a full diagnosis was not given until later (Fries, 1838). He characterized *Marasmius* as forming tough, persistent basidiocarps that had the ability to revive when rehydrated, but later these characters caused confusion with the genus *Collybia* (Fries, 1838). Since its inception, the genus has been characterized in many different ways by different workers. Quélet (1885) followed Fries's concept of *Marasmius*, and used stipe insertion and type of substrate as taxonomic features for infrageneric delimitation.

Patouillard (1887, 1900) was the first mycologist to use microscopic characters to delimit genera in this group of fungi, particularly the structure of the pileipellis cells. The thickness of pileipellis cell wall was used to segregate those genera to two major groups. In the first group with thick-walled pileipellis cells he recognized two genera: *Crinipellis* (with very long, hair-like thick-walled cells) and the new genus *Androsaceus* (with short, ovoid thick-walled cells covered with small warts). *Marasmius* was placed in the second group with four other genera (*Heliomyces*, *Xerotus*, *Hymenogloea* and *Cymatella*) based on their thin-walled pileipellis cells.

Bataille (1919) reported 94 species of *Marasmius* from Europe in an important European monograph. He adopted the classification of Quélet (1885) with some minor adjustments, but completely ignored Patouillard's work.

Many European *Marasmius* species were published by Kühner (1933, 1935) with comprehensive microscopic descriptions. He distinguished nine sections, *Alliatae*, *Androsaceae*, *Epiphyllae*, *Globularinae*, *Gloenemae*, *Hygrometrici*, *Peronatae*, *Ramealinae* and *Rotulae*, based on macromorphological features (stipe attachment and ornamentation), micromorphological features (pileipellis anatomy) and chemical characters (reactions of hyphal walls in Melzer's reagent and in cresyl blue). His classification, based exclusively on European species, was accepted by other mycologists for many years (Singer, 1958b).

Dennis (1951), who worked on *Marasmius* and allied genera from northern South America (Trinidad and Venezuela), used the presence of collarium (a ring of tissue around the apex of stipe to which the lamellae are attached) as an important taxonomic character. This character is very useful to distinguish section *Marasmius* (= *Rotulae* Kühn.) from all other infrageneric groups.

The structure of the pileipellis and amyloidity of the basidiospores were the main characters that Singer (1936) used to distinguish marasmiod genera. He restricted *Marasmius* to species with a hymeniform pileipellis and non-amyloid spores. In subsequent years, Singer conducted extensive research on *Marasmius* and allied genera, especially in the neotropics (Singer, 1942, 1958a, 1960, 1964, 1965, 1976) and Africa (Singer, 1964, 1965). These studies resulted in slight modifications and additions to Kühner's and Dennis' classification systems. Singer's classification concept included 12 sections of *Marasmius*, viz., sections *Alliacei*, *Androsacei*,

Epiphylli, *Fusicystides*, *Globulares*, *Hygrometrici*, *Inaequales*, *Leveilleani*, *Marasmius*, *Neosessiles*, *Scotophysini*, and *Sicci* (Singer, 1986). This infrageneric classification has been followed with minor exceptions by contemporary mycologists, viz., Gilliam (1976), Antonín and Noordeloos (1993), Desjardin and Horak (1997), Desjardin *et al.* (2000), and Antonín (2007).

Recently, *Marasmius* was restricted to only sections *Globulares*, *Hygrometrici*, *Leveilleani*, *Marasmius*, *Neosessiles*, *Scotophysini* and *Sicci* based on a monophyletic lineage that resulted from the analyses of nLSU rDNA datasets as reported by Wilson and Desjardin (2005). Some of Singer's (1976) other sections are now accepted as distinct genera, such as section *Androsacei* = *Setulipes* Antonín (Antonín, 1987), and section *Alliacei* = *Mycetinis* Earle (Wilson and Desjardin, 2005).

2.2 Distribution of *Marasmius*

Species of *Marasmius* are worldwide in distribution with the highest species diversity in tropical regions. *Marasmius* in Europe are relatively well known due to the works of Kühner (1933, 1935), Kühner and Romagnesi (1953), Antonín *et al.* (1992) and the recent European monograph by Antonín and Noordeloos (1993). Likewise, *Marasmius* in North America has been studied extensively due to the works of Gilliam (1975a, 1975b, 1976), Desjardin (1987, 1989, 1990, 1991a, 1991b), Desjardin and Redhead (1987), Desjardin and Petersen (1989a, 1989b, 1989c), Desjardin *et al.* (1992) and Halling *et al.* (1985). In the neotropics, Dennis (1951, 1957) reported a number of *Marasmius* species from the West Indies and Venezuela, and Singer (1958a, 1960, 1965, 1976) reported many species from South America. Pegler (1983, 1987) studied *Marasmius* from the Lesser Antilles and Cuba. More

recently, Desjardin and Ovrebo (2006) reported nine species of *Marasmius* from Panama including three that were new to science. In Africa, early research on *Marasmius* was conducted by Beeli (1928, 1938) and Singer (1964) from the Congo, and Pegler (1977) included many species in his agaric flora of East Africa. Recently, Antonín published a series of papers (Antonín, 2003a, 2003b, 2004; Antonín and Buyck, 2006) on African *Marasmius*, which culminated in a monographic study of tropical African marasmioid species (Antonín, 2007).

In Asia, there were a number of *Marasmius* species reported from temperate habitats due to the works of Berkeley and Curtis (1860), Teng (1996), Mao (2000), Takahashi (1999, 2000a, 2000b, 2002). In tropical habitats, Petch (1947) and Pegler (1986) reported a number of *Marasmius* from Sri Lanka, and Manimohan and Leelavathy (1987a, 1987b, 1989) reported species from South India. In the southern Pacific region, Stevenson (1964) reported 13 species of *Marasmius* from New Zealand. Over twenty years later, Desjardin and Petersen (1989d) published two new species from New Zealand, then in 1997 Desjardin and Horak reported 21 new species out of 56 species that were reported from Papua New Guinea, New Caledonia and New Zealand. In Southeast Asia, Corner (1996) reported 101 species of *Marasmius* from Malaysia, but most of these are not currently accepted in *Marasmius*.

A revision of Malaysian *Marasmius* was conducted by Tan (2008) and these data are expected to be published soon. A preliminary monograph of *Marasmius* from Java and Bali was published by Desjardin *et al.* (2000), reporting 37 species, of which 12 taxa were new to science.

In Thailand, Scamidt (1902) reported three species of *Marasmius* from Koh Chang but those species were later transferred to other genera. Chandrasrikul (1977)

published the first mushroom fieldguide for Thailand and reported only *M. maximus* Hongo from Doi Suthep-Pui National Park. Over a decade later, Soytong (1994) reported 6 species of *Marasmius* in his fieldguide "Mushrooms and Macrofungi in Thailand." Konsab (1998) published "Diversity of Agaric Mushrooms at Huay Kok Ma, Doi Suthep-Pui National Park" and reported 4 species of *Marasmius*. Subsequently, Ruksawong and Flegel (2001) published "Mushrooms and Fungi in Thailand" and reported 5 species of *Marasmius*. Recently, Wannathes *et al.* (2004, 2007) reported a number of *Marasmius* species and their mating systems from Northern Thailand, and Chandrasrikul *et al.* (2008) reported 9 species of *Marasmius* in "Diversity of Mushrooms and Macrofungi in Thailand".

2.3 Ecology of *Marasmius*

Most species of *Marasmius* are decomposer fungi that produce basidiocarps on leaf litter, twigs or dead wood. One species, *Marasmius crinisequi* is known as a plant pathogen, attacking various economically important plants such as cocoa, coffee, rubber, tea and nutmeg crops (Pegler, 1983). No *Marasmius* species are known to be mycorrhizal or lichenized (Singer, 1976). Many temperate species are host or substrate-specific (Antonín and Noordeloos, 1993). Since *Marasmius* is primarily saprotrophic they serve many important ecological roles in debris decomposition, nutrient cycling, soil genesis and as a source of food for myriad molluscs, arthropods and small vertebrates.

2.4 Human uses of *Marasmius*

Marasmius oreades is a well-known edible species of *Marasmius* in Europe and North America, and *M. albogriseus* is widely used as food by the Quichua population of the Department of Cuzco, Mexico (Retnowati, 1999). Because *Marasmius* species are decomposers of lignocellulosic substrates in forest ecosystems, there have been a number of studies published on the ability of *Marasmius* to produce lignin-degrading enzymes. Desjardin (1990) reported on the presence or absence of laccase, tyrosinase and peroxidase in 29 species of *Marasmius* during his study on the morphology of *Marasmius* cultures. *Marasmius quercophilus* produces a number of phenoloxidases, such as laccases, that are used in many eco-friendly technologies such as bleaching of paper pulp, bioremediation, or detoxification of hazardous and toxic xenobiotics in effluent (Tagger *et al.*, 1998; Dedeyan *et al.*, 2000; Farnet *et al.*, 2002). Xylanase is an enzyme that Ratanachomsri *et al.* (2006) reported from a member of *Marasmius* (strain BCC 7928) isolated from Thailand. In addition, a few species were found to have antioxidant properties (Tan *et al.*, 2007).

2.5 Mushroom life cycle

The Basidiomycota is a major lineage of fungi in which the sexual process involves the production of haploid basidiospores borne on club-shape structures called basidia in which nuclear fusion and meiosis occur. They are classified into three classes: Basidiomycetes (mushrooms, puffballs and jelly fungi), Urediniomycetes (rust fungi) and Ustilaginomycetes (smut fungi) (Kirk *et al.*, 2001). The class Basidiomycetes traditionally comprised two subclass: Heterobasidiomycetes (with septate basidia) and Homobasidiomycetes (with single-celled basidia).

The life cycle of typical mushroom (Figure 2.1) traditionally begins at the sexual spore stage. Basidiospores (Figure 2.1-1), each containing a single haploid nucleus, germinate (Figure 2.1-2) and grow into hyphal colonies called mycelium (Figure 2.1-3) that contain a single nucleus in each hyphal compartment. This phase is termed a monokaryon. The monokaryon may rarely produce asexual spores (Figure 2.1-4). The next state of development occurs when the hyphae of two monokaryons of different mating compatibility groups fuse with one another (Figure 2.1-5). Once the two compatible strains have fused by a process termed plasmogamy, subsequent growth occurs as dikaryotic hyphae (Figure 2.1-6). Each dikaryotic cell contains both mating-compatible haploid nuclei intimately associated in pairs, and the dikaryon is capable of indefinite vegetative propagation. The fruiting body (Figure 2.1-7), also termed a basidiocarp or basidiome, is formed from the dikaryotic hyphae. A specialized fertile tissue, the hymenium (Figure 2.1-8), forms on the fruiting body and consists of terminal, spore-bearing cells, the basidia (Figure 2.1-9). Within each of the basidia the two nuclei fuse (karyogamy) to form a diploid nucleus (zygote) (Figure 2.1-10). Meiosis follows immediately to produce four haploid nuclei, which migrate singly into four developing basidiospores (Figure 2.1-11). Sometimes only two spores are produced instead of four, and often these spores contain both compatible nuclei ($n+n$) and are capable of germinating directly into dikaryotic mycelium (Kühner and Lamoure, 1958; Petersen and Hughes, 1999).

2.6 Sexuality patterns of Mushroom

The Homobasidiomycetes contain both self-fertile (homothallic) and self-sterile (heterothallic) species. The majority of homobasidiomycetes (approximately

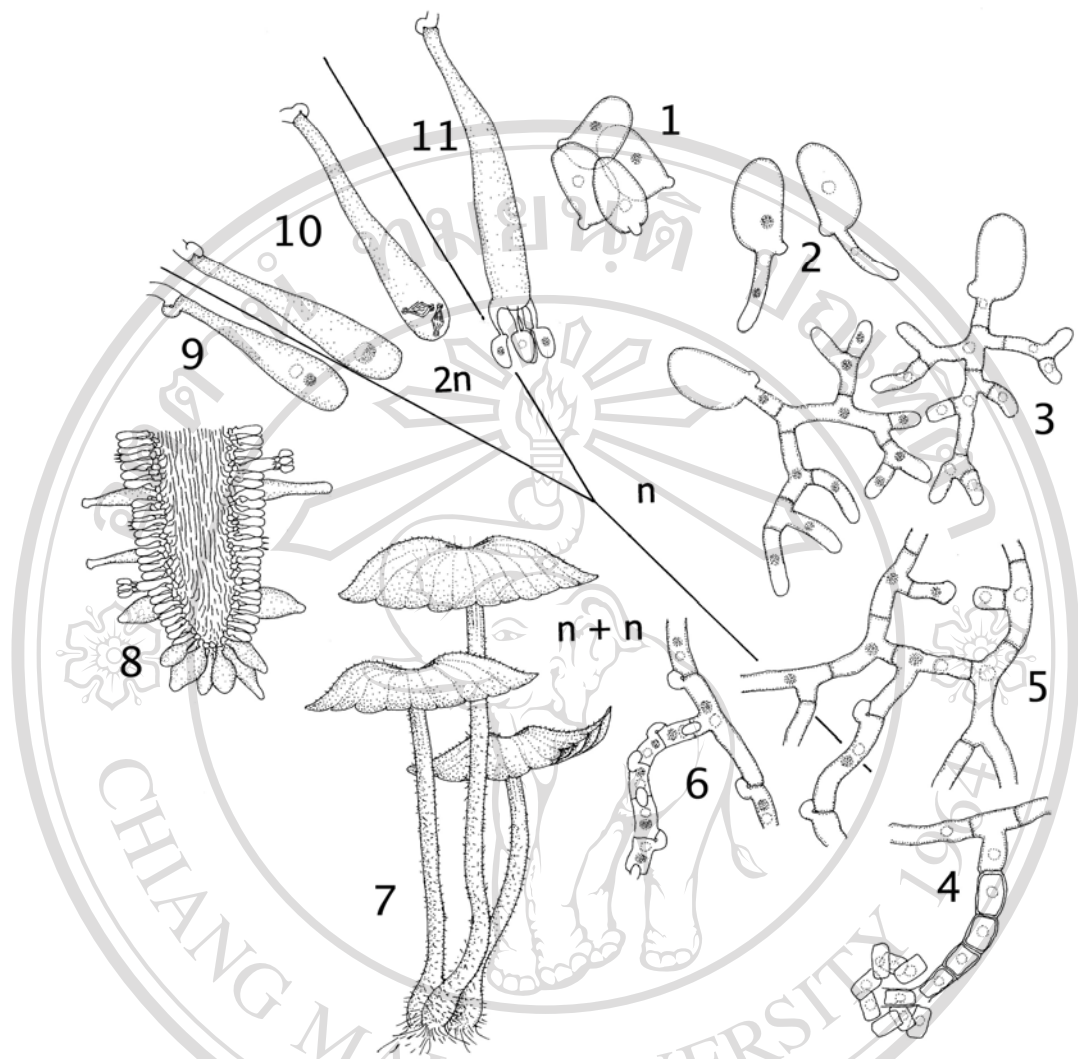


Figure 2.1 Mushroom life cycle. n : haploid monokaryon state; $n+n$: dikaryon state; $2n$: diploid state. 1. Basidiospores prior to germination. 2. Germ tubes grow from basidiospores. 3. Germ tubes branch into germling mycelia. 4. Monokaryotic hyphae may produce asexual spores. 5. Monokaryotic hyphae anastomose to produce dikaryotic hyphae with clamp connections. 6. Dikaryotic hyphae. 7. Under appropriate conditions, the dikaryotic mycelium produces mushroom fruit bodies. 8. A cross-section of lamellae showing sterile cells (cystidia) and basidia with basidiospores. 9. Basidia have two mating-compatible nuclei. 10. Lower basidium–nuclear fusion occurs within the young basidia (karyogamy); upper basidium–meiosis takes place in the basidial initial. 11. Four haploid basidiospores develop on sterigmata of mature basidium (modified from Petersen and Hughes, 1999).

90%) are obligatory out-crossers, while the remaining homobasidiomycetes (10%) is the self-fertile (Whitehouse, 1949). Self sterility is enforced by an incompatibility mechanism, in which specific factors, commonly termed incompatibility factors, determine the pattern of mating in absences of any morphological differentiation. Bipolar or unifactorial incompatibility and tetrapolar or bifactorial incompatibility are the two most common types of mating systems operating in homobasidiomycetes.

About 25% of all investigated species are bipolar. Bipolar species have a single multiallelic gene that controls compatibility, and their basidia give rise to spores of only two different mating types (e.g., A_1 and A_2). In contrast, the remaining 65% of self-sterile species are tetrapolar. Tetrapolar species have two multiallelic genes that control compatibility; their basidia give rise to spores of four different mating types (e.g., A_1B_1 , A_1B_2 , A_2B_1 and A_2B_2). In bipolar species, interbreeding among spore siblings is restricted to 50% (Table 2.1), whereas in tetrapolar species, it is restricted to 25% (Table 2.2) (Raper, 1966).

Table 2.1 Bipolar mating system showing compatibility of two alleles (1 and 2) of the incompatible factor A.

Mating type	A_1	A_2
A_1	-	+
A_2	+	-

+ = compatible mating; - = incompatible

Table 2.2 Tetrapolar mating system showing compatibility of two alleles (1 and 2) of two incompatible factors A and B.

Mating type	A_1B_1	A_1B_2	A_2B_1	A_2B_2
A_1B_1	-	-	-	+
A_1B_2	-	-	+	-
A_2B_1	-	+	-	-
A_2B_2	+	-	-	-

+ = compatible mating; - = incompatible

2.7 Mating study of Mushroom

Kniep (1915) clearly established that clamp connections (Figure 2.2), which he termed conjugate division, form during division of the paired nuclei of dikaryotic mycelium in Basidiomycetes. Subsequently, Bensaude (1918) established the significant fact that clamp connections are never present on monokaryotic mycelium and that monokaryotic mycelium never develops fruiting bodies. Alternatively, fruit bodies form only on dikaryotic mycelium which usually has clamp connections. This observation, that clamp connections are associated with the development of potentially fertile dikaryotic mycelium, lead to the ability to easily conduct studies of mating incompatibility of Basidiomycetes. When two monokaryons are mated, they are sexually compatible if the resultant mycelium bears clamp connections.

In 1920, Kniep described that sexuality in *Schizophyllum commune* is a tetrapolar mating system that is controlled by two pairs of independently segregating factors (A_1 - A_2 and B_1 - B_2). Papazian (1950) reported on the distinct morphology of the contact zone mycelium of the mated monokaryons that occurred in *Schizophyllum*

commune. He observed that the contact zone of different pairs of the four incompatibility types showed various mycelium morphologies (Table 2.3). A) Different *A* and *B* factors, when monokaryons with different alleles at the *A* and *B* loci are mated, a dikaryon resulted. Clamp connections are formed at any particular division and most of the dikaryotic mycelia are capable of fruiting when grown in special conditions favoring fruiting. Morphological differences between the two mated monokaryons do not persist after fusion of the hyphae and the mycelia on both sides are nearly always indistinguish. B) Common *A* and *B* factors, when the mycelia that having identical alleles at both the incompatibility loci are mated, the mycelia meet and usually grow overlapping; Papazian termed this morphology as "overlap". C) Common *B*, when the alleles at the *B* locus are the same and those at the *A* locus are different, the contact zone of both mycelia appeared as a line of poor growth, the hyphae are thin, far apart, and rarely assume an aerial habit. This morphology was termed "barrage". D) Common *A* factors, when the alleles at the *A* locus are the same and those at the *B* locus are different, a mycelium appears normal and appressed to the substrate, but it is thin and has very poorly developed aerial hyphae. This morphology was termed "flat".

Table 2.3 Tetrapolar mating system showing the contact zone morphological differences between the two mated monokaryotic mycelia as described by Papazian (1950).

Mating type	A_1B_1	A_1B_2	A_2B_1	A_2B_2
A_1B_1	O	F	B	+
A_1B_2	F	O	+	B
A_2B_1	B	+	O	F
A_2B_2	+	B	F	O

+ = clamp connection; B = "barrage"; F = "flat"; O = overlap

Fulton (1950) performed similar work with similar results on *Cyathus stercoreus*, and he also reported on the functions of the incompatibility factors. He found out that a pair of factors B_1 - B_2 control nuclear migration during monokaryotic hyphae matings. When the two incompatible mycelia differing in these factors but containing the same A_1 or factors A_2 were paired, nuclear migration occurred and heterokaryotic mycelium which he termed "blotchy" (same as "flat" in Papazian's study) developed. In the combinations of incompatible mycelia that had the same B_1 or B_2 factors, the nuclear migration did not take place. When two incompatible mycelia differing in A_1 or A_2 factors were paired, false clamp connections were observed from the mycelium taken from the contact zone. These false clamp connections indicated that nuclei of two mycelia had entered a cell and formed a temporary dikaryon, although no nuclear migration occurred. These evidences point out that these incompatibility factors must segregate independently of mating type.

The functions of incompatibility factors (A and B) that Fulton had reported were clarified by the work of Casselton and Olesnick (1998). They reported that a tetrapolar mating system is controlled by two multiallelic genetically independent loci that ensure the transmission of the two nuclei of the dikaryotic cell during cell division (Figure 2.2). The genes in loci A are responsible for controlling the pairing of nuclei in the dikaryon, and for the coordination cell division through the formation and septation of clamp cells. The gene at the B locus controls the migration of the nuclei towards the hyphal tip, the dissolution septa and the fusion of clamp cells to ensure a correct dikaryotic state after cell division.

There are scattered species that differ from these predominant patterns of sexuality. A trifactorial system managed by three pairs of independently segregating

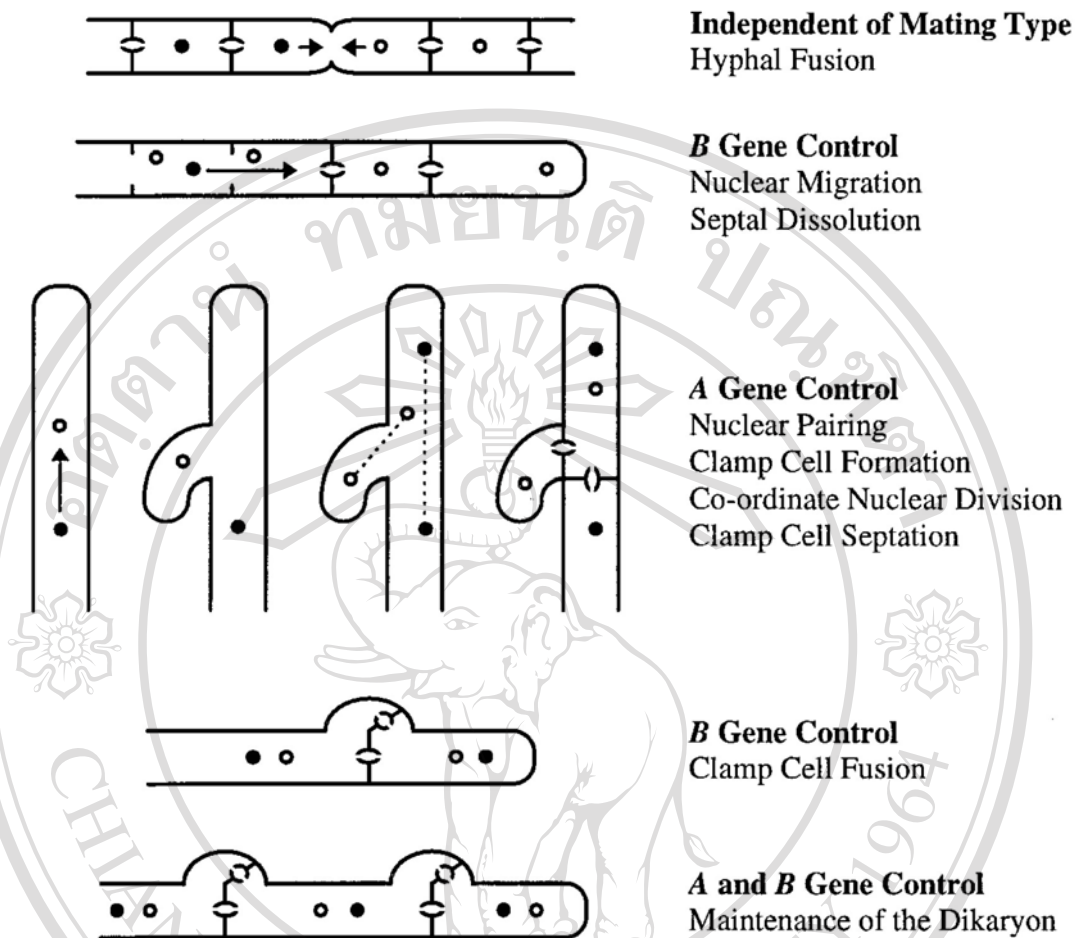


Figure 2.2 Clamp connections form during cell division of dikaryotic hyphae. (from Casselton and Olesnicky, 1998)

factors (A_1 - A_2 , B_1 - B_2 and C_1 - C_2) has been detected in *Psathyrella coprobia* (Jurand and Kemp, 1973). A genetic mosaicism occurs in *Armillaria gallica*, and its life cycle and sexuality pattern are still unclear (Peabody et al., 2000).

2.8 Mating studies in *Marasmius*

Studies on the mating systems operating in species of *Marasmius* have been conducted by mycologists since 1936 and show that there are two mating systems

within the genus, viz., bipolar (unifactorial heterothallism) and tetrapolar (bifactorial heterothallism) (Vandendries, 1936; Yen, 1950; Terra, 1953; Burnett and Evans, 1966; Mallet and Harrison, 1988; Lamoure, 1989; Gordon and Petersen, 1991; Holmer and Stenlid, 1991; Desjardin *et al.*, 1992; Gordon *et al.*, 1994; Petersen 1997; Tan *et al.*, 2007; Wannathes *et al.*, 2007). Gordon *et al.* (1994) examined eleven species of *Marasmius* that represented four sections of the genus, and they found that mating systems are consistent between species at the sectional level (infrageneric taxonomy followed that of Singer 1976, 1986). Members of section *Alliacei*, *Androsacei* and *Marasmius* were tetrapolar, whereas the species of section *Sicci* were bipolar. Subsequently, Petersen (1995) provided an excellent review of mating studies in mushroom systematics, wherein he documented the mating system type of a number of *Marasmius* species belonging to six sections (same infrageneric taxonomy that Gordon *et al.* (1994) followed) of the genus. Members of section *Alliacei*, *Androsacei*, *Epiphylli* and *Marasmius* were consistently tetrapolar, while those of sections *Globulares* and *Sicci* were bipolar.

2.9 Usefulness of mating studies

Mating studies can furnish information valuable to systematics in at least three ways: 1) The use of "species concepts" has recently made its way from the technical/philosophical literature into semipopular journals (i.e. Davis, 1996; Petersen and Hughes, 1999), with one major construct being the "biological species"; 2) regardless of the criteria used to define various "species concepts", it is critical to know whether individuals or representatives of populations can interbreed, and mating systems, together with understanding of barriers to interbreeding (viz. Morell,

1996) are integral to that experimental process (Petersen, 1997); and 3) mating systems (viz., amphithallic, bipolar, tetrapolar) are just as valid as taxonomic characters as are morphological or biochemical characters.

2.10 Phylogenetic studies of *Marasmius*

Since the discovery of the polymerase chain reaction (PCR) by Mullis and Faloona (1987) and its development (Saiki *et al.*, 1988) allowing biologists to sequence DNA from many species in a few days, and the progressive development of analytical options for molecular data, molecular biology is now widely used in fungal systematics. Molecular phylogenetic studies of Homobasidiomycetes have recently been published by Bruns *et al.* (1998), Moncalvo *et al.* (2000), Hibbett *et al.* (1997), Hibbett and Thorn (2001), Hibbett and Binder (2002), Hibbett (2004), Hibbett *et al.* (2007), Matheny *et al.* (2006) and Matheny *et al.* (2007). From these reports, Hibbett (2007) concluded that the Homobasidiomycetes is comprised of 14 major clades that include all fruiting body forms of mushrooms.

A number of genes have been used in the phylogenetic analyses of mushrooms, viz., nuclear ribosomal genes (nu-rDNA), mitochondrial ribosomal genes (mt-rDNA), RNA polymerase II subunits one or two (*rpb1*, *rpb2*), and translation elongation factor (*tlf1- α*). The internal transcribed spacer (ITS) regions and 5.8S ribosomal RNA gene (rDNA) are a good choice of gene for investigating phylogenetic relationships of mushroom at the interspecific level (Boidin *et al.*, 1998) due to the highly variable of ITS regions and the highly conserved of 5.8S rDNA. The ITS regions of rDNA are defined as the unit containing the ITS1 spacer, 5.8S rDNA, and ITS2 spacer (Figures 2.3).

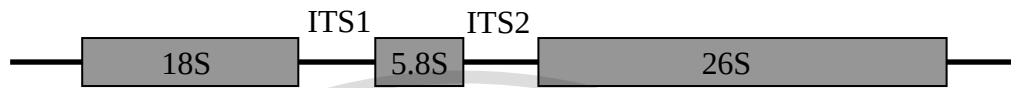


Figure 2.3 Nuclear ribosomal DNA internal transcribed spacer region. (Jobes and Thien, 1997).

Phylogenetic analyses of *Marasmius* based on internal transcribed spacer (ITS), 5.8S, and nuclear ribosomal large subunit (nLSU) DNA sequences data were first generated by Owings (1997 unpubl.) and Owing and Desjardin (1997). They reported that *Marasmius sensu* Singer (1976, 1986) was not monophyletic and was comprised of three well supported lineages. Wilson and Desjardin (2005) confirmed these early results and restricted the genus *Marasmius* to a monophyletic lineage that included only six infrageneric groups.