Ecological Characters of Ectomycorrhizal Fungi and Their Mycorrhizae*

— An introduction to the ecology of higher fungi —

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Introduction

It is well known that a certain species of mycorrhizal fungus can form the ectomycorrhizae with several species of host plants, and that a single species of plant keeps symbiotic relationships with many fungal species (Trappe 1962[13]). The morphological features of mycorrhizae are characteristic depending on the combination of both partners, and moreover the ectomycorrhizae being formed on a plant species are different by each fungal partner (Masui 1927,[1] Zak 1973,[15] Ogawa 1978[7]).

Since Trappe (1967[14]) suggested that the studies concerning ectomycorrhiza should be based on the exact identification of both partners, the descriptions of them became essential to the studies. Strictly speaking, it has been expected that the symbiotic relationships must be testified by the synthesis in pure culture. However, it is almost impossible to practice a series of experiments for the synthesis on all of mycorrhizal fungi because of the difficulties of isolation, pure culture or inoculation of them.

On the other hand, the estimation of mycorrhizal masses and the identification of fungal partners are becoming an important procedure to study ecological roles of mycorrhizal fungi in the forest ecosystem. Therefore it will be expected that the useful methods are proposed for the classification of mycorrhizae and the identification of fungal species by the mycelia or mycorrhizae. It is natural that the methods must be originated from the principles which seem to be reasonable from the mycological points of view.

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General method

Fungal colonies in soil or litter, mycelial structures and the substrates were studied by the following methods. The quadrates with 1×1 m subquadrates were settled on the forest floor. Occurring positions of fruit bodies were marked by pegs for 3 to 5 years and recorded on the map with the distribution of litter and understory vegetations. The areas occupied by fungal colonies of each species were also drawn on the map tracing the occurring positions and the mycelial layers. Mycelia, mycorrhizae, litter and so on were collected beneath the bases of fruit bodies or from the occupied areas repeatedly under different conditions and observed under microscope. If necessary, soil microorganisms were isolated from the colonies or the mycorrhizae by usual methods.

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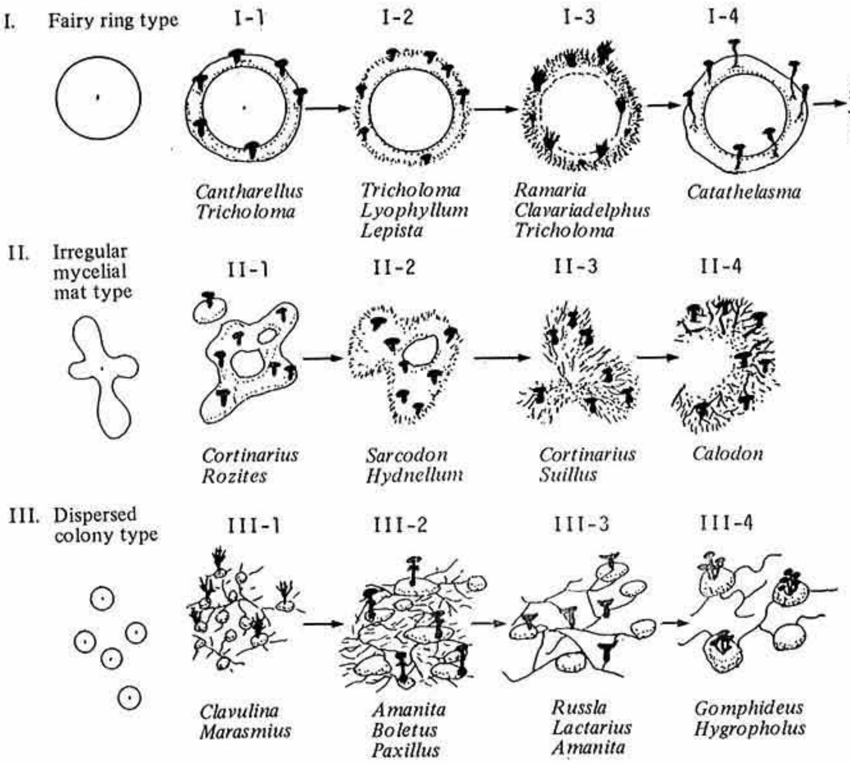


Fig. 1. Life types of higher fungi in field

Results and discussions

1) Colonies of higher fungi in field and their classification

The fungal life types could be classified into 3 types depending on the developmental levels in mycelial structures. Each type was divided into 4 to 5 subtypes by grades of morphological differentiation in mycelial strands and rhizomorphs as shown in Fig. 1.

Fairy ring type I : Fruit bodies are produced originally in a fairy ring. The mycelia grow radially in soil, and do not form any kinds of differentiated mycelial structures such as rhizomorph. In the case of mycorrhizal fungi, their colonies enlarge in circle continuously forming the mycorrhizal masses at the margin of them. Litter decomposers in this type have the ability to utilize various substrates in litter layer. This type could be divided into 5 subtypes according to the mode of mycelial bundle formation.

Irregular mycelial mat type II : Fruit bodies occur sporadically in a certain area. Mycelial mats enlarge irregularly and are not so thick as those in type I. The growth direction of mycelium changes depending on the distribution of substrates. In the case of mycorrhizal fungi, the mycelium grows along roots and forms the cluster of mycorrhizae. Under the favourable conditions the mycelium forms the circular colony. Some litter decomposers occupy larger areas, but their substrates are restricted to a certain kind of leaf litter. This type is divided into 4 subtypes according to the modes of formation of mycelial strands or rhizomorphs.

Dispersed colony type III : Fruit bodies occur gregariously, solitarily or sporadically in larger area. The species in this type form smaller colonies on the specific substrates and inhabit widely in soil. They can move from substrate to substrate by highly organized rhizomorphs in fertile soil with high soil microbial population and organic matters. This life type is most popular among Hymenomycetes. This type is divided into 4 subtypes according to the levels of organization in the structures of rhizomorph.

2) *Descriptions of life types of mycorrhizal fungi*

(1) Fairy ring type I

I–1: Example, *Tricholoma matsutake* : This fungus forms the round and thick colony, Shiro, in B to C horizons with little organic matters. Shiro means fungal colonies or the places producing the fruit bodies in Japanese. The colony grows radially stimulating the growth of pine roots and forming mycorrhizal mass. The mycelium enlarges annually 10 to 15 cm in width and produces the fruit bodies in a fairy ring. Inside the colony the soil changes to powdery structure because of the decompositions of mycelium and mycorrhiza and desiccation of soil. The mycorrhiza formed by *T. matsutake* is witches'-broom shape. The mycelium stimulates not only the growth of leader roots but also the ramification and elongation of lateral roots. There are neither the formation of fungal sheath nor Hartig's net. The color of mycorrhiza is black by the accumulation of tannin in epidermal cells. The hyphae invade into the intercellular spaces of outer cortical cells. Although this mycorrhiza is classified as an ectomycorrhiza, it seems to be parasitic judging from the inner structures of it. The mycorrhiza produces antibacterial and antifungal substances by which soil microorganisms are excluded from the inside of Shiro (Ogawa 1975a,[2] 1975b,[3] 1977a[4] and 1977b,[5] Ohara 1966[11] and 1967[12]).

I–2: Example, *Tricholoma robustum* : The colony is originally round, but it is rare to make a fairy ring. The thin mycelial layer is formed in AC horizon of immature soil. Short mycelial bundles are formed at the margin of the colony. The mycorrhiza is a cluster of black fork shaped ones belonging to witches'-broom shape. The hyphae can not stimulate the growth of leaders but the ramification and elongation of laterals. There are

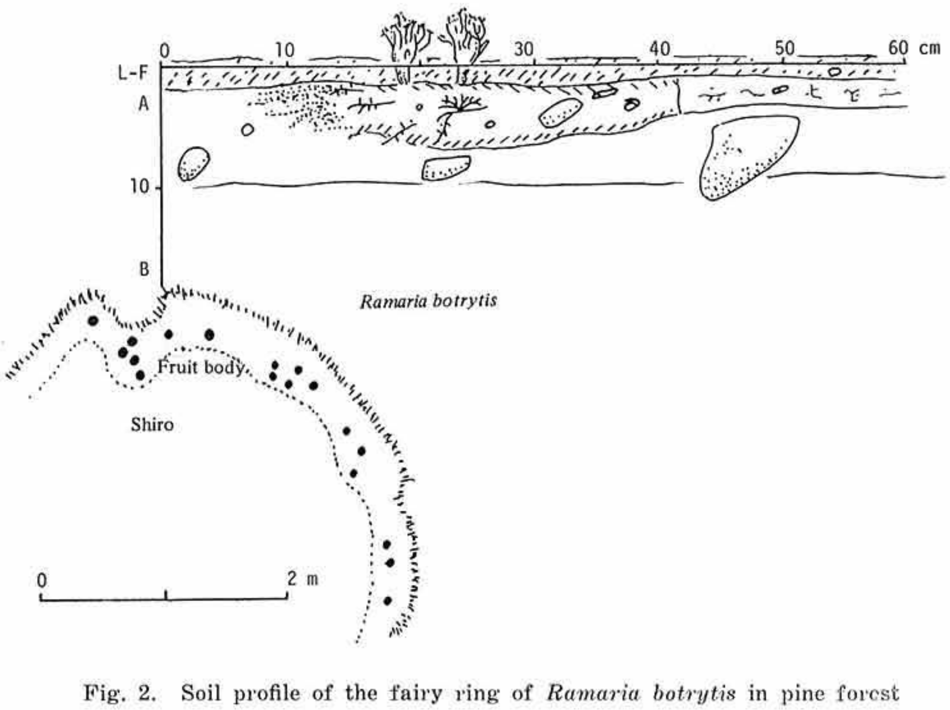


Fig. 2. Soil profile of the fairy ring of *Ramaria botrytis* in pine forest

the formations of thin fungal sheath and Hartig's net. Antibacterial activities of the mycorrhiza is not so high as that in the case of *T. matsutake* (Ogawa 1981[10]).

I-3: Example, *Ramaria botrytis* : The colony is round, and the active zone producing fruit bodies is circular. Mycelial layer consisting of irregular mycelial bundles is formed in AC horizon. The yellowish white mycelial bundles enlarge outwards regularly following the mycorrhizal mass (Fig. 2). After fructification the soil properties change because of decay of mycelium. The mycorrhiza becomes tree branch shape by the promotion of leader's growth and ramification of laterals. The brown mycorrhiza is covered irregularly by mycelium, but there are no formations of fungal sheath and Hartig's net. The activity to exclude soil microorganisms is lower than that in *T. robustum* (Plate 1).

I-4: Example, *Catathelasma ventricosum* : This fungus produces the fruit bodies in a fairy ring with diameter reaching 10 m or more. As the thick and circular mycelial layer is formed 30 to 70 cm in depth of soil in C horizon, the compact mycelial bundles like rhizoids are formed before fructification. The mycorrhiza is in a witches'-broom shape, and there are no formations of fungal sheath and Hartig's net. Originally the life type of this fungus is close to that of *T. matsutake*.

I-5: Some litter decomposers such as *Agaricus*, *Clitocybe* and *Collybia* species are included in this type.

(2) Irregular mycelial mat type II

II-1: Example, *Cortinarius mucifluus* : The fruit bodies occur in a limited area sporadically. The mycelium grows in F and HA horizons forming thick and irregular mycelial mat with gray color. The mycorrhiza which is formed in the mycelial matis fork and pinnate shapes, but the frequency is relatively low. Although Hartig's net is obvious, the fungal sheath is incomplete. There is no changes of soil in the colony.

II-2: Example, *Sarcodon scabrosus* : The fruit bodies occur in a limited area sporadically. The irregular mycelial mat in HA horizon consists of fine mycelial strands with some main hyphae and mycelial mat including numerous mycorrhizae. The mycorrhiza is in a witches'-broom shape in which the laterals ramify frequently and elongate at the bases. There are recognized thin fungal sheath and incomplete formation of Hartig's net.

II-3: Example, *Cortinarius bovinus* : The fungus produces the fruit bodies solitarily or sporadically forming the irregular mycelial mat in F layer. The mycelial layer consists of mycelial strands growing along the roots and forming the tree branch shaped mycorrhiza. This fungus stimulates the growth and ramification of laterals, but the frequency of ramifying is not so high as that of *Sarcodon scabrosus*. The fungal sheath envelops thinly, but the clear Hartig's net formation is observed.

II-4: Example, *Calodon squaveolens* : The fruit bodies occur in lines or gregariously. The mycelial layer consisting of mycelium and rhizomorphs is formed in mineral soil. The rhizomorphs with differentiated inner tissue and short branches grow along roots forming the twisting tree branch or witches'-broom shaped mycorrhiza. Elongated laterals are enveloped by the well developed fungal sheaths, and the clear Hartig's net is formed.

(3) Dispersed colony type III

III-1: Example, *Clavulina cristata* : The fruit bodies occur sporadically. The rhizomorphs grow among leaf litter forming the short and pinnate shaped mycorrhiza sparsely. There are typical fungal sheath and Hartig's net formation.

III-2: Example, *Amanita muscaria* : The fruit bodies occur sporadically or rarely in a fairy ring. The fine and developed rhizomorphs with numerous short branches grow along roots in A to B horizons deeply (Fig. 3). The yellowish brown mycorrhiza is in a coralloid shape with short branching laterals and bears the thick fungal sheath and regular Hartig's net. The mycorrhizae are distributed sparsely (Plate 2).

III-3: Example, *Russla delica* : The fruit bodies occur sporadically. The white and long rhizomorphs grow in litter layer forming the pinnate shaped mycorrhizae sparsely in soil.

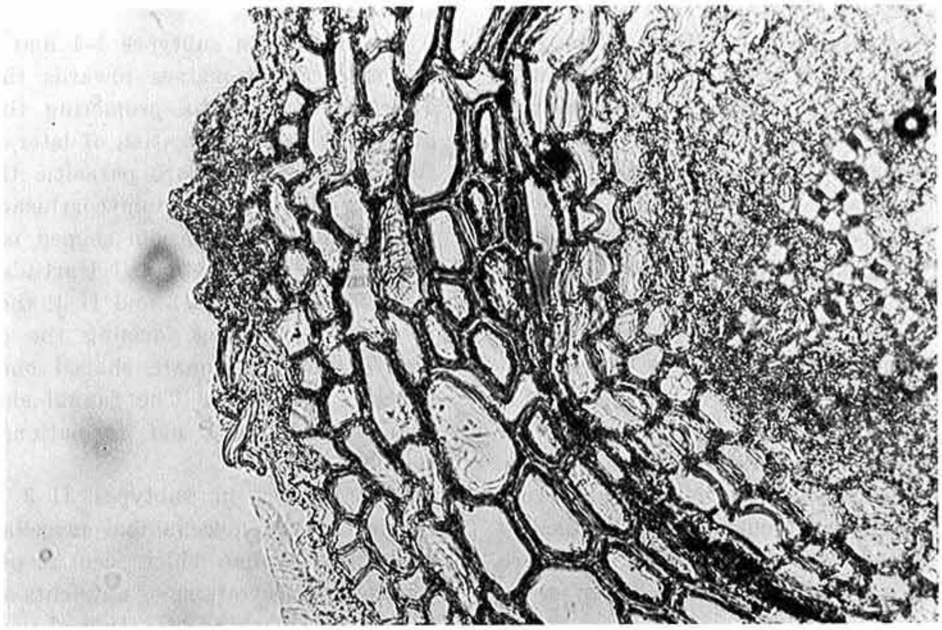


Plate 1. Mycorrhiza of *Pinus densiflora* formed by *Ramaria botrytis*
Top: External appearance of the mycorrhiza
Bottom: Cross section of the mycorrhiza
The formation of fungal sheath and Hartig's net are incomplete.

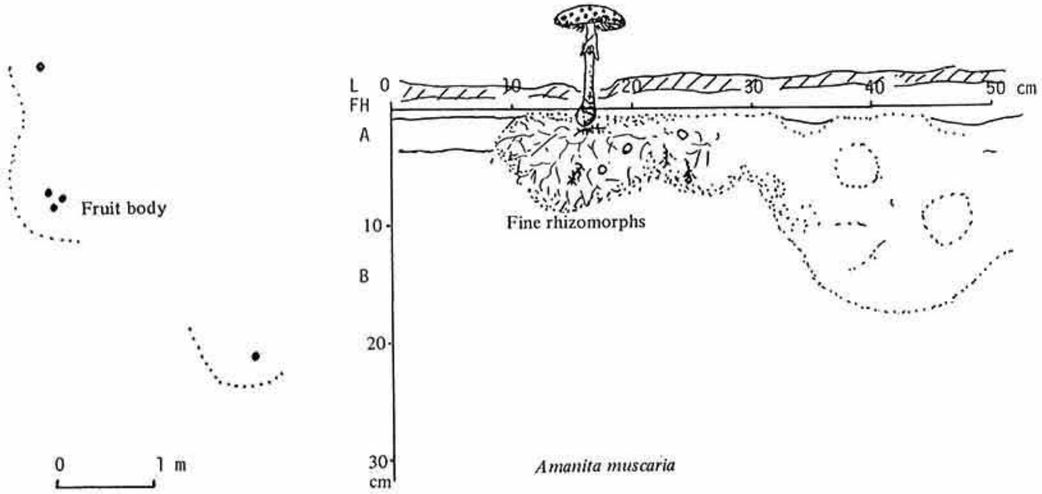


Fig. 3. Soil profile of the colonoy of *Amanita muscaria* in coniferous forest

Fungal sheath is thick and bears ornamental hyphae over the surface, and Hartig's net is obvious. One tip of rhizomorph attaches to the corn of *Abies* and decomposes partly it.

III–4: *Gomphideus tomentosus* : This fungus produces the fruit bodies gregariously forming the cluster of long lived pinnate shaped mycorrhizae in F layer. As the mycorrhiza formation continues for several years on a mother root, the mycorrhiza becomes a coralloid shape. Several rhizomorphs which are highly organized in structures are produced from the mycorrhizae and grow into surrounding soil. The fungal sheath is thick, and the formation of Hartig's net is remarkable.

3) Mycelial structures and mycorrhizae

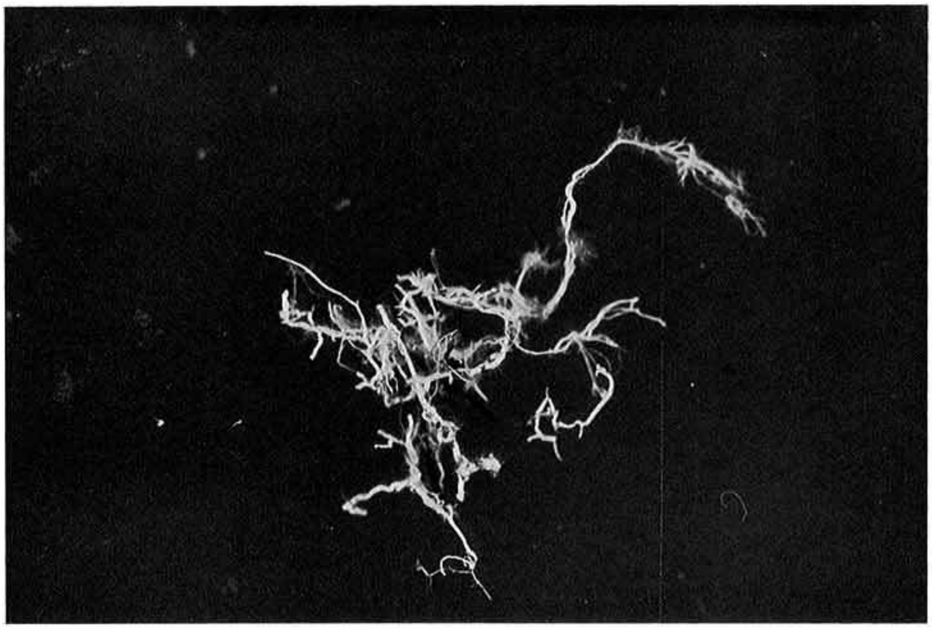
As above mentioned, the more the external appearances develop from mycelial bundles to mycelial starnds and rhizomorphs, the more highly they are organized in the inner structures. There is no differentiated inner structures in the mycelial bundles, but inside the mycelial strands several thick main hyphae surrounded by fine hyphae are observed. Rhizomorphs are divided into two grades of undeveloped and developed ones by the levels of organization in structures. Moreover, the morphological features of mycorrhizae and the modes of hyphal invasion correspond with the levels of development in mycelial structure as shown in Fig. 4.

The species in subtypes I–1 and I–4 form the mycorrhizal masses towards the growth directions of mycelia promoting the growth of leaders and ramification of laterals. These fungi seem to be more parasitic than those forming the typical ectomycorrhizae, because they form witches'-broom shaped mycorrhiza without fungal sheath and Hartig's net.

In subtypes I–2, I–3 and II–1, the mycelial bundles grow along forming the cluster of tree branch or pinnate shaped ones at the margin of colonies. The fungal sheaths are thin, and Hartig's net formations are incomplete.

The colonies in subtypes II–2 and II–3 consist of the mycelia and mycelial strands with main hyphae which seem to play a role for the transportations of nutrients and water. The mycorrhizae are the typical ones of tree branch or pinnate shape which have the organized fungal sheaths and well developed Hartig's net.

The species in subtypes III–1, III–2 and III–3 form the undeveloped rhizomorphs which have no organized tissues except main



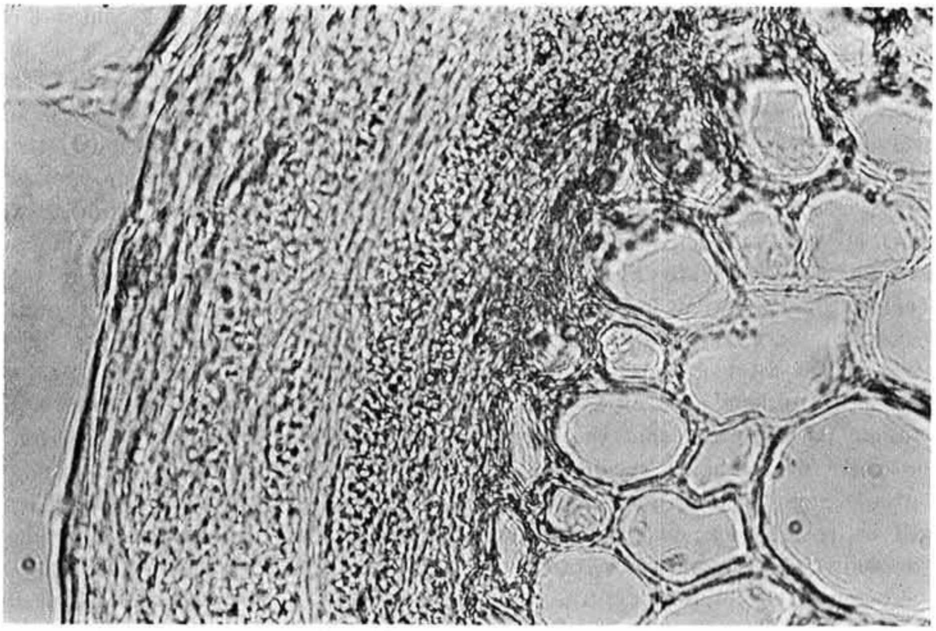


Plate 2. Mycorrhiza of *Abies veitchii* formed by *Amanita muscaria*
Top: External appearance of the mycorrhiza
Bottom: Cross section of the mycorrhiza
The roots surface is covered with thick fungal sheath and typical Hartig's net is formed

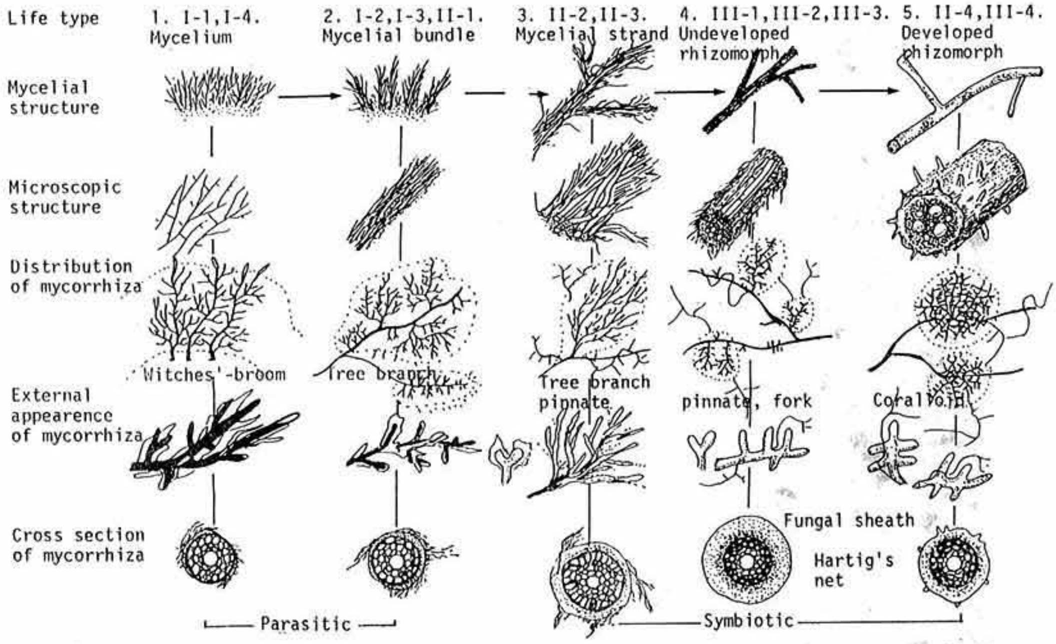


Fig. 4. Relations among mycelial structure, distribution of mycorrhiza and the morphology

and coating hyphae. The rhizomorphs extend in soil forming the fork or pinnate shaped mycorrhizae with the organized fungal sheaths and Hartig's nets.

The species in subtypes II–4 and III–4 produce the well-developed rhizomorphs in which some tissues like epidermis, parenchyma and vascular bundle are discernible. The coralloid or tree branch shaped mycorrhizae are formed in a certain area. Fungal sheaths and Hartig's nets are typical among ectomycorrhizae.

It is certain from these results that the morphological features of mycorrhizae have been fixed genetically depending on the characters of fungal partners and that the fungi has formed their mycorrhizae specific to a species, a group in genus or a genus. Therefore, it will be possible on most of species or genera to identify the fungal partners from their mycorrhizae or mycelial structures.

4) Habitats and distribution of mycorrhizal fungi in forest

Habitats of the species in type I are restricted in mineral soil with low microbial population and organic matters. Some aerobic speices in subtypes I–2 and I–3 inhabit in AC horizon, but most of species in this type form the thick mycelial layers deeply in B to C horizons. The colonies of these species tend to be formed at the primary stage of forestation, for example in pine forests (Ogawa 1978)[7] and secondary deciduous forests. In aged stands, they lose the chance to invade into forest soil because of the maturation of surface soil and increase of antagonists. The mycorrhizae in this type are distributed in the limited areas occupied by mycelia.

The mycelial layers of species in subtypes II–1 and II–2 enlarge in HA horizon where the fine roots are growing with high frequency. The fungi in these subtypes inhabit in wet site or in dry site depending on the characters of them (Ogawa 1977).[6] Only the species in subtype II–1 occupy most of area in the pure stand of *Pinus thunbergii* planted on sand dune (Ogawa 1979).[8] The mycelial layers in subtypes II–3 and II–4 are formed in F to A horizons being controlled by the distribution of roots. The species in type III have wide habitats from Ao to B horizons of fertile soils rich in organic matters. These fungi also coexist at the same place with each other growing by rhizomorphs which have the resistibility against antagonists.

The florae of higher fungi in forest are dependent on plant species composition, soil properties and soil microbial florae and also continuously variable following the development of forest ecosystem (Ogawa 1980).[9] Therefore, some mycorrhizal fungi of which ecological characters have been well known are able to be used as an index to reflect changes of soil conditions and microbial florae in forest.

Taxonomically there are several interesting problems in these ecological characters. A species has its own ecotype even under different conditions, and a group in a genus shows the mostly similar life type at least belonging to the same original life type. It is expected that there are some problems concerning the phylogeny of higher fungi among these facts. It is desirable that the higher fungi should be classified considering their ecological characters because their heterotrophic lives have evolved being influenced by the means to obtain their substrates.

Summary

The ecological characters of higher fungi were investigated in field. Their life types could be classified to 3 major types according to the modes of mycelial growth under natural conditions; Fairy ring type I, Irregular mycelial mat type II and Dispersed colony type III. These types were divided into 13 subtypes by the levels of development in mycelial structures such as rhizomorph. The species without any differentiated mycelial structures except mycelial bundles form witches'-broom shaped and parasitic mycorrhizae. The ones with mycelial strands form the tree branch or witches'-broom shaped and symbiotic mycorrhizae. The ones with rhizomorphs form the typical mycorrhizae in spite of rod, fork, pinnate and coralloid, or the cluster of them. The species without rhizomorphs form their colonies mainly in mineral soils from HA to C horizons occupying the larger areas. The ones with rhizomorphs inhabit widely under various soil conditions.

Key words: Fungal ecology, Classification of ectomycorrhizae, Fungal life type.

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