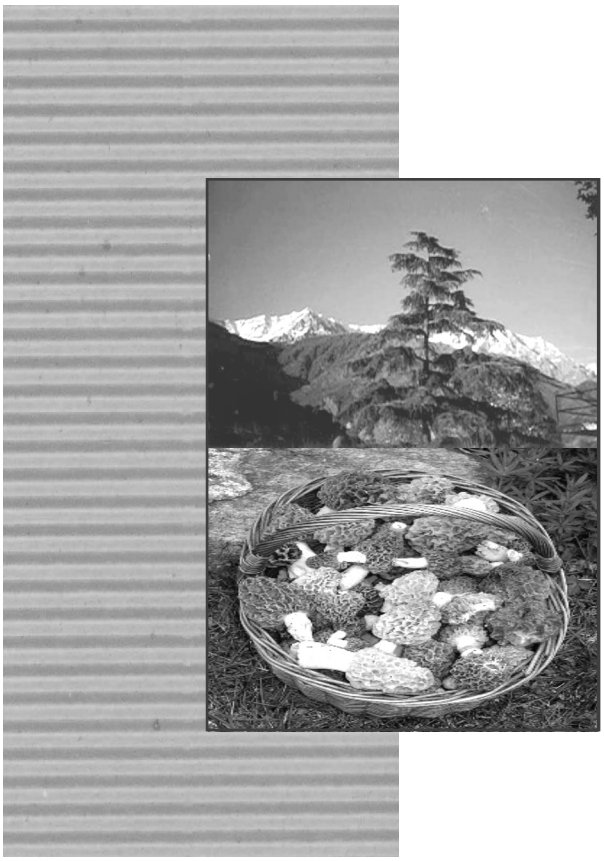
CHAPTER

# 2 The Life Cycle Pattern



## CHAPTER OUTLINE

- The Ascocarp
- Ascospores and Mycelium
- Heterokaryon Formation
- Sclerotial Development
- Ascocarp Development
- Cultivation Aspects

Different aspects of morel biology have been well worked out all over the world. Orientation of every study has been towards development of technology for artificial cultivation of morels. But foolproof method for commercial cultivation of morels still eludes scientists. The life cycle, however, is well studied and various steps in the life cycle seem to be as follows: The ascocarp, the morel of commerce, is the fructification that appears in nature and is the article of commerce. It has sterile ridges and fertile grooves or depressions. The latter bears hymenium which is lined by asci containing ascospores. Ascospores on germination produce mycelium, which becomes heterokaryotic, forms sclerotia and then produces ascocarps. The 'unseen' thread that binds the different events in the life cycle in morels is still not completely traced, though lot of work has been done on individual 'events', which is being reviewed here. Once the 'missing link' is discovered, the morels, no doubt, will be 'booming' and 'mushrooming' in the homes.

## THE ASCOCARP

The fructification or ascocarp in *Morchella* is stipitate, hollow and differentiated into pileus (cap) and stipe (stalk). The colour of the pileus varies from dirty greyish-white to dark-brownish depending upon the species, age of fruiting body, and the type of vegetation. Pileus constitutes the fertile portion of the ascocarp. It is hollow and fleshy. The honeycombed surface of pileus gives the appearance of a sponge. When young, the surface of pileus is quite smooth, and as it grows, there appears a network of ridges and grooves or depressions (pits) on the surface due to unequal growth of the hymenial surface. The ridges are sterile while the pits are fertile.

Pileus is variously shaped in *Morchella* species. In *M. angusticeps,* pileus is elongated, sub-globose, or elongated with slightly subconic apex and adnate at the base; in *M. conica,* pileus is elongated, more or less conical in shape, acute at the apex, subobtuse, also adnate at the base; in *M. crassipes,* pileus is more or less conical in shape and adnate at the base; in *M. deliciosa,* pileus is elongated to sub-globose, obtuse at the apex, adnate to the stipe base; *M. esculenta,* has sub-globose ovate or elongated pileus attenuated upwards but obtuse at the apex and adnate to the stipe at the base; in *M. semilibera,* the pileus is conic to elongated, free at the base with acute or obtuse apex light brown in colour; in *M. tibelica* also the pileus is elongated and conical with conic apex and adnate base stipe; and in *Morchella simlensis*, the pileus is globose or elongated with obtuse apex and adnate base stipe.

## ASCOSPORES AND MYCELIUM

The hymenium which lines the pits bears numerous asci. These asci are cylindrical, sub-cylindrical with obtuse apex. Each ascus contains eight ascospores. The ascospores are ellipsoid, uniseriate, yellowish in mass, hyaline singly, smooth and eguttulate but with external oil drops at each end. The asci are positively phototrophic. The ascospores are wind disseminated. Falling on a suitable substratum (moist humus), each ascospore germinates to produce new mycelium. The ascospore that happens to fall on unsuitable substratum will perish (Shad, 1989). Mycelium is inconspicuous and subterranean. It grows few inches deep in soil. The mycelial colonies are white, turning white-brown with age.

direct hyphal growth from a germinating spore to the ungerminated spore and suggested that heterokaryon formation could occur at this stage in the life cycle.

Hervey *et al.* (1978) crossed several hundred pairs of single ascospore isolates of *Morchella esculenta.* They observed the build up of aerial hyphae at the interaction interface in all non-self combinations and termed this as "barrage-like build up". They observed that either ascus fusion nuclei must be heterozygous for certain genetic factors or that asci of a single ascocarp do not at all arise from a single pair of nuclei. They further hypothesized that heterokaryosis must occur at some time in the life cycle because of the observed segregation patterns of two morphological cultural characteristics (flat and fluffy) among progeny of a single fruiting body.

Volk and Leonard (1989 b) taking clue from reports of Fincham *et al.* (1979) on *Neurospora* and *Podospora* that heterokaryosis is under the control of multiple genes, including in some cases, the mating type genes, wanted to further characterize the barrage type build up of aerial hyphae cytologically, culturally and genetically. They showed that *Morchella* is capable of forming a heterokaryon between monoascosporus strains. In cultural evidence, they noticed that at the point of mating of hyphae raised from spores of the same species in the same Petri dish, an aerial bridge of hyphae [referred to as "barrage-like build up of hyphae" by Hervey *et al.* (1978) and retermed as "mycelial meld" by Volk and Leonard (1989)] was formed when two colonies met in majority of the cases followed by deposition of a dark brown pigment into the medium. The cultural characteristics and mycelial interactions between two genetically dissimilar cultures suggested presence of heterokaryotic phase in the life cycle of morels. But whether heterokaryotic phase is essential in the morel life cycle was not demonstrated. Further, no mycelial meld was noticed for reaction between the mycelia from the same spore or between genetically identical sister spores. Two different mutations isolated in this study were used to show genetic complementations in the heterokaryons. Interspecific hyphal fusion has also been reported among *M. esculenta, M. crassipes,* and *M. deliciosa* but not with *M. semilibera* and *M. angusticeps.*

Cytological studies of aerial mycelial meld demonstrated nuclear pairing in presumptive heterokaryon. Volk and Leonard (1989b) suggested that mycelial meld is a point at which heterokaryosis takes place in the morel, and evidence obtained indicates that a stable heterokaryosis takes place in the morel and a stable heterokaryon is formed in the melds. But whether or not this heterokaryon with its paired nuclei is associated with sexual reproduction or is strictly a vegetative trait remains to be elucidated. Ability to process mutants from the meld reaction through sclerotia and to the fruiting body would reconcile this problem (Volk and Leonard 1989b).

Volk and Leonard (1990) maintain that there is no firm evidence that nuclear pairing is a natural phase of the morel life cycle or even that two pairing nuclei are in fact different. However, this nuclear pairing is a common feature of forced heterokaryotic mycelia in *Morchella* and is also seen in certain sclerotia and in the sterile cells of the fruiting body, providing a possible link between heterokaryon, the heterokaryotic sclerotium and fruiting body formation. Buscot (1993) also observed no evidence of mating types in morels.

Volk and Leonard (1989) postulated that generations of forced heterokaryons under laboratory conditions and their processing through sclerotia to fruiting body should lead to reproducible and more dependable method for morel cultivation. Sehgal and Sharma (2007)

In the subsequent studies on *Morchella esculenta*, Volk and Leonard (1990) observed that sclerotia formation in mycelial culture occurs when it is grown at low temperature (4°C) or on a complete medium (glucose peptone yeast extract, mineral salt medium plus 2% compost sheep manure) or when nutrient depletion occurs (such as complete colonization of a Petri dish).

The development biology of sclerotia formation in different groups of fungi has been reviewed by Wiletts and Bullock (1992), but unfortunately morel sclerotia are not included in their review. Studies on nutritional requirements of sclerotia formation have been done only by a few workers.

Small 1-2 mm sclerotial initials begin to form after 7-10 days growth which subsequently expand to form a single large sclerotium. Sclerotia are considered mature when radial expansion ceases and the pigmentation attains a dark brown colour. Sclerotia are the terminal type formed from the repeated branching of a terminal hypha. However, Amir *et al.* (1992) maintain that *Morchella esculenta* sclerotia initials appear laterally to the main leading hypha and thus can be classed as lateral type.

Volk and Leonard (1990) further observed that during the formation of sclerotia, the cells round up to form varied and unusual shapes with thick walls and remain multinucleate, store nutrients and accumulate oil droplets. A cross-section of sclerotia reveals a series of compacted, isodiametric cells with very thick wall, able to tolerate adverse conditions such as low temperature and desiccation.

Intensive studies on morphology and physiology of *Morchella esculenta* during sclerotial formation were carried out by Amir *et al.* (1992, 1993, 1994, and 1995 a, b). One of the critical requirements for culturing *Morchella* spp. in a two-layer system is the production of large sclerotial biomass in the poor substrate. In order to understand the process and factors involved, Amir *et al.* (1992) devised an improved experimental system—split-plate procedure. In this method, Petri dishes (9 cm in diameter) were divided into two by a plastic barrier to prevent diffusion. One side of this split-plate contained nutrient poor medium (Noble Agar GNA), while the other side contained nutrient rich medium (PDA, DM, etc.). Inoculum placed on the poor side grew towards the rich side, ultimately producing sclerotia mainly on the poor medium. They also studied the effect of addition of salts, sugar and PEG and resultant water potentials in the poor medium. While salts inhibited the production of sclerotia, sugars especially hexoses and hexitols enhanced it. They concluded from their experiments that two conditions are required for the production of the medium—a turgor potential in the hyphae conducive to morphogenesis and the type of sugar added to the poor medium. Maximum sclerotium formation was obtained on the noble agar side (GNA) of the plate when 0.5 M hexose was added.

Amir *et al.* (1993) identified six major stages in the growth of sclerotia in *M. esculenta*. The first two stages refer to the colonization of the plate by the hyphae and the other four stages to the formation and development of sclerotia on GNA side. The most prominent changes on the GNA side occurred when the hyphae on the DM side reached the end of the plate. Restriction of the linear growth stimulated the development of new branches from tertiary branches.

Restriction of the linear growth also reserved the cytoplasmic stream when it started flowing from the DM side to the GNA side—point of origin of sclerotial initials. This reverse

production, was reduced or delayed suggesting that these processes are competitive and distinction between 'flat' and 'fluffy' may not be absolute. The earlier observation (vide Buscot 1993) that morel sclerotia form only after the total available nitrogen has been depleted from the medium would suggest that limitation on supply of this nutrient may fundamentally affect the balance between aerial mycelium formation and sclerotium formation. The ability to shift from one pattern of development to another depending on nutrient availability may be significant during natural colonization process (Kaul, 1997).

## ASCOCARP DEVELOPMENT

Fructification of mushroom is believed to progress through four stages: initiation leading to the formation of primordia; maturation during which primordia develop into mature sporocarps; sporulation and autolysis or disintegration (Manachere, 1974). Buscot (1989) carried out field observations on growth and development of *Morchella rotunda* and *Mitrophora semilibera* in relation to forest soil temperature. He found that maturation in both lasted for 3-4 weeks. During this time ascocarp development progressed in three different sub-stages:

(i) Pre-emergence—ascocarps are still covered by litter. They are small (2-15 mm) and develop a distinct stipe and a furrowed hymenium. Pre-emergence may last for 2 or 3 weeks during which the growth rate is very slow (1-2 mm every 2 days).

(ii) Emergence—there is sudden increase in ascocarp growth rate. The ascocarps rapidly emerge from the litter and reach two-thirds of their final size within 24 hours which gives the impression that fruiting bodies develop overnight.

(iii) Final maturation—after rapid expansion, the rate of ascocarp development decreases and finally fruiting bodies reach their mature size and pigmentation and begin to sporulate.

Buscot (1989) further obtained data, which correlates the initial extent of spring time reheating of soil with ascocarp maturation. Volk and Leonard (1990) obtained the development of fruiting body from tiny primordium to mature size in incidental culture with begonias (*Begonia tuberhybrida*). The primordium is at first a tiny white fungus–like mass of hyphae. The hymenium differentiates at the very apex, enlarging and pigmenting as the primordium grows into a mature fruiting body (c.f. Kaul, 1997).

## CULTIVATION ASPECTS

Mycologists want to cultivate morels just like shiitake or button mushroom and there have been numerous attempts to achieve success ever since morels have been consumed. There are various reports in literature which mention the attempts to cultivate morels. Initially, attempts have been made to reproduce natural conditions under which the fungus grows. Ramsbottom (1953) cites that the probable earliest mention to grow morels artificially was perhaps by J.G. Gleditsch in 1753. He mentions that countrywomen of Neo Marchia noticed appearance of morels at burnt sites. This observation led to burning of areas for morel production. But this practice had to be abandoned as it resulted in frequent forest fires. That morels appear on burnt grounds abundantly has been reported subsequently by a number of workers (McCubbin, 1913; Krieger, 1967; Moser 1949 a, b and Backer and Matkin, 1959).

laboratory at San Francisco State University (USA), he obtained 16 mature ascocarps from 8 cultivations. Experiments were conducted in walk-in growth chamber maintained at 15-18°C and 85% relative humidity with daily light exposures. He used a sterilized substrate of cooked wheat barries at 50% moisture. Inoculum was obtained from stipe tissue or ascospore culture. After the growth of mycelium, axenic conditions were not maintained. Ascocarp development was preceded by vegetation hyphae, conidia and sclerotia.

Subsequently two patents describing a process for the cultivation of morels have appeared in USA (Ower *et al*., 1986 and Ower *et al*., 1988). Royse (1995) commenting on morel cultivation maintains that these patents have revealed some of the processes involved in predictable production of sporocarp, but attempts to practice the invention have met with limited success. The commercial production of morels (in USA only) is a relatively recent development and only one company is producing morels on a commercial scale. Royse (1995) further provided the following details of the process under trial:

"Commercial cultivation involves mainly the production of sclerotia and early over-wintering stage of mushroom. Briefly 'nutrient primed' sclerotia are produced in soil placed on a layer of sterilized wheat. The production of sclerotia requires about 18-24 days under ideal conditions. At maturity the sclerotia are harvested, soaked in clean water for 24 hours and distributed into a thin layer of pasteurized 'nutrient poor' soil mix. The sclerotia germinate via the production of mycelium. After the mycelium has spread throughout the soil, a continuous (24-hour duration) fine mist of clean water is provided to stimulate the formation of ascocarps."

At present, there are five partners (Neogen, Domino's, Skandigen of Sewden, Kuhn Champignon of Switzerland and the Salk Institute Biotechnology Associates of La Jolla, CA) in the 'Company M'. The company has built pilot plant in Michigan and is expected to sell morels in the limited markets very soon. Many people still believe that Company M cannot grow morels. However, Volk (1990) has categorically mentioned that he visited their growth chambers and witnessed approximately fifty morels emerging from each plant containing soil and sclerotia. As such, there should not be any doubt that morels cannot be domesticated.

A number of problems still remain to be solved before it is accepted as a satisfactory commercial process. Consistent fruiting, competitive weed moulds, poor yield and small size are some of these problems seeking immediate attention. Further research is still needed to understand the environmental and genetic factors involved in the regulation of fruiting.