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# ASCOCARP ONTOGENY AND A NATURAL CLASSIFICATION OF THE ASCOBOLACEAE*

J. van Brummelen
*Rijksherbarium, Leiden*

(With 1 diagram)

Ascocarp ontogeny has proved to be a very useful basis for the classification of the Ascobolaceae. A scheme of interrelationships between the sections of *Ascobolus* and *Saccobolus* is given. It is suggested that ascocarp ontogeny will play a prominent part in recognizing series of supra-specific taxa within other natural families of the Discomycetes, e.g. Thelebolaceae and Pyronemataceae.

Among the Pezizales the Ascobolaceae and their allies include many objects suitable for developmental studies. The coprophilous species in particular can often be easily isolated and grown on artificial media.

Since Yu's experiments (*1954*) on factors concerning the most favourable conditions for ascospore-germination in some coprophilous species of *Ascobolus*, several species have been studied that could not be cultured before. The development of Discomycetes that do not produce fruit-bodies in culture is usually incompletely known. Publications on the sexuality, compatibility and cytology of such species that have been cultured contain a great deal of information from which many data on ascocarp ontogeny can be deduced. The development of the sporophytic part of the ascocarp in particular has drawn the attention of investigators in these fields of research. Unfortunately identifications can only rarely be verified. Studies devoted to the ontogeny of both gametophytic and sporophytic parts of the ascocarp of Discomycetes are very rare.

Corner's classical publications (*1929a, 1929b, 1930a, 1930b, 1931*) laid a basis for a comparative morphology of Discomycetes with special emphasis on the dynamic aspects of ascocarp ontogeny. This, however, did not result in an increased interest in the problems and significance of ascocarp ontogeny of Discomycetes until the last decennium. Recently the Ascobolaceae and their allies have been the subject of ontogenetic studies by Gamundi & Ranalli (*1963, 1964, 1966, 1969*), van Brummelen (*1967*), and Durand (*1971*).

## Methods

In developmental studies of Discomycetes the results of three methods should be combined to obtain a complete structural and functional image of ascocarp ontogeny.

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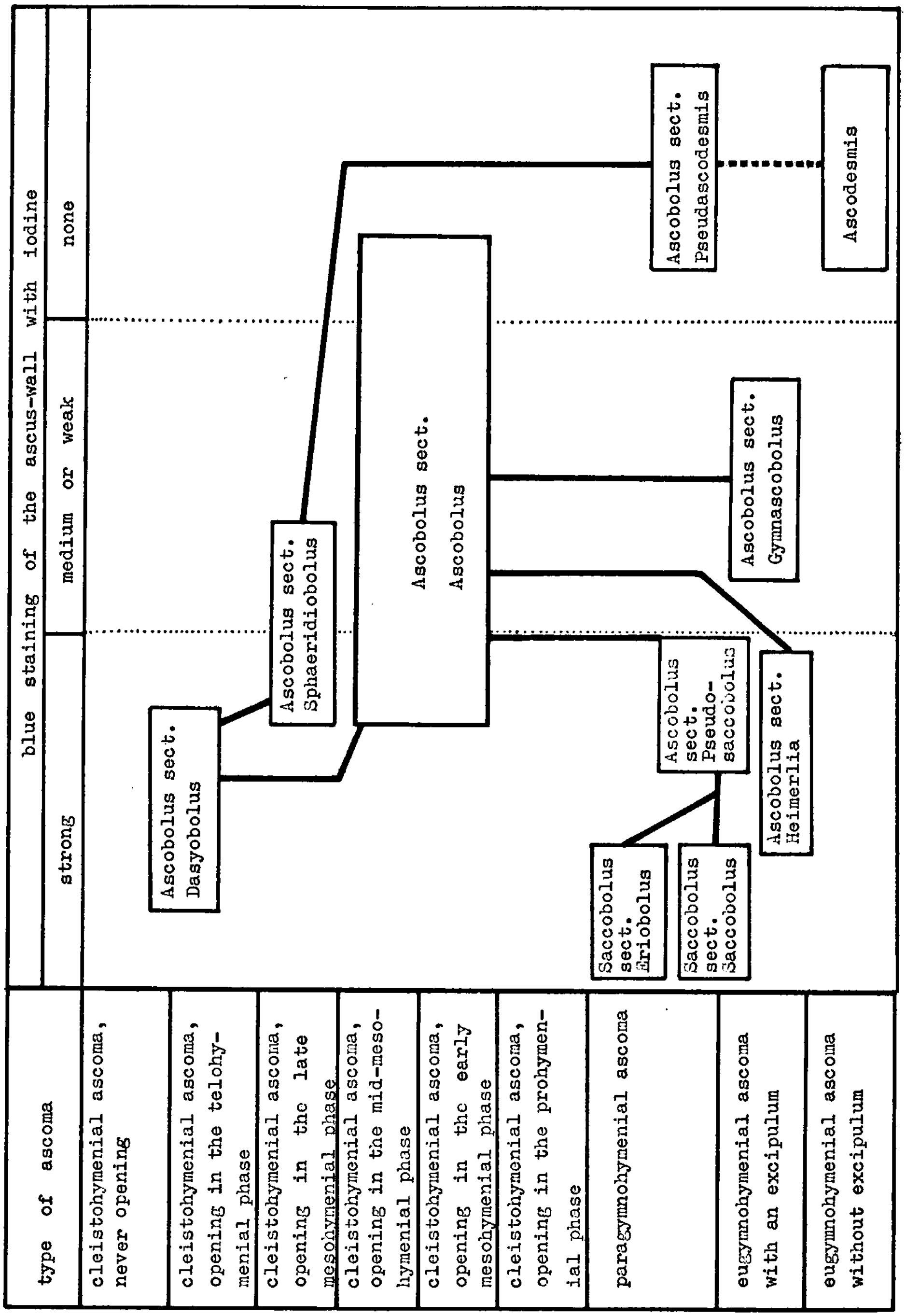
DIAGRAM I


blue staining of the ascus-wall with iodine
strong
medium or weak
none
type of ascoma
cleistohymenial ascoma, never opening
cleistohymenial ascoma, opening in the telohymenial phase
cleistohymenial ascoma, opening in the late mesohymenial phase
cleistohymenial ascoma, opening in the mid-mesohymenial phase
cleistohymenial ascoma, opening in the early mesohymenial phase
cleistohymenial ascoma, opening in the prohymenial phase
paragymnohymenial ascoma
eugymnohymenial ascoma with an excipulum
eugymnohymenial ascoma without excipulum
Ascobolus sect. Dasyobolus
Ascobolus sect. Sphaeridiobolus
Ascobolus sect. Ascobolus
Ascobolus sect. Pseudascodesmis
Ascodesmis
Ascobolus sect. Gymnascobolus
Ascobolus sect. Pseudosaccobolus
Ascobolus sect. Heimerlia
Saccobolus sect. Eriobolus
Saccobolus sect. Saccobolus

Besides a study of vital and stained microscopic preparations of whole mounts of the earliest stages (initiation) extensive investigations of series of microtome sections showing different stages are indispensable (topographic ontogeny). Omission of this method has led to erroneous interpretations and unacceptable generalizations. In several cases hyphae of various origin can be differentiated by staining. A third method (Corner's hyphal analyses) is an attempt to trace hyphae from their origin to their end or vice versa through their elaborate organization in the fruit-body. In hand-made sections or oriented fragments of tissue, elements are separated by gently pressing the cover-glass or pulling the hyphae with needles. Unfortunately, this method also does not help very much in unravelling the often perplexingly intricate structures of the early stages of the ascocarp.

## ASCOCARP ONTOGENY IN ASCOBOLACEAE

The first indication of ascocarp initiation is the development in the mycelial hyphae of weakly to strongly curved and swollen branches. Of these archicarps one or more terminal or subterminal cells differentiate into multinuclear ascogonia. Antheridia are also often found. In certain species parthenogenetic or apogamous processes have been reported.

From the base of the archicarp or from adjacent mycelial hyphae, and shortly before or after the differentiation of ascogonia, thin hyphae arise. These branch and form an incomplete or continuous sheath around the archicarp. This primary sheath is the beginning of the gametophytic part of the ascocarp. In open types of development, i.e. with gymnohymenial ascomata, the investing hyphae of the primary sheath have only a restricted growth in that they do not form an entire sheath over the hymenium during further development. In closed types of development, i.e. with cleistohymenial ascomata, the investing hyphae produce a continuous primary sheath. Thus small spherical bodies are formed. Usually there is a more or less centrally placed archicarp with a strongly swollen ascogonal apparatus in each of them, surrounded by intricate investing hyphae. Often the cells of the primary sheath undergo a change in becoming inflated and thick-walled, while their growing activity slows down.

Observations in species of *Ascobolus* with cleistohymenial ascomata have revealed that at a certain stage of development, when the primary sheath has reached a thickness of only a few layers of cells, a secondary sheath develops within it. The same has been observed in species of *Thelebolus*, while Durand (*1971*) described a similar development in a species of *Lasiobolus*. The hyphae of this sheath are relatively narrow and have dense cytoplasm. They arise near the base of the archicarp. Unfortunately it was not possible to establish with certainty from what cells they originate.

As a result of the active growth of hyphae of the secondary sheath within the primary sheath tangential forces are exerted on the peripheral layers. Depending on the structure and growth activity of the hyphae of the primary sheath different cortex-textures will result. Peripheral cells may become flattened, or break up into small groups. During later development the process results in smooth and rough surfaces respectively. Both kinds are found in cleistohymenial ascomata of *Ascobolus*, *Thelebolus*, and *Lasiobolus*.

Peripheral gaps are also filled by interstitial growth of new elements from the inner layers. During further development hyphae of the secondary sheath form the medulla in the lower part and the palisade of paraphyses in the upper part of the young ascocarp.

There is a striking resemblance between the development of cleistohymenial ascomata in *Ascobolus* and its allies and the developmental scheme given by Doguet (*1955*) for perithecia in *Melanospora*.

From the ascogonia ascogenous hyphae grow upward. Their first cells are multinuclear. Soon branches are sent out whose cells are dikaryotic. On reaching the thin layer of small plasm-rich cells at the base of the paraphyses these branches spread centrifugally, with frequent sympodial ramification. Their cell-divisions are accompanied by conjugate nuclear divisions. The croziers, terminally formed on this sympodial system, grow up from between the bases of the paraphyses and give rise to the asci. The incipience, development, and ripening of the asci proceeds in a centrifugal direction; the oldest being in the centre of the hymenium.

Further development of the ascocarp is mainly brought about by intercalation of new elements and subsequent strong inflation of asci. Only in species of *Ascobolus* sect. *Gymnascobolus* is there a submarginal secondary growing zone (cf. Corner, *1929a*).

The types of development in Pezizales are distinguished by the developmental phase in which the hymenium becomes exposed. In an earlier study (van Brummelen, *1967*) two sets of descriptive terms were introduced.

During ascocarp ontogeny five phases were distinguished in respect to the ripening of the hymenium. These chronological phases were named (1) a r c h i h y m e n i a l phase: before the initials of the hymenium are present; (2) p r o h y m e n i a l phase: paraphyses are present but no croziers are as yet formed; (3) m e s o h y m e n i a l phase: the hymenium is in progress of ripening, but no asci have as yet ripened; (4) t e l o h y m e n i a l phase: mature asci are present and normally ascospores are discharged; and (5) p o s t h y m e n i a l phase: the hymenium becomes overripe or obsolate and decomposes.

With regard to the hymenial development two main types of ascomata were distinguished. (I) C l e i s t o h y m e n i a l ascomata in which the hymenium is enclosed, at least during its early development. Ascomata of this type may be further subdivided according to the hymenial phase when they open to expose the hymenium. (II) G y m n o h y m e n i a l ascomata: the hymenium is exposed from the first until the maturation of the asci.

Two subgroups of the latter were recognized: (a) p a r a g y m n o h y m e n i a l ascomata in which the ascogonium is overarched by investing hyphae of limited growth that do not form a continuous sheath and (b) e u g y m n o h y m e n i a l ascomata with a fully exposed ascogonium.

By the use of these unambiguous terms a more detailed differentiation in developmental types is possible than before. Of the seven types recognized (van Brummelen *1967*: *pl. 17*) no less than six occur within the genera *Ascobolus* and *Saccobolus*.

## Classification

In my opinion the genera *Ascobolus* and *Saccobolus* form a very homogeneous and natural group of operculate Discomycetes, which can be given the rank of family (cf. Rifai, *1968*). Especially the existence of a violet episporial pigment of vacuolar origin is a unique character which unites these fungi. Between the Ascobolaceae and other coprophilous Pezizales with protruding asci the relations are more remote and less clear.

For a subdivision of the Ascobolaceae in taxa of lower rank the following structural characters proved to be of importance: the shape of ascocarps, asci and ascospores, the cortical texture, the ascospore-arrangement, and the reaction of the ascus-wall with iodine.

If the taxa of the Ascobolaceae are classified according to these structural criteria only, the resulting groups of species each show the same type of ascocarp ontogeny.

Exactly the same groups will be arrived at if the species are concatenated in series of over-all similarities (classification 'par enchaînement').

By classifying the Ascobolaceae according to the ontogeny of the ascocarp, supplemented with some of the structural criteria mentioned, a very useful classification is obtained which reflects empirical relations.

The genus *Ascobolus* is distinguished by the mutually free ascospores, which are not arranged in a regular package during any phase of maturation. In *Saccobolus* the ascospores are firmly or loosely united into a cluster according to a more or less regular pattern of arrangement.

In *Ascobolus* seven sections were recognized (van Brummelen, *1967*: 63): section *Dasyobolus* with cleistohymenial ascomata that do not open before the telohymenial phase; section *Sphaeridiobolus* with cleistohymenial ascomata opening in the late mesohymenial phase and globular ascospores with rounded warts; section *Ascobolus* with cleistohymenial ascomata opening in the early or mid-mesohymenial phase; section *Pseudascodesmis* with paragymnohymenial ascomata, the habit of *Ascodesmis*, and the ascus-wall not staining blue with iodine; section *Pseudosaccobolus* with para- or eu-gymnohymenial ascomata, the habit of *Saccobolus* and the ascus-wall staining deep blue with iodine; section *Heimerlia* with small eugymnohymenial ascomata and the cortex scarcely developed; and section *Gymnascobolus* with eugymnohymenial ascomata with an active submarginal growing zone.

*Saccobolus* was divided into two sections, section *Saccobolus* and section *Eriobolus*, both with paragymnohymenial or more rarely eugymnohymenial ascomata. These two sections, although slightly differing in ascocarp ontogeny, are distinguished on the basis of structural characters, such as pigmentation and ascospore-arrangement.

A tentative scheme of interrelationships between the sections of *Ascobolus* and

*Saccobolus* is given in the diagram on page 390. In this scheme the sections are arranged from the top downwards according to their ascocarp ontogeny from fully closed to fully open. In the same sequence there is a decreasing growth-activity of the hyphae of the primary sheath, especially in the archihymenial phase.

## CONCLUSION

Ascocarp ontogeny has proved to be a very useful basis for the classification of the Ascobolaceae. It may also help in recognizing series of supra-specific taxa within other natural families of Discomycetes. The Thelebolaceae and the Pyronemataceae in particular are promising objects in this respect.

It would be of great importance to the taxonomy of Discomycetes to include in principle the stages of ontogenetic development in the comparative study of characters.

In the past extreme types of ontogenetic development (*Ascobolus immersus*, *Thelebolus stercoreus*, and *Ascodesmis*) have been the starting-point of far-reaching speculations on relationships within the Ascomycetes.

We must always be on guard, especially in ontogenetic and morphological studies, not to base generalizations on the study of a single or only a few taxa.

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