Edited by
Karl Esser

# THE MYCOTA

A Comprehensive Treatise on Fungi
as Experimental Systems for Basic and Applied Research

## Physiology and Genetics

Selected Basic and Applied Aspects

## XV

Second Edition

Timm Anke
Anja Schüffler
*Volume Editors*

Springer

# The Mycota

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K. Esser

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# The Mycota

A Comprehensive Treatise on Fungi as Experimental Systems for Basic and Applied Research

Edited by K. Esser

XV

*Physiology and Genetics*
Selected Basic and Applied Aspects
2nd Edition

Volume Editors:
Timm Anke and Anja Schüffler

Springer

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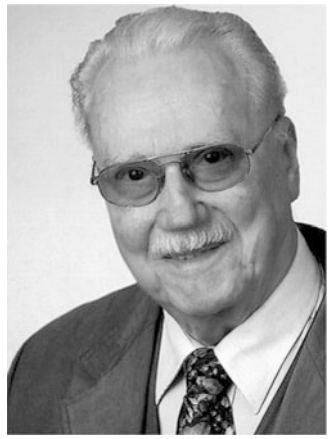

**Karl Esser**
(born 1924) is retired Professor of General Botany and Director of the Botanical Garden at the Ruhr-Universität Bochum (Germany). His scientific work focused on basic research in classical and molecular genetics in relation to practical application. His studies were carried out mostly on fungi. Together with his collaborators he was the first to detect plasmids in higher fungi. This has led to the integration of fungal genetics in biotechnology. His scientific work was distinguished by many national and international honors, especially three honorary doctoral degrees.

**Timm Anke**
(born 1944) studied biochemistry at the University of Tuebingen where he got his PhD with a dissertation on the biosynthesis of fungal siderophores. In 1973, he joined the group of Fritz Lipmann at the Rockefeller University in New York City where he was investigating the biosynthesis of valinomycin, a streptomycete ionophore. After his return to Tuebingen in 1975, he started to build up a group searching for new antibiotics from basidiomycetes within the framework of Hans Zaehners Collaborative Research Center (SFB 76) focusing on the chemistry and biology of microorganisms. In 1981, he became full professor of biotechnology at the University of Kaiserslautern from which he retired in 2010. In addition, he headed the Institute of Biotechnology and Drug Research IBWF e. V. in Kaiserslautern from 1998 to 2010. One of his outstanding achievements in the field of antibiotic research is the discovery of the strobilurins, a major class of agricultural fungicides for which he was awarded the Karl Heinz Beckurts Prize in 1996.

**Anja Schüffler**
(born 1981) studied biology at the University of Kaiserslautern. Her PhD focused on the characterization of fungal natural products with antimicrobial activity. In 2010, she received a DFG fellowship and joined Prof. Fenical's group at the Scripps Institute for Oceanography in San Diego, where she studied antibacterial natural products from streptomycetes. In 2011, she started a research fellowship at the Institute of Biotechnology and Drug Research (IBWF, Kaiserslautern). Her scientific work focuses on bioactive natural products and assay development, the biosynthesis of secondary metabolites as well as the isolation and taxonomy of fungi of diverse habitats.

# Series Preface

Mycology, the study of fungi, originated as a sub discipline of botany and was a descriptive discipline, largely neglected as an experimental science until the early years of this century. A seminal paper by Blakeslee in 1904 provided evidence for self-incompatibility, termed "heterothallism," and stimulated interest in studies related to the control of sexual reproduction in fungi by mating-type specificities. Soon to follow was the demonstration that sexually reproducing fungi exhibit Mendelian inheritance and that it was possible to conduct formal genetic analysis with fungi. The names Burgeff, Kniep and Lindegren are all associated with this early period of fungal genetics research.

These studies and the discovery of penicillin by Fleming, who shared a Nobel Prize in 1945, provided further impetus for experimental research with fungi. Thus, began a period of interest in mutation induction and analysis of mutants for biochemical traits. Such fundamental research, conducted largely with Neurospora crassa, led to the one gene:one enzyme hypothesis and to a second Nobel Prize for fungal research awarded to Beadle and Tatum in 1958. Fundamental research in biochemical genetics was extended to other fungi, especially to *Saccharomyces cerevisiae*, and by the mid-1960s fungal systems were much favored for studies in eukaryotic molecular biology and were soon able to compete with bacterial systems in the molecular arena.

The experimental achievements in research on the genetics and molecular biology of fungi have benefited more generally studies in the related fields of fungal biochemistry, plant pathology, medical mycology, and systematics. Today, there is much interest in the genetic manipulation of fungi for applied research. This current interest in biotechnical genetics has been augmented by the development of DNA-mediated transformation systems in fungi and by an understanding of gene expression and regulation at the molecular level. Applied research initiatives involving fungi extend broadly to areas of interest not only to industry but to agricultural and environmental sciences as well.

It is this burgeoning interest in fungi as experimental systems for applied as well as basic research that has prompted publication of this series of books under the title The Mycota. This title knowingly relegates fungi into a separate realm, distinct from that of either plants, animals, or protozoa. For consistency throughout this series of volumes, the names adopted for major groups of fungi (representative genera in parentheses) are as follows:

*Pseudomycota*

| | |
|---|---|
| Division: | Oomycota (*Achlya, Phytophthora, Pythium*) |
| Division: | Hyphochytriomycota |

*Eumycota*

| | |
|---|---|
| Division: | Chytridiomycota (*Allomyces*) |
| Division: | Zygomycota (*Mucor, Phycomyces, Blakeslea*) |
| Division: | Dikaryomycota |
| Subdivision: | Ascomycotina |
| Class: | Saccharomycetes (*Saccharomyces, Schizosaccharomyces*) |
| Class: | Ascomycetes (*Neurospora, Podospora, Aspergillus*) |
| Subdivision: | Basidiomycotina |
| Class: | Heterobasidiomycetes (*Ustilago, Tremella*) |
| Class: | Homobasidiomycetes (*Schizophyllum, Coprinus*) |

We have made the decision to exclude from The Mycota the slime molds which, although they have traditional and strong ties to mycology, truly represent nonfungal forms insofar as they ingest nutrients by phagocytosis, lack a cell wall during the assimilative phase, and clearly show affinities with certain protozoan taxa.

The series throughout will address three basic questions: what are the fungi, what do they do, and what is their relevance to human affairs? Such a focused and comprehensive treatment of the fungi is long overdue in the opinion of the editors.

A volume devoted to systematics would ordinarily have been the first to appear in this series. However, the scope of such a volume, coupled with the need to give serious and sustained consideration to any reclassification of major fungal groups, has delayed early publication. We wish, however, to provide a preamble on the nature of fungi, to acquaint readers who are unfamiliar with fungi with certain characteristics that are representative of these organisms and which make them attractive subjects for experimentation.

The fungi represent a heterogeneous assemblage of eukaryotic microorganisms. Fungal metabolism is characteristically heterotrophic or assimilative for organic carbon and some nonelemental source of nitrogen. Fungal cells characteristically imbibe or absorb, rather than ingest, nutrients and they have rigid cell walls. The vast majority of fungi are haploid organisms reproducing either sexually or asexually through spores. The spore forms and details on their method of production have been used to delineate most fungal taxa. Although there is a multitude of spore forms, fungal spores are basically only of two types: (1) asexual spores are formed following mitosis (mitospores) and culminate vegetative growth, and (2) sexual spores are formed following meiosis (meiospores) and are borne in or upon specialized generative structures, the latter frequently clustered in a fruit body. The vegetative forms of fungi are either unicellular, yeasts are an example, or hyphal; the latter may be branched to form an extensive mycelium.

Regardless of these details, it is the accessibility of spores, especially the direct recovery of meiospores coupled with extended vegetative haploidy, that have made fungi especially attractive as objects for experimental research.

The ability of fungi, especially the saprobic fungi, to absorb and grow on rather simple and defined substrates and to convert these substances, not only into essential metabolites but into important secondary metabolites, is also noteworthy. The metabolic capacities of fungi have attracted much interest in natural products

chemistry and in the production of antibiotics and other bioactive compounds. Fungi, especially yeasts, are important in fermentation processes. Other fungi are important in the production of enzymes, citric acid, and other organic compounds as well as in the fermentation of foods.

Fungi have invaded every conceivable ecological niche. Saprobic forms abound, especially in the decay of organic debris. Pathogenic forms exist with both plant and animal hosts. Fungi even grow on other fungi. They are found in aquatic as well as soil environments, and their spores may pollute the air. Some are edible; others are poisonous. Many are variously associated with plants as copartners in the formation of lichens and mycorrhizae, as symbiotic endophytes or as overt pathogens. Association with animal systems varies; examples include the predaceous fungi that trap nematodes, the microfungi that grow in the anaerobic environment of the rumen, the many insect associated fungi, and the medically important pathogens afflicting humans. Yes, fungi are ubiquitous and important. There are many fungi, conservative estimates are in the order of 100,000 species, and there are many ways to study them, from descriptive accounts of organisms found in nature to laboratory experimentation at the cellular and molecular level. All such studies expand our knowledge of fungi and of fungal processes and improve our ability to utilize and to control fungi for the benefit of humankind.

We have invited leading research specialists in the field of mycology to contribute to this series. We are especially indebted and grateful for the initiative and leadership shown by the Volume Editors in selecting topics and assembling the experts. We have all been a bit ambitious in producing these volumes on a timely basis and therein lies the possibility of mistakes and oversights in this first edition. We encourage the readership to draw our attention to any error, omission, or inconsistency in this series in order that improvements can be made in any subsequent edition.

Finally, we wish to acknowledge the willingness of Springer-Verlag to host this project, which is envisioned to require more than 5 years of effort and the publication of at least nine volumes.

Bochum, Germany — Karl Esser
Auburn, AL, USA — Paul A. Lemke
April 1994 — *Series Editors*

# Volume Preface

More than 120,000 different fungal species have been described, and it is estimated that there exist more than $1.5 \times 10^6$ species. Fungi have adopted many different ways of living in very diverse habitats as saprophytes, pathogens, symbionts or endophytes. Fungi and their products are used for the fermentation and processing of food and feeds, for biological control and for the production of vitamins and amino acids. Some of their secondary metabolites are used in medicine, e.g. as antibiotics, immunosuppressants, cholesterol-lowering drugs or agrochemical fungicides. Recently, progress in the field of mycology has been substantial due to new methodological approaches and technologies, many of them DNA-based, strongly adding to the motivation to compile a new volume of *Mycota XV Physiology and Genetics: Selected Basic and Applied Aspects*.

Chapter "Fruiting Body Development in Ascomycetes" provides a general overview about the morphology and development of fruiting bodies in ascomycetes with discussion of regulatory networks such as signal transduction pathways, protein degradation mechanisms as well as transcriptional regulators and chromatin modifiers. Chapter "Fungal Inteins: Distribution, Evolution and Applications" summarizes the current knowledge of inteins, their occurrence, evolution and application. Inteins are internal protein sequences which are excised from a precursor protein (extein) whose N- and C-termini are subsequently ligated to yield two stable proteins, the mature protein and the intein. In the interaction of yeasts, killer toxins play an important role. Their structures, modes of action and resistance as well as possible applications are discussed in chapter "Yeast Killer Toxins: Fundamentals and Applications". Chapter "The Fungal MCC/Eisosome Complex: An Unfolding Story" deals with the fungal MCC/eisosome complex which plays an important role in plasma membrane organization and diverse plasma membrane-associated processes. In chapter "The Genus Periglandula and Its Symbiotum with Morning Glory Plants (Convolvulaceae)", the enigma of why ergot alkaloids are equally present in fungi (*Clavicipitaceae*) and higher plants (Convolvulaceae) is addressed and solved. Chapter "Volatiles in Communication of Agaricomycetes" presents a comprehensive survey on communication activities of *Agaricomycetes* on all organismal scales and community levels in which signalling by fungal volatile organic compounds (VOCs) is recognized. The substantial progress in elucidating the lifestyle, metabolism and genetics of endophytic fungi is addressed in the chapter "Endophytic Fungi, Occurrence, and Metabolites". Basidiomycetes are a rich source of unique secondary metabolites in most cases not found in other fungi. The chapter "Secondary Metabolites of Basidiomycetes" offers a survey of new compounds isolated since 2008. Chapter "Identification of Fungicide Targets in Pathogenic Fungi"

covers the current molecular targets of antifungal compounds and discusses future directions of fungicide research. Helminths can pose serious problems to animal and human health. It is therefore quite remarkable that fungi produce low molecular weight compounds specifically interfering with reactions not present in mammalian hosts, thus paving the way for selective nontoxic medications or agrochemicals. For the discovery of avermectin, successfully used in the therapy of roundworm parasites, Prof. Satoshi Omura, author of chapter "Helminth Electron Transport Inhibitors Produced by Fungi", was awarded the Nobel Prize in Physiology or Medicine in 2015. Chapter "Cyclic Peptides and Depsipeptides from Fungi" describes the occurrence, structures and biological activities of peptides and depsipeptides produced by fungi and discusses the importance of these compounds as lead compounds for agricultural and pharmaceutical applications.

In chapter "Polyketide Synthase–Nonribosomal Peptide Synthetase Hybrid Enzymes of Fungi", recent efforts in engineering-selected fungal species to make them amenable to efficient genetic modifications for identifying genes responsible for the biosynthesis of secondary metabolites are addressed. This review also discusses how the engineered fungi are used in deciphering the mechanism of natural product biosynthesis, primarily through heterologous reconstitution of biosynthetic pathways of interest. Fungal polyketides are among the prominent fungal metabolites. As addressed in chapter "Biosynthesis of Fungal Polyketides", their biosynthesis is increasingly well understood at chemical, biochemical and genetic levels, thus offering a chance to obtain sufficient quantities of complex but potentially valuable therapeutics. The mycotoxins ochratoxin, citrinin and patulin are often found as contaminants of foods. New results concerning the regulation and the simultaneous occurrence of ochratoxin, citrinin and patulin producing penicillia in certain habitats are presented in chapter "Aspects of the Occurrence, Genetics and Regulation of Biosynthesis of the Three Food Relevant Penicillium Mycotoxins: Ochratoxin A, Citrinin and Patulin".

We do hope that readers enjoy reading this volume of *The Mycota*. We are very grateful to the contributing authors, whose expertise and efforts have made this project possible. We thank Dr. Andrea Schlitzberger of Springer Verlag for her support and engagement during the preparation of this volume.

Kaiserslautern and Mannheim, Germany — Timm Anke
October 2017 — Anja Schüffler

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# Fruiting-Body Development in Ascomycetes

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CONTENTS



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## I. Introduction

Fruiting bodies are multicellular structures, which protect the products of meiosis, the sexual spores. They occur during the sexual life cycle of the *Dikarya*, a group that encompasses the ascomycetes and basidiomycetes (Hibbett et al. 2007; Peraza-Reyes and Malagnac 2016) (Fig. 1). However, only filamentous species show the development of fruiting bodies, while yeasts never exhibit comparable structures.

In this chapter, which is an extension and update of a previous review in this series (Pöggeler et al. 2006b), we will give an overview of the development of fruiting bodies in ascomycetes, including an outline of some model ascomycetes, which have been used to study fruiting-body development at the molecular level. Further, we will summarize factors that can either be environmental or endogenous, which control this process. Finally, regulatory networks will be mentioned that govern fruiting-body development. This includes signal transduction pathways, protein degradation mechanisms, and transcriptional regulatory networks. Ultimately, we observe that novel experimental approaches such as quantitative mass spectrometry, functional genomics, or super resolution microscopy have begun to improve our knowledge about the mechanistic

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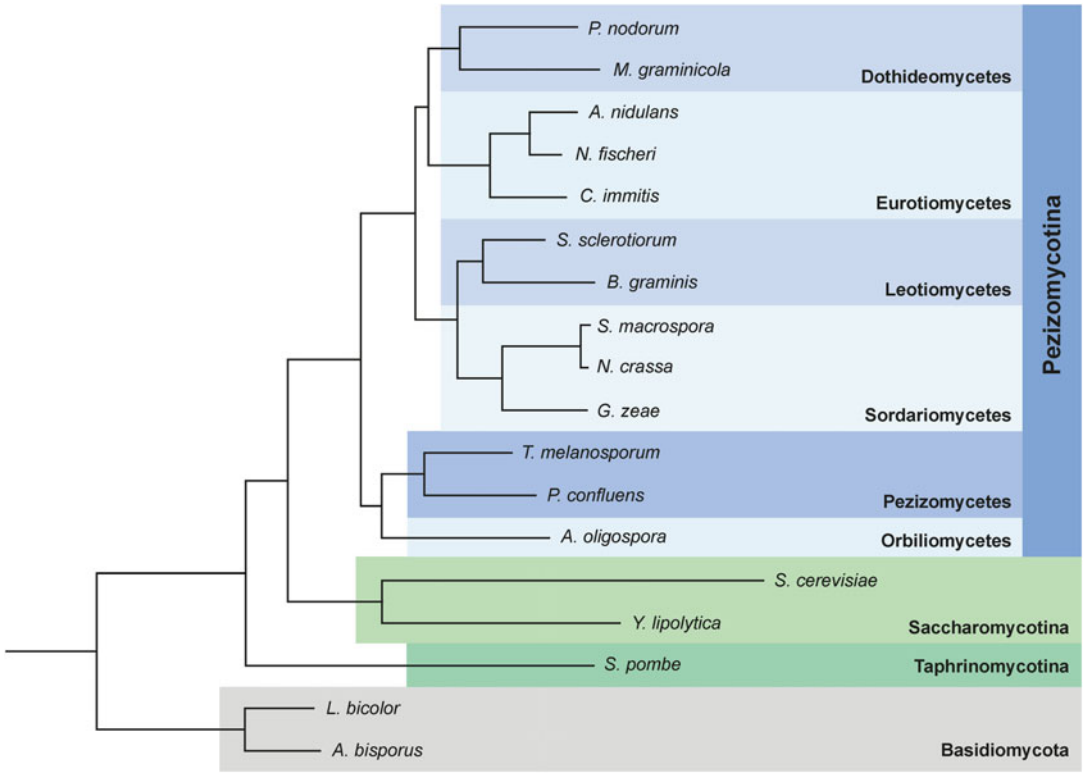


**Fig. 1** Phylogenetic tree of *Ascomycota*. Characteristic species are given as examples. Branch lengths are proportional to genetic distances [adapted from Traeger et al. (2013)]. Species used to construct the phylogenetic tree: *Agaricus bisporus*, *Arthrobotrys oligospora*, *Blumeria graminis*, *Coccidioides immitis*, *Aspergillus nidulans*, *Gibberella zeae*, *Laccaria bicolor*, *Mycosphaerella graminicola*, *Neosartorya fischeri*, *Neurospora crassa*, *Phaeosphaeria nodorum*, *Pyronema confluens*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Sclerotinia sclerotiorum*, *Sordaria macrospora*, *Tuber melanosporum*, *Yarrowia lipolytica*. *S. pombe*, *L. bicolor*, and *A. bisporus* served as outgroups

processes that lead to the formation of multicellular structures.

## A. Fungal Sexual Development

Fungi propagate either asexually or sexually. Asexual propagation is characterized by mitotic divisions, and as a result, endospores within sporangia or exospores like conidia are generated. In contrast, sexual propagation is characterized by karyogamy and meiotic divisions, and fungi share this feature with most other eukaryotes. Generally, sexual reproduction is thought to be the source of genetic diversity. During meiotic divisions, recombination occurs between chromosomes of two heterogenetic mating partners (Peraza-Reyes and Malagnac 2016). As a result of meiotic divisions, fungi produce four haploid spores, which may be doubled or multiplied by one or several postmeiotic mitoses.

The ascus is the meiosporangium of the Ascomycota. These sac-like sporangia carry the ascospores, the products of meiosis. In mycelial ascomycetes, asci are usually formed inside developmentally complex fruiting bodies that are called the ascomata or ascocarps. In contrast to filamentous ascomycetes, ascospores of unicellular ascomycetes (yeasts) are never found in fruiting bodies. The development of fruiting bodies is a rather complex cellular process that requires special environmental and genetic conditions, which control

the expression of developmentally regulated genes. Fruiting bodies are highly complex structures, which contain several different tissues protecting the asci. For example, 15 different cell types were recognized in fruiting bodies of the Sordariomycete *Neurospora crassa* (Bistis et al. 2003). For a coordinated fruiting-body development, enzymes involved in cell wall biogenesis and metabolism are required, as well as genes responsible for the cytoskeleton structure and organization. Here we will mention some representative examples, and a more detailed description on this subject can be found in our previous review (Pöggeler et al. 2006a). The *ami1* gene from *Podospora anserina*, for example, is necessary for nuclear positioning, most likely by regulating components of the dynein pathway. This gene was shown to be responsible for male fertility, and deletion results in a delayed formation of fruiting bodies in the corresponding mutants (Bouhouche et al. 2004). The outer shell of the fruiting body, the peridium, is an essential structure to protect the meiosporangia with the ascospores. The peridium consists of bundles of filamentous cells, and their cell walls have three main constituents, namely, chitin, mannan, and β-glucan. Though the related biosynthetic pathways have intensively been investigated, it has not been demonstrated with certainty that the corresponding genes are preferentially expressed in fruiting-body tissues. For *Sordaria macrospora* functional analysis of the class VII (division III) chitin synthase gene (*chs7*) has shown that it is dispensable for fruiting-body formation, but the corresponding mutant displayed sensitivity toward cell wall stress (Traeger and Nowrousian 2015). Another result comes from *Tuber borchii*, where three genes for chitin synthesis were investigated. Albeit they are constitutively expressed in vegetative mycelium, they show a differential expression in sporogenic or vegetative tissue of the fruiting bodies (Balestrini et al. 2000). In contrast, several chs mutants from *N. crassa* and *Aspergillus nidulans* show severe defects in perithecial development (Fajardo-Somera et al. 2015).

Important pigments of the cell walls are melanins. They are synthesized either through the DHN (1,8-dihydroxynaphthalene) or the DOPA (L-3,4-dihydroxyphenylalanine) pathways. Some can also be generated by the L-tyrosine degradation pathway (Langfelder et al. 2003). One of the best-characterized melanin biosynthetic pathways is the DHN melanin pathway, which has been verified for many members of the Pezizomycotina. Melanins stabilize the cell wall and provide protection against UV light-induced DNA damage. An investigation with *S. macrospora* showed that expression of melanin biosynthesis genes is correlated with fruiting-body development. For example, melanin gene expression is highly repressed in submerged cultures, where no sexual development occurs. Similarly, sterile mutants of *S. macrospora* showed only reduced transcript levels of melanin biosynthesis genes (Engh et al. 2007). Finally, mutants with a defect in melanin biosynthesis from *Ophiostoma piliferum* and *Podospora anserina* showed defects in the formation of fruiting bodies. These observations are consistent with early reports for *N. crassa*, *P. anserina*, and *Tuber* species, where correlation between melanin biosynthesis and the reproductive cycle was suggested (Hirsch 1954; Esser 1966; Prade et al. 1984; Ragnelli et al. 1992; Teichert and Nowrousian 2011).

Very important proteins of the cell wall are the hydrophobins and lectins. Although they have mainly been characterized in higher basidiomycetes, where they are implied in mushroom formation, they have also a function in the Pezizomycotina. Cryparin, a class II hydrophobin, was found mainly in the cell walls of fruiting bodies from the chestnut blight pathogen, *Cryphonectria parasitica*. Deletion mutants lacking the cryparin gene were unable to generate wild-type-like fruiting bodies. Thus, this pathogen needs hydrophobins for its fitness under natural conditions (Kazmierczak et al. 2005).

On the genetic level, there are many genes regulating the sexual cycle of ascomycetes. Important master genes involved in the general control of sexual development are part of the mating-type loci. They have been found so far in all ascomycetes, irrespective of whether they produce fruiting bodies or not. Their regulatory role during the sexual cycle has been thoroughly

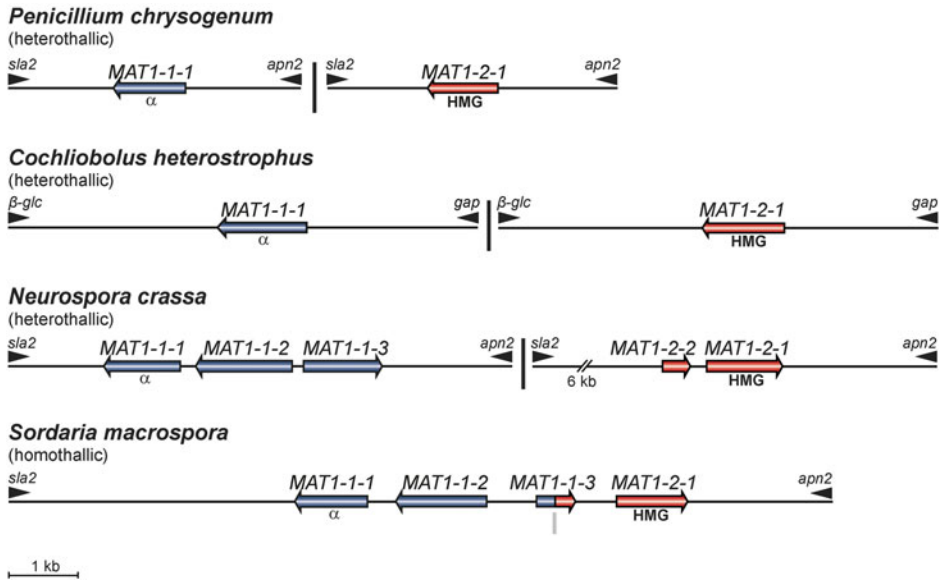


**Fig. 2** Examples of mating-type loci of heterothallic members of the Dothideomycetes (*Cochliobolus heterostrophus*) (Wirsel et al. 1998), Eurotiomycetes (*Penicillium chrysogenum*) (Böhm et al. 2013, 2015), and Sordariomycetes (*N. crassa*). For comparison the mating-type locus encoding four open reading frames from the homothallic fungus *S. macrospora* (Sordariomycetes) is shown. The flanking regions often carry conserved genes, such as *sla2* (cytoskeleton assembly control factor) and *apn2* (DNA lyase). An exception is the MAT locus from *C. heterostrophus* with the following flanking genes: GAP, GTPase-activating protein; ß-Glc, ß-glucosidase. Abbreviations: "α" and "HMG" indicate genes encoding transcription factors with conserved DNA-binding domains

studied in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, which produce asci, but no fruiting bodies. The role of mating-type genes during fruiting-body development is by far less well understood, although some studies have shown that mating-type genes are directly involved in fruiting-body development (Nelson and Metzenberg 1992; Pöggeler et al. 1997) (see also Sect. IV.C.1). In general, two types of fungal breeding systems are distinguished. Heterothallism involves two individuals with opposing mating types, while homothallism refers to sexual reproduction by selfing. In the latter case, individual strains do not need a mating partner to propagate sexually. Pseudohomothallism finally can be considered to be an exceptional type of heterothallism. The term was used for species that contain asci with four ascospores, each carrying two nuclei with opposite mating-type genes. Thus, after germination, these resulting heterokaryotic mycelia can undergo selfing. This type of breeding system is found, e.g., in *P. anserina* or *Neurospora tetrasperma*.

Usually the mating-type loci of heterothallic species contain dissimilar sequences, albeit they are located at identical chromosomal positions. Thus, mating-type loci do not represent alleles of a given gene but rather dissimilar DNA sequences which are called idiomorphs. MAT loci from the Pezizomycotina carry one or more open reading frames of which at least one codes for a mating-type transcription factor (TF). In general, the MAT1-1 locus of heterothallic species contains one to three open reading frames, while only a single gene is found in MAT1-2 loci. In contrast to baker's yeast, species of the Pezizomycotina carry no silent mating-type loci. Thus, mating-type switching as observed in yeast does usually not occur in heterothallic filamentous ascomycetes.

Mating-type loci encode TFs that are directly involved in the sexual life cycle. Figure 2 displays the general structure of mating-type

loci from members of the Eurotiomycetes, the *Dothideomycetes*, and the *Sordariomycetes*. The *MAT1-1-1* gene encodes a TF that is characterized by an α DNA-binding domain, while the *MAT1-2-1* gene codes for TFs with a high-mobility group (HMG) DNA-binding domain. A detailed description of mating-type locus-encoded TFs is given in Sect. IV.C.1.

### B. Fruiting-Body Morphology

During their sexual life cycle, filamentous fungi of subdivision Pezizomycotina generate fruiting bodies that were historically used for their taxonomic classification. Current classification systems that rely on molecular data show that these conventional classifications contain non-monophyletic groups (Schoch et al. 2009; Ebersberger et al. 2012). However, different fruiting-body morphologies are important traits in fungal ecology, and the foremost common types of fruiting bodies (Esser 1982) are described below (Figs. 3 and 4).

1. **Cleistothecia** are closed, spherical fruiting bodies that distribute the ascospores after disintegration of the peridium of the fruiting bodies. Typically, members of the Eurotiomycetes such as *Aspergillus fumigatus*, *Aspergillus nidulans*, and *Penicillium chrysogenum* generate cleistothecia.
2. **Pseudothecia** are spherical fruiting bodies that contain cavities (loculi) that contain the gametangia. Spores are actively discharged through openings which arise from local lysis of the peridium. Pseudothecia are, for example, found in the Dothideomycetes, e.g., *Venturia inaequalis*.
3. **Perithecia** are closed flask-like fruiting bodies that look similar to the pseudothecia. Within perithecia, sterile hyphae are found that enclose the generative tissue (hymenium). The hymenium generates asci with usually eight ascospores, which are actively discharged from the perithecium through a preformed opening, the ostiole. Perithecia are typical fruiting bodies of members of the Sordariomycetes, such as *N. crassa*, *P. anserina*, and *S. macrospora*.
4. **Apothecia** are open to cup-shaped fruiting bodies that have a hymenium layer on their surface carrying the asci. The spores are actively discharged, and examples of species that have apothecia are *Ascobolus immersus*, *Pyronema confluens*, and *Morchella* sp. within the Pezizomycetes and *Botrytis cinerea* within the Leotiomycetes.

## II. Systems to Study Fruiting-Body Development

Fruiting-body development has been studied in a wide range of different ascomycetous species. Here we describe four model systems, which were used intensively for investigations on fruiting-body development. Further, we will mention some emerging model ascomycetes that were used recently for studying specific aspects of the sexual life cycle, including fruiting-body formation.

### A. *Neurospora crassa*

The model fungus *Neurospora crassa* is a heterothallic species of the *Sordariaceae* and has a rather complex sexual life cycle. In general, two mating types can be distinguished, which are called “A” (MAT1-1) and “a” (MAT1-2). Both strains generate macro- and microconidia, which can be considered as male gametangia. In addition, both strains form female gametangia that are called ascogonia. Female gametangia are surrounded by supporting hyphae, which after 2–3 days generate a protoperithecium (young fruiting body). During fertilization, the female gametangium generates a distinct uptake hypha called trichogyne. This trichogyne will fuse with male gametes, which can be macro- or microconidia as described above. Alternatively, a fusion with vegetative hyphae has also been described. However, self-fertilization of each strain is prevented by an incompatibility mechanism. Thus, trichogynes can only fuse with male gametes from an opposite mating-type partner. In summary, the protoperithecium with a trichogyne from

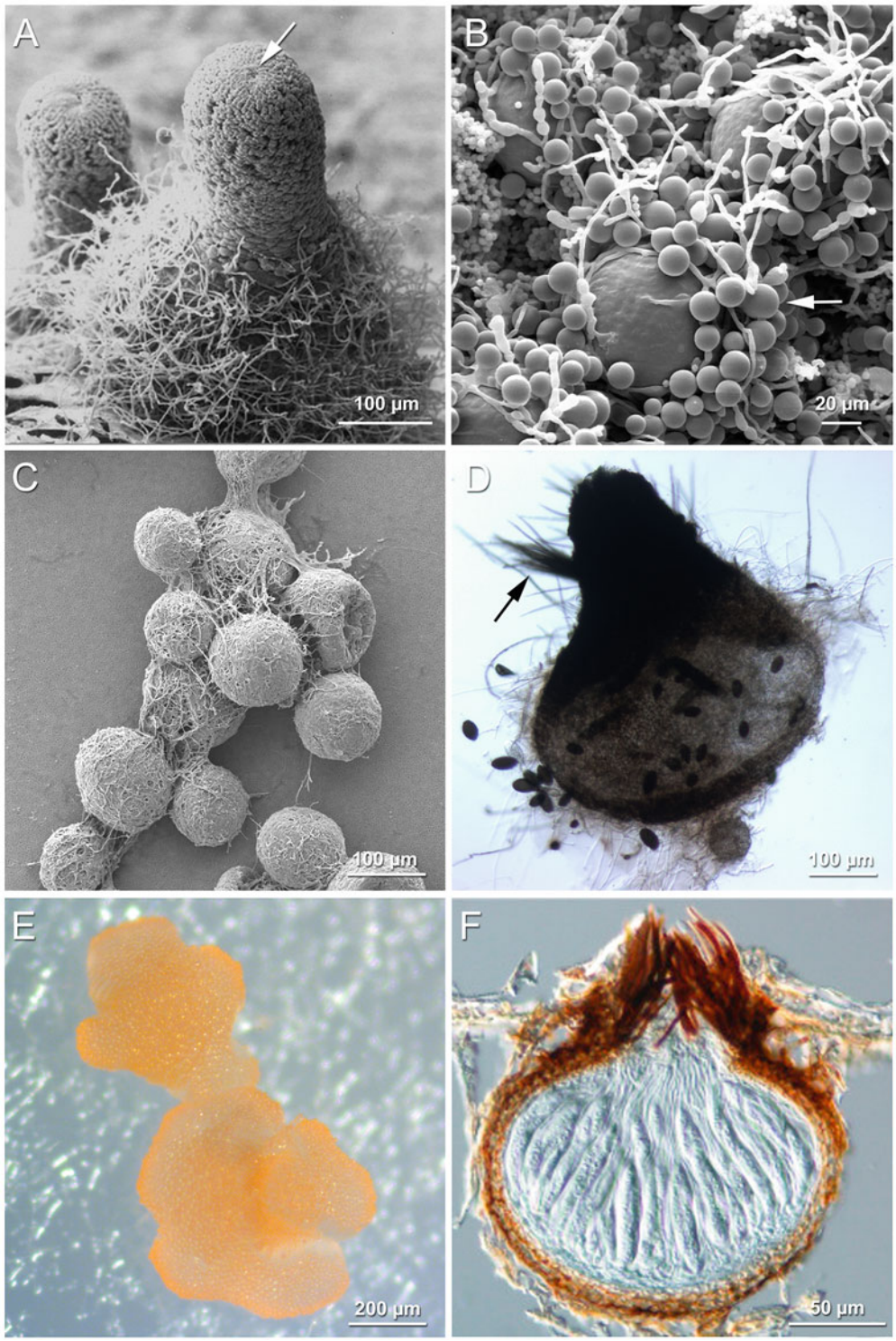


**Fig. 3** Typical fruiting bodies of ascomycetes. (**a**) Perithecia of the homothallic Sordariomycete *S. macrospora*; the arrow points to the ostiolum that is used to discharge the eight-spored asci. (**b**) Cleistothecia from

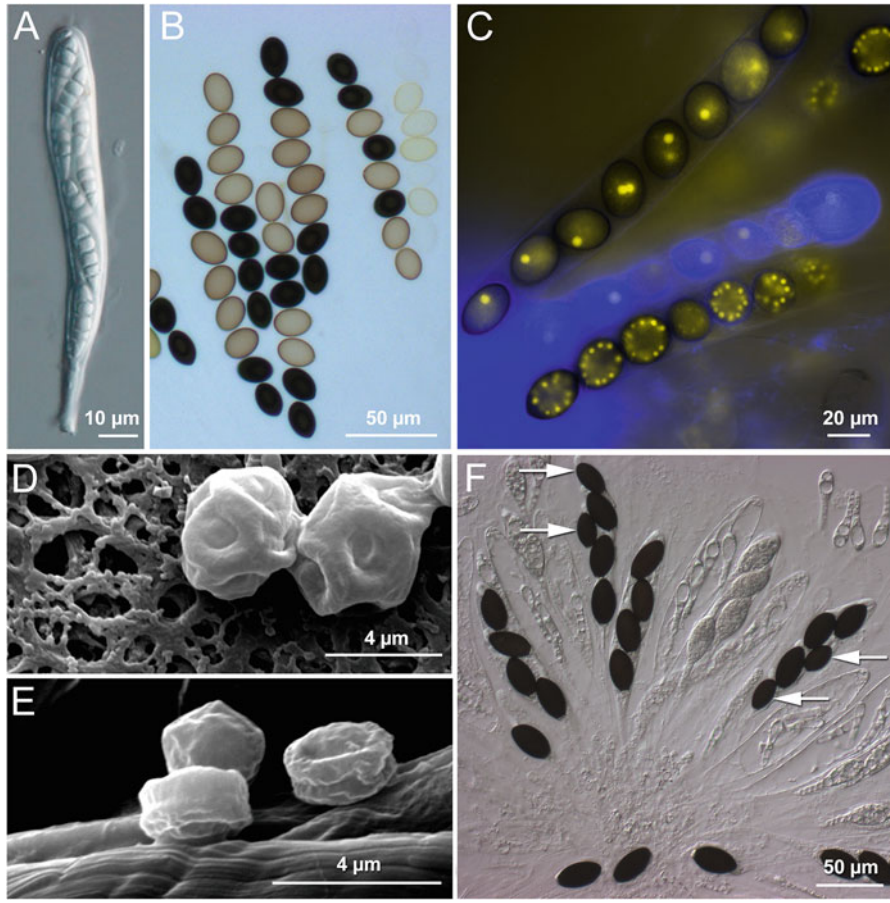


**Fig. 4** Asci from different fungi of the Pezizomycotina. (**a**) Ascus from the Dothideomycete *Keissleriella quadriseptata* [from Tanaka et al. (2015)]. (**b**) Asci from *Sordaria macrospora*, obtained from a cross between a wild type (black spores) and a spore color mutant (lu with yellow spores). (**c**) Fluorescence microscopy of *S. macrospora* asci and ascospores. YFP-tagged histones label nuclei. In the upper ascus, clearly one to two nuclei are visible in each ascospore, while the lower one shows ascospores with several nuclei, which appear after several mitotic divisions. (**d**) Asci from the fungus *E. crustaceum*. (**e**) Ascospores from *E. crustaceum*. (**f**) Asci from *Podospora anserina* contain four spores or five spores. Usually, each ascospore carries two nuclei; however, in rare cases, asci contain smaller spores with only a single nucleus. Arrows indicate small spores with only a single nucleus compared to the regular ascospores with two nuclei

an "A" strain can only be fertilized by a nucleus of an "a" strain and vice versa. The fusion of the male gamete with the trichogyne will lead to the induction of the dikaryotic phase. During this phase, two genetically different nuclei exist within one cell. After several conjugated divisions, ascus development will start with the formation of ascogenous hyphae, which generate the so-called crozier cell, which undergoes conjugated divisions resulting in three cells,

**Fig. 3** (continued) A. nidulans with small surrounding Hülle cells. (**c**) Cleistothecia from the homothallic fungus *Eupenicillium crustaceum*. (**d**) Perithecia from *Podospora anserina* show typical hairs (arrow) at the neck of the perithecia. (**e**) Apothecia from *P. confluens*. (**f**) Pseudothecium from the Dothideomycete *Keissleriella quadriseptata* [from Tanaka et al. (2015)]. (**a**), (**b**), and (**c**) are scanning electron micrographs and (**d**)–(**f**) light microscopy; (**b**) courtesy of G. Braus (Göttingen, Germany); (**e**) from Traeger et al. (2013); (**f**) copyright from Elsevier Press

two basal and one upper cell. The two nuclei in the upper dikaryotic cell undergo karyogamy followed by meiosis (Peraza-Reyes and Malagnac 2016). In *N. crassa*, a postmeiotic mitosis follows before spore formation starts. Thus, each ascus contains eight linearly ordered ascospores. After maturation, perithecia have a size of about 300 μm, while ascospores have a size between 15 and 30 μm. Importantly, ascospore germination occurs only after a heat shock. Fruiting-body formation in *N. crassa* was investigated in diverse genetic, biochemical, and molecular studies (Davis 1995).

### B. *Podospora anserina*

*P. anserina* is a coprophilic fungus with a pseudohomothallic mating system, which shows similarities to the life cycle of *N. crassa*. The mating-type strains are designated "+" (MAT1-2) and "−" (MAT1-1). However, there are some distinct differences compared to *N. crassa*. As male gametes, microconidia, but no macroconidia, are generated that germinate under specific physiological conditions. Secondly, the asci usually contain only four spores, which are generated as a result of specific nuclear distribution mechanisms. After meiosis and postmeiotic mitosis, spore-wall formation covers two genetically distinct nuclei. Usually one nucleus carries the "+" and the other the "−" mating type. With a frequency of about 3%, five- or six-spored asci are generated. They carry either two or four smaller spores that carry only a single nucleus. These spores can be used to generate haploid mycelial isolates (Scheckhuber and Osiewacz 2008; Peraza-Reyes and Malagnac 2016).

### C. *Sordaria macrospora*

*S. macrospora* is a coprophilic fungus that is taxonomically closely related to the above-described species *N. crassa* and *P. anserina*. The life cycles of all these ascomycetes are very similar, although *S. macrospora* has a homothallic mating system. In contrast to *N. crassa* however, *S. macrospora* does not generate macro- or microconidia, and thus, only the sexual cycle contributes to the propagation of this fungus. The sexual cycle can be completed in the laboratory within 1 week, since ascospores require no heat shock or resting period for germination (Esser and Straub 1958). The sexual cycle starts with the formation of ascogonia. However, so far the molecular mechanisms leading to the formation of the dikaryotic hyphae are not understood. After karyogamy of two nuclei in the abovementioned crozier cells, meiosis will follow to generate the meiotic products as a source for ascospore formation. Similar to ascus formation in *N. crassa*, meiosis is followed by a postmeiotic mitosis. As a result, eight ascospores within a single ascus are derived from a single dikaryotic mother cell.

As mentioned above, sexual reproduction is a source of genetic diversity. Usually strains of opposite mating types from heterothallic species (e.g., *N. crassa* or *P. anserina*) are used for conventional genetic recombination studies. However, it has been shown for many species that recombination can also occur between two strains of a homothallic species. In these cases, the strains are distinguished by at least a single mutation. Homothallic species such as *S. macrospora* and other *Sordaria* species are used for conventional genetic analysis (Teichert et al. 2014a).

### D. *Aspergillus nidulans*

*A. nidulans*, which is like *S. macrospora* a homothallic species, was used extensively to study genetic recombination and fruiting-body formation. The sexual cycle starts with the formation of ascogonia and later dikaryotic hyphae, a process, which is probably very similar to the life cycle of *S. macrospora*. Within cleistothecia, spherical asci are generated containing eight ascospores. These octades are unordered and thus distinguished from the ordered asci of the abovementioned species. In recent years, several factors controlling cleistothecia formation were studied extensively, such as the velvet complex (Bayram and Braus 2012) (see Sect. IV.C).