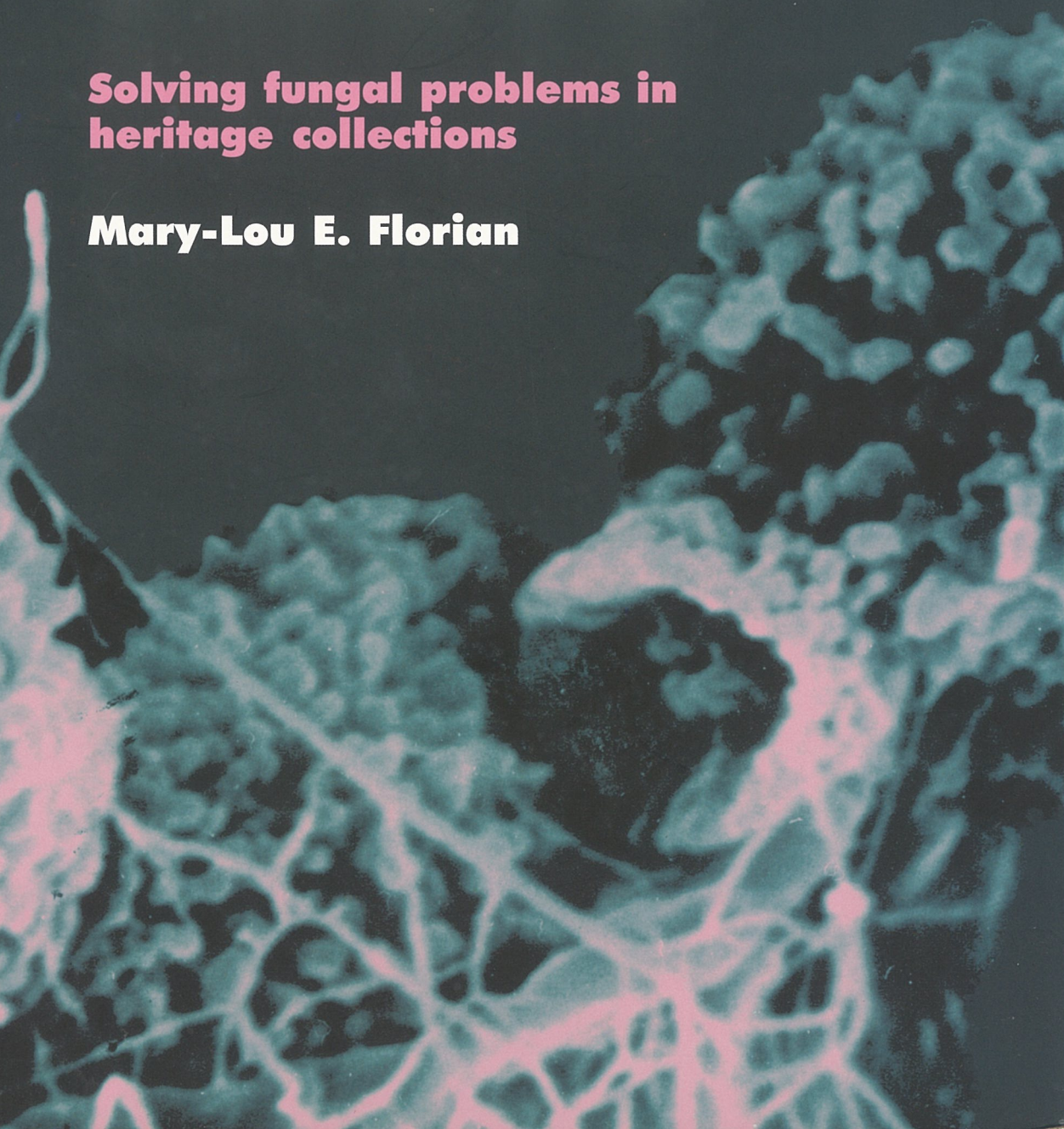
FUNGAL FACTS

Solving fungal problems in heritage collections

Mary-Lou E. Florian



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1 Introduction

1.1 The manual

Each person has their own 'mouldy' experiences. Some may have been involved in major fungal infestations which have occurred because of water damage caused by fires, the weather or plumbing problems; some may have been involved in small infestations of only one specific object; while others may have experiences of anything in between. In all experiences, the overriding concerns when dealing with mouldy materials are safety issues relating to staff health hazards and the preservation of the precious objects. Understanding the relevant aspects of the fungi's biology is the basis for determining logical and safe methods of control, eradication and prevention.

This book is organized to allow you to build logically (by reading the chapters sequentially) a knowledge base that will enable you to solve fungal problems related to heritage objects, regardless of the environment or material, or how small or large the infestation. Health hazards caused by fungal infestations are a major concern and this issue is addressed where relevant throughout the text, and specifically in the final chapter. Questions are raised that need to be answered when assessing and dealing with any fungal problem.

1.2 Chapter 2: What are fungi? Classification and nomenclature

It is necessary to have a vocabulary that describes the fungi involved in infestations related to heritage material. This first chapter explains the scientific naming of fungi and where the species with which we are concerned fit in the kingdom of fungi. It also illustrates and describes the life cycles of typical fungi to enlarge the vocabulary and give us an understanding of which stage we have to deal with.

The identification methods for determining the species are also discussed. Identification of the species takes time and is often determined only after the infestation has been dealt with. When an infestation is encountered, all health precautions have to be in place *before* it is known whether the problem is caused by a hyperallergenic or a toxic species. In the event of a fungal infestation, the steps to take are:

- confine it
- stop its growth
- eradicate it
- prevent it from happening again.

The value of identification after the fact is often just as a document. If the fungal infestation occurs in an environment in which the common known toxic species are suspected, then stringent personal protection is required.

This chapter demonstrates that the common surface fungi belong to a group called the conidial fungi because they reproduce vegetatively by conidia.

1.3 Chapter 3: Where do they come from?

The air carries of all kinds of fungal structures (conidia, hyphal fragments and sexual spores) which land on surfaces. Any material exposed to the air will be contaminated with many different fungi, but only specific species will develop because the moisture and other aspects of the material of the object and the environment have met their growth requirements. Only those that are viable can grow. This chapter describes the origin of these airborne fungal structures and shows how the amounts vary with seasons, geographical location and between indoor and outdoor air. Most importantly, it demonstrates their cosmopolitan nature. Apart from airborne fungal structures, the material of heritage objects may become contaminated during their manufacturing process, e.g. papermaking, or by some treatments, e.g., mouldy starch paste, or from an adjacent mouldy object. Relevant cases are discussed.

1.4 Chapter 4: The culprit, conidium: what does it look like and what does it do?

The main fungal species of relevance to us are the conidial fungi. It is mainly their conidia – incorrectly called spores – which land on materials and develop into a fungal colony that are our concern. Hyphal fragments are also a concern as they can develop into fungal colonies. Conidia are complex physiological structures designed for survival of the species with their own food material and built-in mechanisms to respond to the environment, i.e. they will only grow where they have a chance of survival. In this chapter, the secrets of the conidia are disclosed and their relevance in prevention are explained.

Throughout the book there are questions for which we do not have the answers, such as the longevity of conidia, and more research is needed.

1.5 Chapter 5: Germination and vegetative growth of fungi: why is it happening and can we control it?

What are the environments of storage areas or exhibits, in museums, historic sites, art galleries, archives, etc., that can support fungal growth? Indoor uncontrolled environments may experience extreme seasonal temperature and humidity changes that can initiate fungal growth – in heritage buildings it is recognized by the musty, mouldy odour emanating from leather, paper, rugs, heavy drapes, etc. These materials are very adsorptive and can adsorb sufficient water vapour to support fungal growth. This rarely occurs in controlled environments unless there has been a mechanical breakdown of the air controls or water is present from broken plumbing or a leaky roof. The process of adsorption and its relationship to water vapour (relative humidity) in the air are discussed.

In both uncontrolled and controlled environments, microenvironments can exist which can support fungal activity, the more common being:

- the surfaces of cold walls
- behind bookshelves or other objects that prevent circulation of air
- in a cardboard box on a cement floor
- on windowsills subject to condensation
- rooms in which the floors or walls have been washed.

In these microenvironments of high relative humidity (RH), adsorptive materials of heritage objects can adsorb enough water to allow fungal infestation.

Cold storage units (not freezers) are a potential source of high RH from water deposited on

the floor from condensers or from mechanical breakdown causing a temperature rise – a disaster in the making. If the infestation is not noticed immediately this usually results in fungal growth on everything in the unit including walls, shelves and heritage objects. Before any problems are detected, the air circulation spreads the conidia from the first few colonies, contaminating everything. In time, water will condense on adsorptive and non-adsorptive surfaces, and fungal growth will appear everywhere.

In this chapter, because of the importance of water, all aspects of the water relationships between the environment, the material and the fungus are explained, including hysterical hysteresis!

The environment does not always influence fungal activity. For example, two heritage objects stored side by side, both made of leather – one becomes mouldy but the other does not. Why? The environment is common to both objects therefore it must be differences in their materials. There may be a chemical fungicide present or a chemical which can alter the vapour pressure of the water so that it cannot be utilized by the fungus. This characteristic of water is called its water activity. Apart from water, the other aspects of the environment that influence fungal activity – nutrients, light, temperature and the gases – are also discussed. But the key to prevention and control of fungal growth is water in materials. The bottom line – the limiting aspect of fungal growth – is the amount of water in the materials, not the RH.

1.6 Chapter 6: What's in a fungal infestation and what is its effect on the materials of heritage objects?

So the growth has stopped and the next step is collection recovery. Before you can decide on logical methods of collection recovery you must know what you are removing. We know that there are the fungal structures which can be removed mechanically but some metabolic products are still present. Research is needed to determine the influence of these metabolic products over the longer term on the substrate, paper, textile, etc. For example, the brown fox fur colour in fungal fox spots on old paper does not develop until at least approximately 23 years after the first appearance of fungal growth. The discoloration is mainly the biofilm left by the fungal growth. Should it have been removed 23 years ago?

If a colour is visible, we need to know where it is: is it in the fungal structures, in the material fibres or excreted between the fibres? We also need to know its chemical nature so it can be solubilized and removed. Conservation methods used for collection recovery will be based on the answers to these questions. Unfortunately, there are still more questions than answers. The effects of fungal growth on the material of the heritage object must be understood. Most surface fungi do not digest the substrate; they use only soluble nutrients present because of dust, soiling, treatments or natural deterioration of the materials. Many surface conidial fungi have the enzymatic ability to digest cellulose causing structural loss in the cellulose fibres of plants, in some textiles, paper and wood but this occurs only when in damp environments for a prolonged period of time. The changes in these materials due to these fungi are also reviewed.

1.7 Chapter 7: Collection recovery – removing fungal structures from heritage objects

Now that we know what is there and why, the composition of the material of the heritage object and the effects of infestation, we are ready to remove them and prevent reinfestation. The most important aspect of any activity dealing with fungal infestations is to prevent the contamination of other heritage objects and the environment in which staff work. This requires specific actions to prevent the release of conidia and fungal fragments into the air or heritage objects coming into contact with contaminated cleaning tools, gloves, packing

paper, etc. The procedures are discussed in detail under aseptic techniques and draws on the approaches used in mycological research laboratories.

Often, because of the lack of disaster planning, funds or the overwhelming size of the infestation, time is needed to get procedures in place to deal with the infestation. Thus temporary storage of the fungal-infested material is necessary, ranging from refrigeration to freeze-drying. These are interactive treatments which will influence the materials of the heritage objects – they may encourage fungal activity or, conversely, kill it. All types of temporary storage treatments are discussed in detail.

Although fungicides are discussed, they are not recommended – there are more logical alternatives. The important point to remember is that even dead fungal structures have to be removed. This book only discusses fungal questions – it is up to the professional conservator to decide on the most applicable method of cleaning heritage objects in accordance with conservation standards and ethics. Therefore I offer only a review of reported methods – such as vacuuming, low-oxygen environments, irradiation etc. – to highlight their effects on the fungi and materials being cleaned.

1.8 Chapter 8: Monitoring for air quality and surface contamination in museums and archives: a review

Air monitoring of any facility in which heritage collections are housed should be undertaken seasonally. This provides a baseline to which subsequent analyses can be compared and is similar to insect monitoring – if there is a deviation from the norm there is a problem. Apart from seasonal monitoring, when a major infestation is discovered monitoring should be carried out immediately (and also after the clean-up) to determine if the norm has been reached. As this is a new and developing field the subject is dealt with as a separate chapter.

When an infestation is found, the air should be sampled in order to obtain information about the viable fungi in the air – the airspora. However, this information is of no use for health hazard interpretation – for health considerations, the sampling must be of the bioaerosol, which includes not only viable fungal structures but also the presence of the allergenic material in the air. Dead hyphae, conidia and fragments of the cell walls of fungi and bacteria act as allergens causing allergic and respiratory problems in some vulnerable people; only a few species are toxic. For both allergenic or toxic reactions, large doses over a long period of time are usually required. This allergenic material is also called antigenic because when someone is exposed to it the body produces antibodies to fight it, so anyone suspected of having been exposed to antigenic materials can be tested for the presence of specific antibodies.

In major fungal infestations caused by free water, bacteria are usually found in the water which may also be a source of allergens. Details about these allergens and methods of monitoring for them are discussed. We now have appropriate methods for determining the presence of these potential health hazards, but there are still more questions than answers about their health effects.

1.9 Chapter 9: Prevention, disaster preparedness and summary

Many easily accomplished methods of prevention are available, ranging from simple maintenance and dust removal to inspection of newly acquired heritage objects and seasonal air monitoring.

Objects, when they first come into the museum or any other heritage collection may, in their past, have supported a fungal infestations and still carry fungal structures. Such objects have the potential to be a health hazard and to support a fungal infestation and/or contaminate other collection objects.

Collection managers are now aware of the need for inspection of objects coming into museum storage and similar facilities. How-

ever, in the past when mouldy objects or unopened boxes were stored and not inspected until years after acquisition, when finally discovered it was often assumed that the infestation was due to their old storage environment. Records at the time of acquisition will help to nullify or verify this.

Prevention is the logical approach as we can no longer use residual biocides that remain active for years, not only destroying the microbes but also presenting a hazard both to the materials of the heritage object and people.

As already mentioned, this book is concerned with fungal questions. Disaster preparedness is an activity that should be undertaken prior to an infestation and should be specific to each situation. The plans should be drawn up in reference to this and the answers to our fungal questions. There are many scenarios on which to draw and the literature is extensive. Therefore this topic is only briefly reviewed.

In summary, the questions that I have tried to answer demonstrate that there are many aspects to a fungal infestation: the origin of the fungal species; the causative fungal species and their biology; the interaction on the materials of the object; the potential health hazards to staff; removal of the fungal structures from the infested materials; decontamination of objects and areas; prevention of reinfestation. Understanding each one of these aspects requires specific knowledge. It is the purpose of this book to present this relevant information. Suggestions are offered, but the final approach to your problem will be based on the specific problem you are trying to solve. You must be able to decide if a problem exists, assess the problem and, from your knowledge, respond appropriately to it.

2 What are fungi? Classification and nomenclature

2.1 Classification: introduction

2.1.1 Nomenclature

The naming of organisms – biological nomenclature – is an essential step to ensure that when an organism is under discussion everyone knows exactly which organism is being discussed. All organisms are classified into related groups: the largest grouping is the kingdom, i.e. plant, animal, true fungi etc. This grouping is divided into smaller groups of organisms with common characteristics, and then further subdivided into smaller groups with more common characteristics until finally the unique small group: the species. The names and sequence of the main categories are: kingdom, phyla, orders, families, genera, species. The categories from kingdom to species of one species demonstrates its evolutionary pathway or hierarchy.

Each species is a group of individuals with genetic and structural similarities and each has a unique name. The name of a species is a Latin binomial nomenclature, e.g. *Aspergillus repens* (usually expressed in italics). The first epithet, *Aspergillus*, is the generic name (a genus – a collection of similar or related species). The second epithet, *repens*, represents one particular group of unique individuals in the genus. The two-word combination is unique for that species. The hierarchy for *Aspergillus repens* is shown in Box 2.1.

2.1.2 Prokaryotes and Eukaryotes

The basic division of life forms is between the **Prokaryotes** and the **Eukaryotes**. The Prokaryotes – bacteria and cyanobacteria – are the earliest forms of life. These are single-celled organisms with a single chromosome free in the cytoplasm. There are no organelles (mitochondria or plastids) in the cytoplasm. Reproduction occurs by simple cell division, by fission or budding.

The Eukaryotes, the remaining organisms, are single cell and multicellular organisms that contain two pairs of chromosomes inside a

Box 2.1 FUNGAL FACTS
Classification categories

The following shows the classification categories of a common surface fungus, the green mould on oranges, *Aspergillus repens*.

Kingdom – Eumycota
Phylum or division – Dikaryomycota
Subphylum – Ascomycotina
Class – Ascomycetes
Order – Eurotiales
Family – Eurotialaceae
Genus – *Aspergillus* spp.
Species – *Aspergillus repens*

membraned nucleus in each cell. Cellular organelles, mitochondria and plastids, are present. In the mitochondria, the cell's energy source, adenosine triphosphate is produced. Also in the cell are found plastids which contain chlorophyll and other pigments, enzymes or stored food. In growth cell division (mitosis) two sets of chromosomes are duplicated and divided into two cells. Sexual reproduction involves the formation of sex cells, gametes (in humans, female eggs or male sperm) with a single set of chromosomes by a cell division called meiosis. A cell with two sets of chromosomes is said to be diploid and a cell with one set of chromosomes, haploid. Gametes are combined to form a new cell called a zygote with a mixture of two sets of chromosomes from two individuals. All the above structures in fungi are illustrated in the life cycles shown in Section 2.2.4. In higher plants and animals the dominant stage is diploid, i.e. man, lion, starfish etc., but in lower plants (algae) and fungi, the dominant stage is haploid.

Organisms are divided into five kingdoms:

- *Monera* (archaeobacteria and true bacteria), the single-celled Prokaryotes and the Eukaryotes
- *Protoctista* (ciliates, flagellates, microsporidia and three phyla of flagellated fungi)
- *Plants* (that photosynthesise)
- *Animals*
- *Eumycota* (true fungi)

2.1.3 The fungi in the kingdoms Protoctista and Eumycota

Fungi classification has undergone radical changes since the 1960s. Prior to that date, the fungi were included in the Plant Kingdom, but they are now classified in their own kingdom, Eumycota. To make things more complex, two

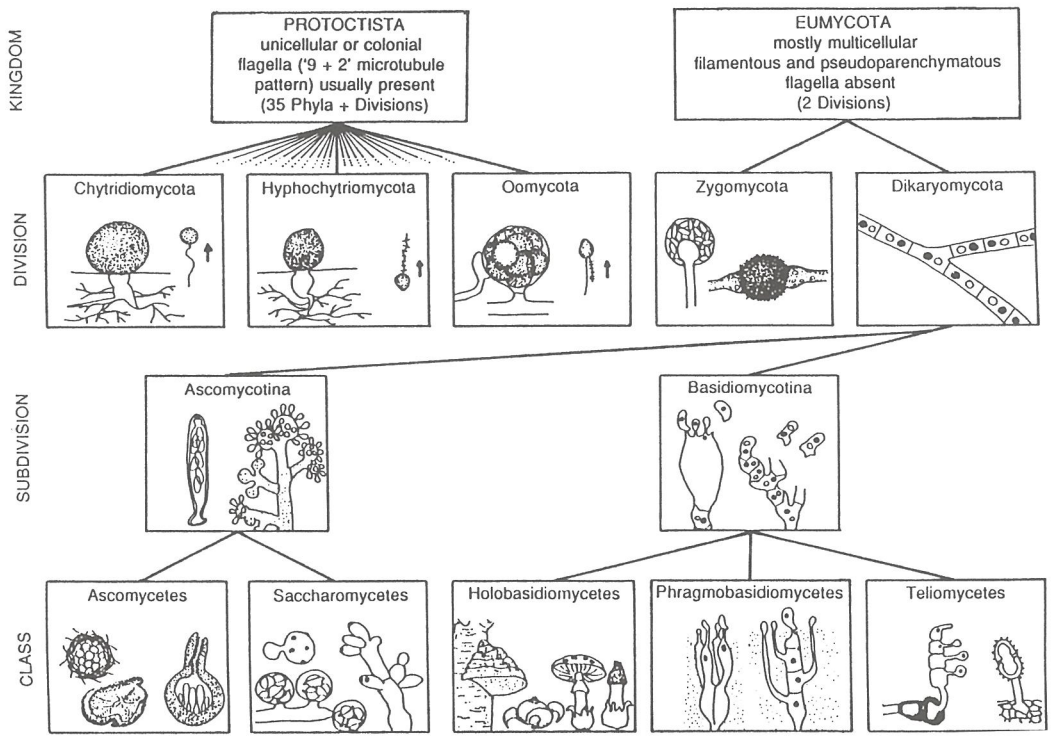

Figure 2.1 Diagnostic chart of fungi in the two kingdoms, Protoctista and Eumycota. The problem fungi for heritage objects are in the class Ascomycetes (from Kendrick 1992).

kingdoms, the Protoctista and the Eumycota (see Fig. 2.1) contain fungal species, the reason being that fungi are defined functionally or ecologically, not phylogenetically. True fungi are eukaryotic, heterotrophic (cannot make their own complex carbon molecules required for nutrition), adsorptive organisms that have a diffuse, branched, tubular vegetative body of hyphae, reproduce by spores and do not have flagella. This describes a way of life rather than a phylogenetic line, which describes some Protoctistan as well as the true fungi, the Eumycotan fungi. The Protoctistan may or may not have hyphae, have a single-celled flagellated stage and spores, and an amoeboid stage in their life cycle. Historically, because they reproduce by spores, they were included in the fungi but now, because of their flagellated and amoeboid stage, they are placed with the Protoctistans.

The Protoctistan include slime moulds and the fungal water moulds, white rusts, downy mildews, and damping-off fungi. The Protoctista are not found in relation to heritage object deterioration or airborne surface infestations. Figure 2.1 is a diagnostic chart of the Eumycota (true fungi) and Protoctista, and illustrates the major differences between the two kingdoms.

2.2 The true fungi (Eumycota) classification

Classification is an evolving discipline. With the new methods of establishing relationships between organisms with DNA, new relationships are realized. These are updated daily on the websites for classification (http://tolweb.org/tree/). Hawksworth *et al.* (1995), a dictionary of fungi, compares the proposed classification of fungi covering the years 1969–1995 of which Kendrick (1992) is one. The unifying feature in all is the grouping of the fungi relevant to this book under Ascomycetes, Basidiomycetes and Zygomycota. Kendrick's book (also available on his website www.bryce@mycolog.com) has been used as the major reference for this section on classification.

2.2.1 Overview

Eumycota is divided into two phyla (divisions): the phylum **Zygomycota** (conjugation fungi) and the phylum **Dikaryomycota** (Ascomycotina and Basidiomycotina) shown in Figure 2.1.

The Zygomycota reproduce by sexual fusion (conjugation) of somatic cells. They have wide, thin-walled, hyphal tubes which are coenocytic (without septations or divided into cells) and require moisture-saturated environments for growth. Some species, e.g. *Mucor*, *Rhizopus* (common bread mould – for details of its life cycle see Section 2.3), have been isolated from the surfaces of heritage objects. They have airborne spores and utilize sugar and starch, therefore they do not damage protein and cellulosic materials.

Box 2.2 shows the classification of some common surface fungi in the kingdom Eumy-

Box 2.2 FUNGAL FACTS
Categories and characteristics of fungi in the kingdom Eumycota

Phylum Zygomycota – mildew, black bread mould fungi (aseptate, coenocytic hyphae, stolons, sporangiophore, sporangium, spore, conidia, fusion, zygospore)

Phylum Dikaryomycota (septate hyphae)

- Subphylum Ascomycotina – hyphae septate, conidia, ascospores, ascus
  - Class Ascomycetes (anamorphs and teleomorphs) – hyphae septate, conidia, ascospores, ascus
    - Genus – *Aspergillus, Penicillium, Eurotium* etc.
      - Species – *Aspergillus clavatus, Penicillium chrysogenum, Eurotium repens*
  - Class Saccharomycetes – yeast
- Subphylum Basidiomycotina – hyphae septate, basidiospores, basidium
  - Class Holobasidiomycetes (basidium not divided by septate)
  - Class Phragmobasidiomycetes (basidium with three transverse septa)
  - Class Teliomycetes (basidia develops from diploid teliospore)

cota. The fungal species of major concern to us are found in the phylum Dikaryomycota, which is divided into the two subphylums, **Ascomycotina** (Ascomycetes) and **Basidiomycotina** (Basidiomycetes). Their hyphae are narrow and septate (divided into cells by cross-walls called septa), and they live in a wide ecological range. Most reproduce sexually by the fusion of nuclei which occurs in unique hyphae called dikaryotic hyphal cells. They are heterotrophic in nutrition, and given a nitrogen source they are able to utilize most of their own amino acids for making proteins. The majority of fungi that produce surface growth on heritage objects are found in the Ascomycotina; the main wood deteriorating fungi are in the Basidiomycotina.

In older literature (see Box 2.3) another subphylum – called variously the **Deuteromycotina**, Deuteromycetes, fungi imperfect or conidial fungi – is included. This is a group of conidial fungi in which the sexual stage (teleomorph) has not been observed. These fungal species manifest only asexual reproduction and are therefore called anamorphs. When both stages are present they are called holomorphs. Deuteromycota is a form division: they are mainly Ascomycetes and a few Basidiomycetes which have lost their ability to reproduce sexually (or do so modestly). Over the years of mycological research and new molecular methods for building DNA profiles for identification, many of these anamorphs have been connected to their teleomorph in Ascomycotina and Basidiomycotina. Because of this, most Deuteromycotina anamorphs are considered to belong to Dikaryomycota.

Despite our knowledge of their ancestry, it is still necessary to set up a single classification for the conidial fungi anamorphs. Thus the Deuteromycota, as an anamorph class, is an artificial but convenient taxon using conidial head characteristics, i.e. **hypomycetes** (open conidial head) or **coelomycetes** (closed conidial head). The majority of identified fungal species related to infested heritage objects belong to this conidial fungal subphylum. Their identification is based on their culture growth responses, conidia shape, ornamentation, colour, and method of formation, conidiophore shape, etc. The teleomorphs are identified by the shapes of the sexual stage, the ascospores and ascomata (structure-bearing ascospores) shape etc.

In many older mycology textbooks the classification shown in Box 2.3 is used.

Box 2.3 FUNGAL FACTS
Old classification

Kingdom – Planta

Phylum or division – Eumycophyta

Classes:

- **Phycomycetes** – aseptate, coenocytic hyphae, stolons, sporangiophore, sporangium, spore, conidia, fusion, zygospore
- **Ascomycetes** – hyphae septate, conidia, ascospores, asci
- **Basidiomycetes** – hyphae septate, conidia, ascospores, asci. basidiospores, basidium
- **Deuteromycetes** – hyphae septate, conidia, sexual reproduction absent

2.2.2 Subphylum Ascomycotina and Basidiomycotina

The class Ascomycetes

The Ascomycetes are divided into 44 orders which are separated by their ascocarp morphology and their biology. Only a few of these orders (discussed below) contain species that are commonly encountered on heritage objects.

Order Dothideales

This order contains *Cladosporium* (anamorphs) and *Alternaria* spp. which are the commonest cosmopolitan airborne contaminants originating from dead herbaceous plants. The conidia are multicellular and easily identified (see Fig. 4.4, p. 32).

Order Onygenales

This species can digest keratin, cellulose and live in dung. The keratin digester (such as *Tricophyton* and *Microsporium*) may cause skin

diseases in humans, such as ringworm and athlete's foot. Others can digest keratinacious hair, horn, feathers and keratinized epidermal cells. A species has been cultured from deteriorated hair on natural history specimens (Hawks and Rowe 1988), but it would have originated in the natural environment of the animal, not in a contaminant from the museum environment. The ascomata, containing sexual ascospores, often bear highly characteristic branched appendages that can make identification easy (shown in Fig. 2.2). The cellulosic digesters are

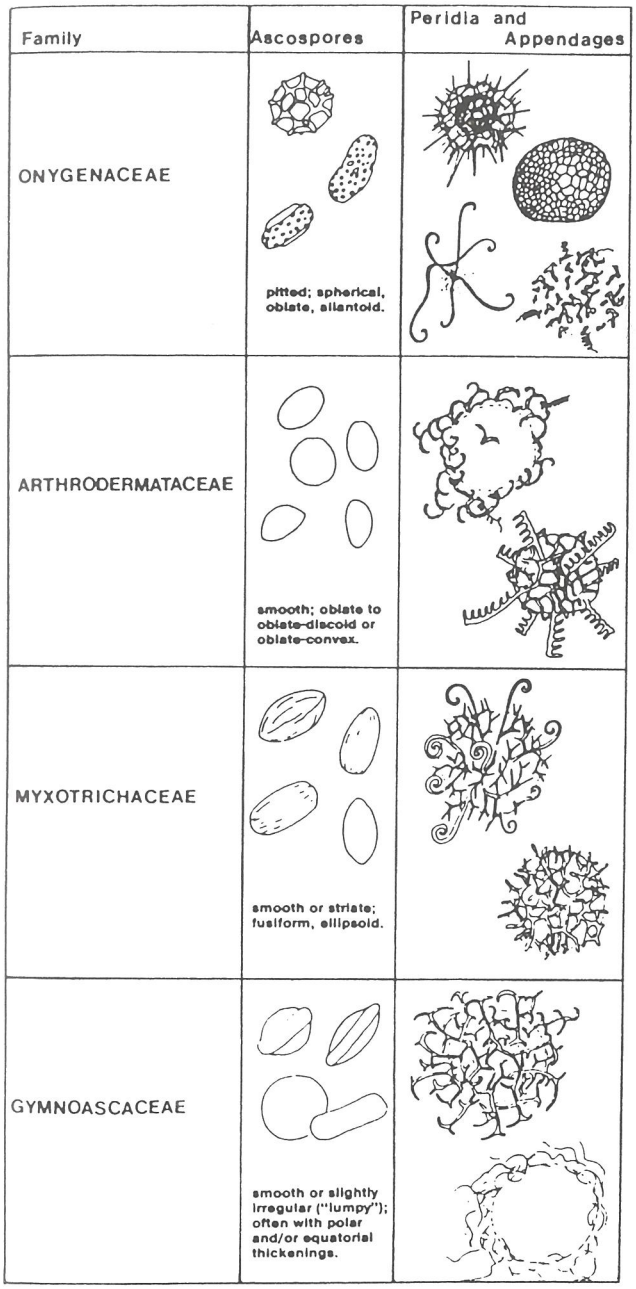

Figure 2.2 Ascospores and ascocarp wall (peridium) ornamentation and appendages of the families of Onygenales. Some of these ascocarps may be found on archaeological terrestrial buried heritage objects (from Kendrick 1992).

common soil fungi digesting plant material, paper, and straw related to the soil. They could cause damage to cellulosic and keratin artifacts in damp soil burial.

Order Sordariales

This order contains *Neurospora, Sordaria*, and *Chaetomium* species which have been isolated from surfaces of heritage objects. *Neurospora* and *Sordaria* grow on surfaces, and both are teleomorphs which produce asci that shoot the ascospores out at maturity.

Chaetomium species have asci that are deliquescent. The ascospores form a mass of coiled dichotomously branched hairs on top of the ascoma (shown in Fig. 2.3). It is a cellulolytic species and can damage cellulolytic textiles and paper fibres. It has been isolated on paper that has been subjected to dirty water damage. Anamorphs are usually encountered on surfaces but *Chaetomium* is one exception – it is commonly found as a teleomorph. The teleomorphs are encountered when materials have become soiled with dirty water or from terrestrial burial.

Chaetomium has *Botryotrichum* anamorphs; both have been isolated from heritage objects (see Table 3.5, p. 25).

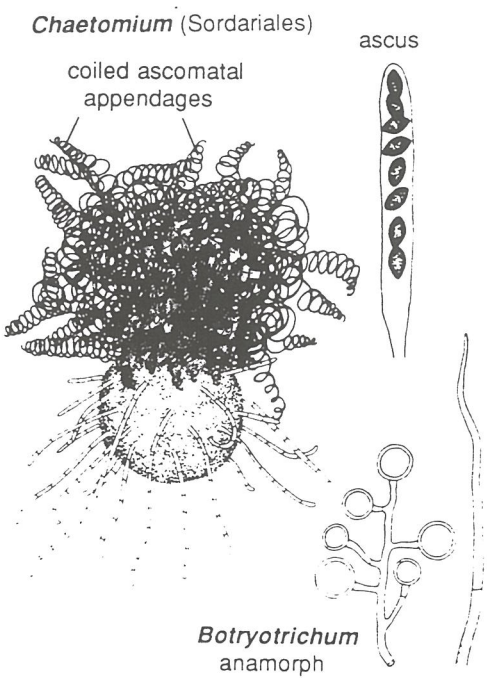

Figure 2.3 Ascoma with ascomatal curled appendages and an ascus with eight ascospores of *Chaetomium* sp. teleomorph and its anamorph *Botryotrichum* (from Kendrick 1992).

Order Eurotiales – the main culprits

This order contains the most successful of all the anamorphic conidial fungi, i.e., *Eurotium*, *Aspergillus* and *Penicillium* species – the common blue and green moulds. They readily produce masses of airborne conidia. These are the common surface fungi found on artifacts and archival materials, and are also common food contaminants. They are the prime colonizers and decomposers of plant debris and are the producers of antibiotics, e.g. penicillin and immunosuppressant cyclosporine, and other commercially used metabolic products such as citric acid.

Eurotium are anamorphs of the teleomorphs of *Aspergillus*. Rarely are the teleomorphs encountered on heritage objects infested with surface fungi. *Eurotium* species of this genera have been identified as the causative fungus in old fox spots on paper. Anamorphs are separated into two anamorph classes: an artificial but convenient taxon, class hypomycetes, *Stachybotrys, Eurotium, Aspergillus* and *Penicillium*, etc.; and the class coelomycetes. The hypomycetes contain the majority of species of concern to us; the coelomycetes are plant pathogens and produce their conidia in plant tissue.

Life cycle of Eurotiales conidial fungi

The general pattern of the life cycle of the fungi (see Fig. 2.4 for the *Aspergillus* sp.), starts with a conidium that germinates on the surface of materials. Germinating conidia produce a germination tube (Fig. 2.5) that develops into a hypha which initiates vegetative growth. Hyphae in conidial fungi are thread-like (average 5 microns thick), multicellular structures that grow by tip elongation and profuse branching. A group or mass of hyphae is called the **mycelium** (Fig. 2.6). In conidial fungi the

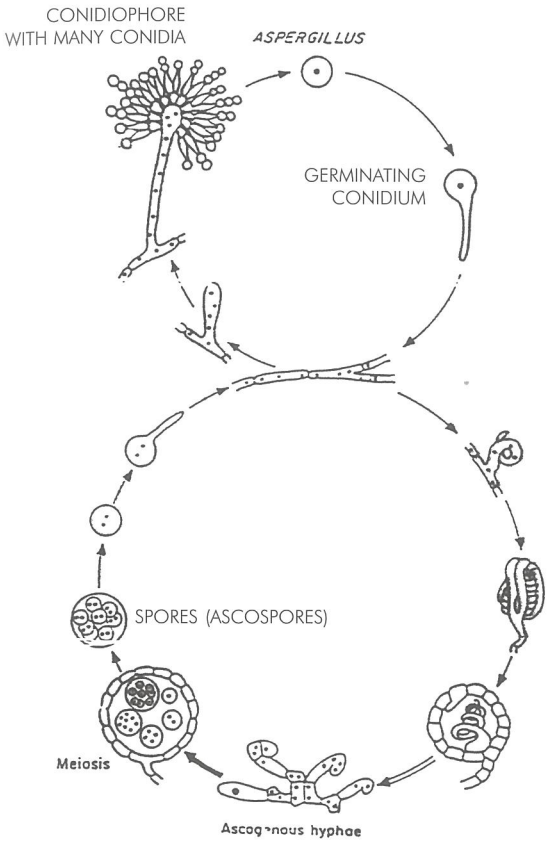

Figure 2.4 The life cycle of *Aspergillus* sp., a typical conidia fungus. The top circle represents asexual reproduction and the formation of conidia which are dispersed by air. The bottom circle represents sexual reproduction and the formation of spores (ascospores) which are usually formed inside the material on which it is growing (after Burnett 1976).

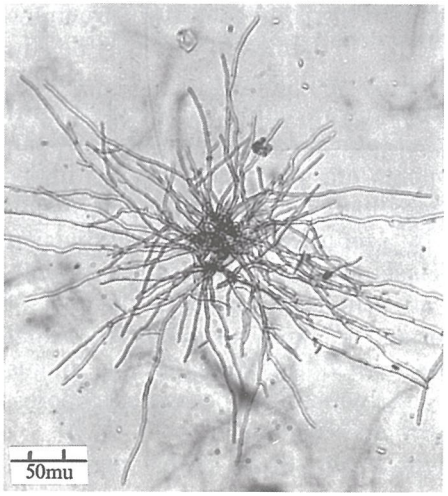

Figure 2.5 A circular fungal colony of branching hyphae developed from one conidium after 24 hours' growth (photomicrograph by M-L. Florian).

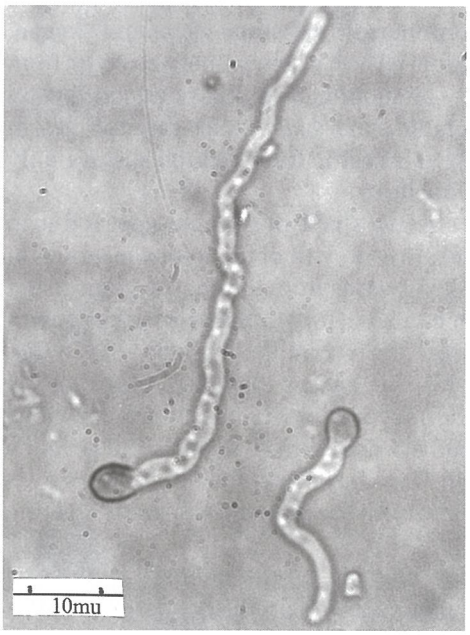

Figure 2.6 Germinating pigmented conidia and colourless germination tubes (photomicrograph by M-L. Florian).

mycelium may take the form of a diffuse, felted network in a substrate, like a mouldy spot on jam. The substrate is the material on which the fungus grows, i.e. jam, orange skin, paper, leather, textile, etc., into the vegetative stage (mycelium).

The mycelium produces hyphae which produce masses of asexual conidia that become airborne. The process of differentiation of hyphae into asexual conidia is called conidiation. The conidia are formed without fusion of nuclei and thus are genetically identical (clones). The conidia usually form on the surface of the mycelium and are seen as coloured dusty circles (see Section 4.1). We have all seen this green growth on fruit, such as an orange. The conidia are picked up by air currents and eventually settle on and initiate growth on surfaces.

Conidia are usually spherical structures about 5–50 μm in diameter. They may be single-celled or a group of cells of low metabolic activity. They have a reproductive and survival function, and are the main unit of dispersal. Sexual spores are produced, usually under adverse growing conditions, by cell fusion followed by a reduction division cell division called **meiosis**, which allows genetic variability. They are commonly formed in the body of the thallus and are rarely airborne. The sexual spores are named according to the fungus subdivision to which they belong: ascospores, phycospores and basidiospores.

The hyphae can adsorb simple sugars and amino acids, and secrete enzymes. The enzymes digest simple polypeptides, carbohydrates, and fats in the dead organic substrates into free amino acids, simple sugars, glycerol and fatty acids. These nutrients are adsorbed into the hyphae along with substrate water and oxygen. They do not adsorb water vapour or oxygen from the air. The moulds or mildews which live on dead organic material are called saprophytes, in contrast to parasitic fungi, which survive only on a living host.

The circular shape of a fungus spot is due to the concentric growth of the hyphae (shown in Fig. 2.5), outwards from the central germinated spore or conidia. Growth may also occur downwards into the substrate. Hyphae may worm themselves in spaces between fibres or by secreting enzymes which digest the amorphous material between fibres – such as the grounds in paper. They may digest the fibres themselves and then adsorb the digested substrate, creating holes.

Under adverse environmental conditions some fungal species produce **sclerotia** which look like black fly spots in the fungal spot. Sclerotia are made up of an organized mass of pigmented hyphae and stored nutrients. They function in vegetative propagation. The fungal spots in cotton textiles are commonly caused by the sclerotia of *Cladosporium* spp. The sclerotia are difficult to remove because they are attached to and between the textile fibres.

Class Saccharomycetes – the yeasts

Most yeasts are anamorphs. Their vegetative structure is single cells (shown in Figs 2.1, 2.2 and 2.7), which go through a process of asexual reproduction by budding. The yeasts of importance to heritage objects are those that cause enzyme hydrolysis of mainly starch and sugar-based adhesives in wet papers. Bacteria are often associated with yeast in water-soaked objects. The bacteria will also develop in free water and hydrolyze protein grounds, sizes and adhesives.

Figure 2.7 *Rhodotorula*, a red yeast. This is a single-celled fungi that reproduces by budding, forming conidia. Isolated from surfaces of leather and in paper (after Kendrick 1992).

2.2.3 Subphylum Basidiomycotina (Basidiomycetes) – the wood digesters

The Basidiomycetes have many features in common with the Ascomycetes: chitinous hyphal walls, regular septate hyphae, produce conidia, and the presence of a dikaryon phase but differ in sexual dikaryiotic hyphae morphology and spore development (see Fig. 2.8 which illustrates the difference). The Ascomycetes have hyphae with reflexed tips called crosiers, and the Basidiomycetes have connections between

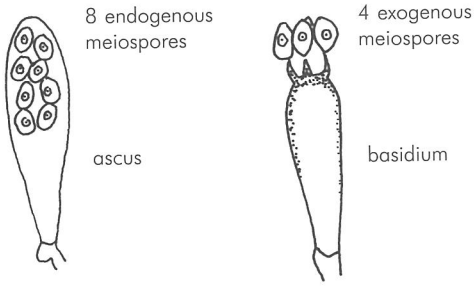

Figure 2.8 Comparison of the ascus of Ascomycetes and basidium of Basidiomycetes (after Kendrick 1992).

somatic cells called clamp connections. In the Basidiomycetes, the reproductive meiospores (basidiospores) are formed externally on a basidium, whereas in the Ascomycetes the meiospores (ascospores) are produced internally in an ascus.

The wood-destroying fungi which cause most structural internal wood damage belong the Basidiomycetes. Most of these species produce large bracket or shelf-like structures in which the reproductive basidiospores are formed and eventually become airborne. There are three classes of Basidiomycetes: the mushrooms and polypores; the jelly fungi; and the rusts and smuts. The main Basidiomycetes responsible for causing structural wood damage are in the two families: *Polyporaceae* (*Poria, Polyporus and Ganoderma*) and *Coniophoaceae* (*Serpula lacrymans*) the dry rot fungus, and *Coniophora*, the cellar fungus which have fungal species that can destroy structural wood. These fungi have evolved to be saprophytes on wood (to recycle fallen branches or logs in forests), but they are just as efficient at recycling structural wood if the wood is allowed to become damp for long periods of time. Figure 2.9 illustrates the basidiocarps of some wood-destroying fungi.

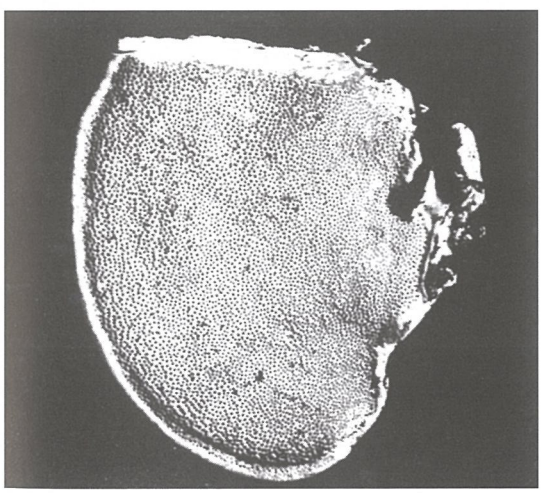

Figure 2.9 The underside of a bracket or shelf basidioma of family, *Polyporaceae*. They occur in dead forest debris and structural wood in use. It is leathery and corky.

2.2.4 Comparison of life cycles of some fungi isolated from heritage objects

The following diagrams of common fungi illustrate the differences in their life cycles.

Figure 2.10 shows the life cycle of *Mucor* sp., called black bread mould because it is a very common contaminant of bread and produces black heads of conidia. *Aspergillus* sp. (Fig. 2.4)

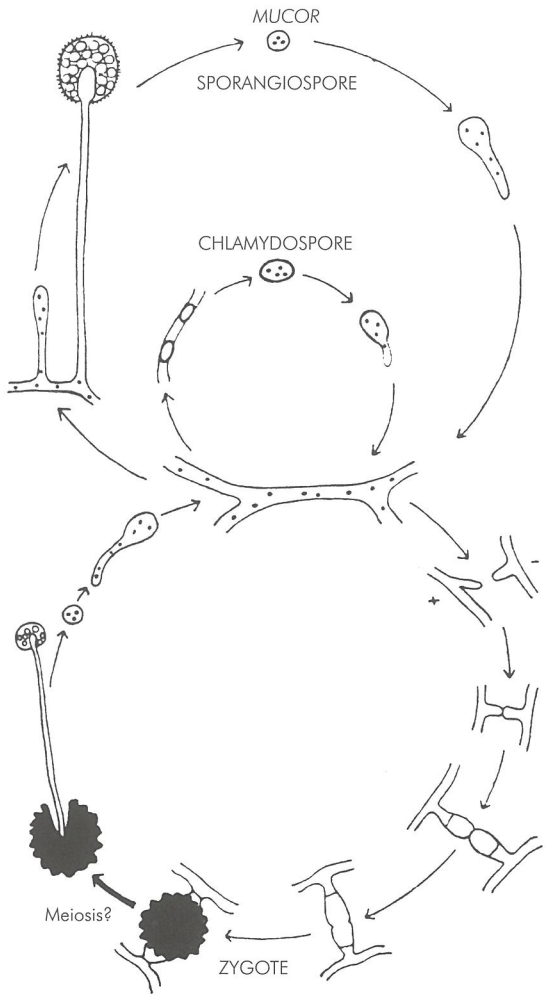

Figure 2.10 Life cycle of *Mucor* sp., black bread mould. The top circle represents the asexual phase and the bottom the sexual phase. It is classified in the Phycomycetes in phylum Zygomycota because it has sporangiospores not conidia, and zygotes not ascocarps as the Ascomycetes (after Burnett 1976).

and *Neurospora* sp. (Fig. 2.11) are both common airborne surface contaminants on heritage objects. Figure 2.12 shows the simple life cycle of yeasts. They do not have a filamentous hyphae but produce single cells that bud for asexual reproduction, and asci with ascospores for sexual reproduction. Figure 2.13 shows the life cycle of *Coprinus*, the common ink-cap gill mushroom. The life cycle of the structural wood-destroying fungi is similar to *Coprinus*.

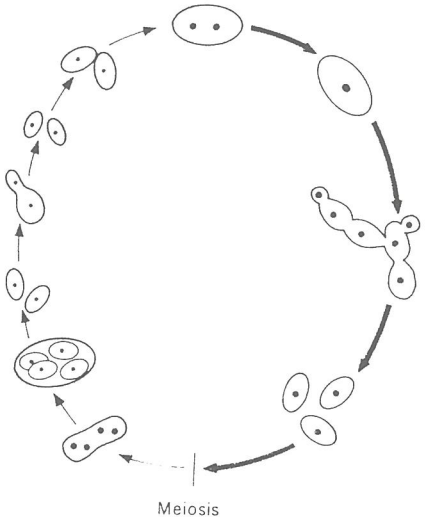

Figure 2.12 The life cycle of *Saccharomycetes* yeast. It is an Ascomycete which does not form a mycelium. It reproduces asexually by budding of the single cells. Sexual reproduction, fusion of cells followed by meiosis, results in the formation of the ascus with four ascospores (from Burnett 1976).

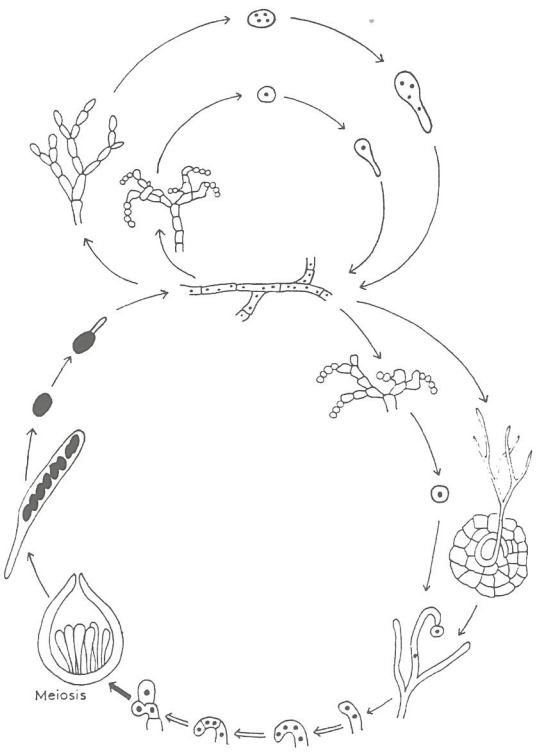

Figure 2.11 The life cycle of *Neurospora*, an Ascomycete, the red bread mould. The top circle represents asexual reproduction and the bottom, sexual reproduction. Eight dark ascospores are produced in the ascus. The germination of the ascospore produces a mycelium on which are developed asexually macro or microconidia. Nuclei in hyphae from these two types of conidia fuse to give rise to the sexual stage. Reduction division, meiosis occurs in the formation of the ascospores (after Burnett 1976).

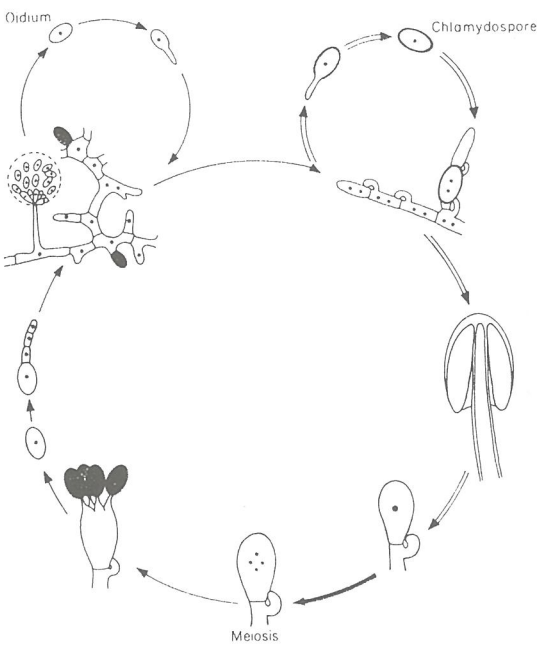

Figure 2.13 Life cycle of *Coprinus*, a Basidiomycete, a common ink-cap gill mushroom. Its life cycle consists of asexual stages (at the top) and the sexual stage which involves the formation of the basidia each with four basidiospores. These are located on the gills of the mushroom (basidioma). Asexual reproduction occurs in the soil usually by the chlamydospores. In polypore fungi that attach to structural wood, the basidiospores are formed in the pores of the shelf basidioma (after Burnett 1976).

2.3 Identification methods

2.3.1 Culturing

The classical method is to culture the fungal structure (shown in Fig. 2.14). Using a sterile technique, small samples of mycelium (or just conidia or spores) are placed on sterile media in a sterile petri plate. The media contains agar to make it solid at room temperature, as well as nutrients, proteins, starch, sugars, buffering salts, and chemicals to adjust the water activity (available water for fungal growth), etc. The recipes for the media may be adjusted to grow certain species or species with specific physiological requirements. The acidity of the media may be adjusted to eliminate bacterial contaminants. Liquid cultures can also be prepared in flasks or test tubes without agar. The petri plates or test tubes are placed in incubators at specific temperatures for growth.

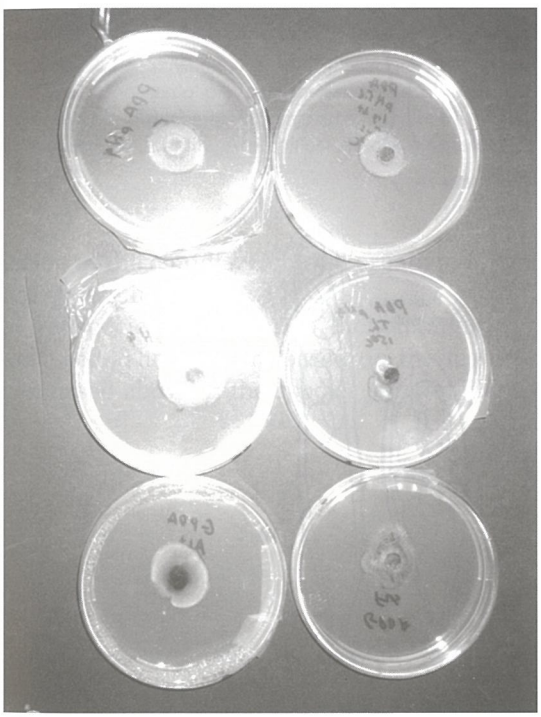

Figure 2.14 Petri plates of cultured fungal species. Each plate contains an agar-based media with added nutrients, i.e., PDA (potato dextrose agar) and NA (nutrient agar). The pH is usually adjusted. These plates were incubated at 20°C. The central circle is an inoculum, a plug of the cultured fungus (photograph by M-L. Florian).

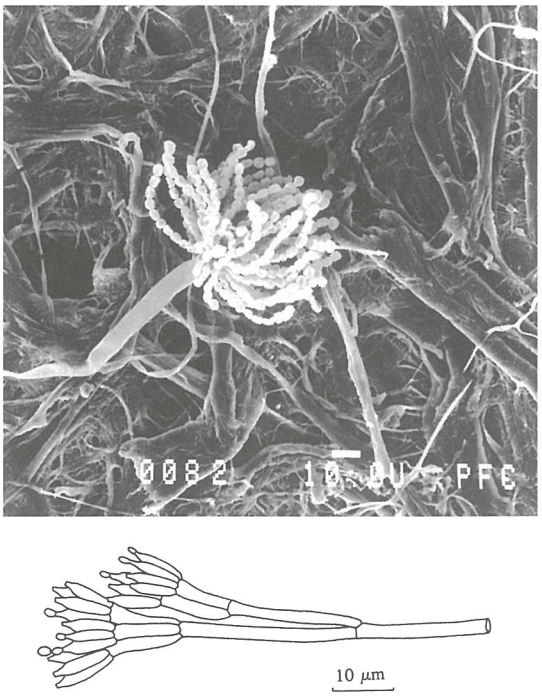

Figure 2.15 Conidiophores and conidia of *Aspergillus* sp. with its ball-shaped tip from which the conidia are formed, top, and *Penicillium* sp., bottom, with a branched tip (after Florian 1998).

Identification of these cultured species includes microscopic examination of conidiophores (see Fig. 2.15) and conidia, and the visual observation of the type of mycelial growth and the colour of the conidia if anamorphs are present. If sexual ascospores and ascocarps (see Fig. 2.16) are present, their structure and shape also assist in identification. As well as structural features, the physiological response to the specific media used and parameters on the environment used in culturing are observed.

In the food industry where food, such as wheat and other grains, is usually tested for contaminants, a new method (Kozakiewicz 1989b) is being developed to identify the common species – using scanning electron microscope (SEM) observation of the ornamen-

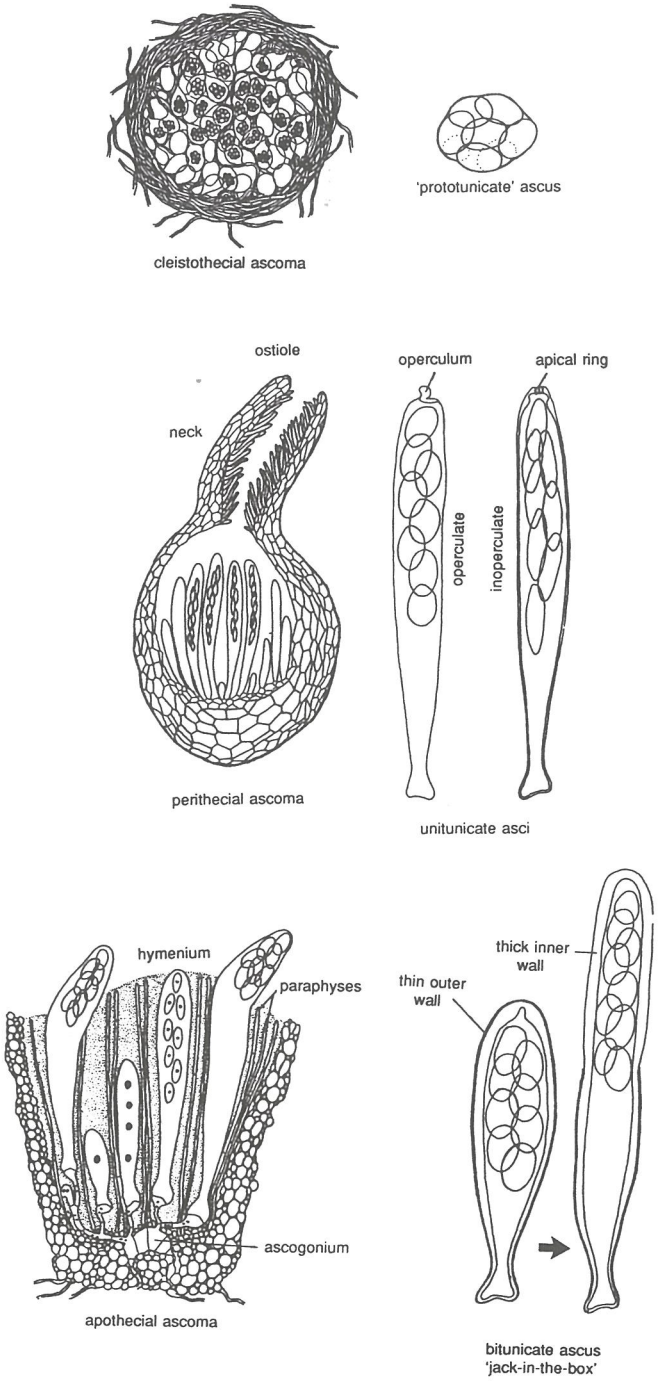

Figure 2.16 A comparison of the three different types of ascoma of the Ascomycetes and the differences in their asci (after Kendrick 1998).

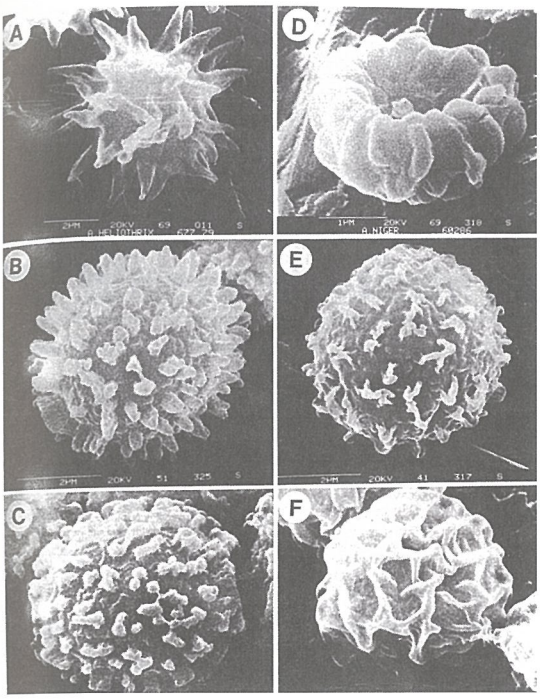

Figure 2.17 SEM micrographs illustrating five of the conidial ornamentation categories: A, echinulate, B, aculeate, C, tuberculate, D, verrucose, E, F, lobate-reticulate, used to identify *Aspergillus* species (Kozakiewicz 1989b).

tation of the conidia. Conidial ornamentations are shown in Figure 2.17.

For species identification, dichotomous keys are used. The keys are based on the features discussed above. If the species you are identifying is not represented in the key you are using, this presents a major problem. Other difficulties arise because some reproductive structures, which are required for identification, may not have developed.

When sampling surfaces of fungal-infested heritage objects, cultures can be a major problem because the sample from a surface of say a piece of old paper, will be contaminated by many airborne fungal species. The one (or ones) that grow may not be the causative species because the causative species may no longer be viable; may be inhibited by the growth of other contaminants; or may not be able to utilize the specific culture media used.

2.3.2 Molecular taxonomy and immunology

Molecular taxonomy methods are now also being developed for fungal identification. These involve enzyme activity, base sequences in DNA, immunoassays using antibodies, and electrophoresis known as SDS-PAGE (sodium-dodecylsulphate polyacrylamide gel electrophoresis), based on separating proteins by molecular size (for details of these methods see the Bibliography: Identification methods of fungal species).

Molecular methods also have inherent problems. If a sample from a surface is analyzed, in all probability structures from several species will be found and it is difficult to determine which one is the causative species.