EDIBLE AND MEDICINAL MUSHROOMS

TECHNOLOGY AND APPLICATIONS

EDITED BY DIEGO CUNHA ZIED
AND ARTURO PARDO-GIMÉNEZ

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Technology and Applications

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Preface

The term *Mushrooming*, or mushroom cultivation, refers to the intentional and directed production of mushrooms as a substitute for wild collection in fields and forests with a harvest under defined conditions of growing, resulting in strict quality control and food safety without risk of consumption of poisonous or toxic species, and with guaranteed benefits from fungi.

Although knowledge about the cultivation of edible and medicinal mushrooms is practically the same throughout the world, there are significant differences between countries and even within the same country. These are primarily associated with different socioeconomic conditions. In this way, just as there are large-scale growers, other smaller-scale plants act as a complement to the family economy, while very basic and rustic facilities coexist with others that operate on a high technological level.

This book involves a multidisciplinary approach that includes aspects of agriculture and agronomy, microbiology, biology, biotechnology, chemistry, environmental management, food technology, and health, among others. With a global and collaborative purpose, the book consists of 22 chapters written by 28 authors, from 15 different countries, who are recognized experts in the different areas that compose this activity. We thank them all for their participation.

The different areas of the science of cultivation are approached, so the book can serve as a tool for researchers, professors, technical specialists, and growers, and as an introduction for both students and anyone interested in the world of *mushrooming* knowledge as a business opportunity or out of simple curiosity.

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1

Mushrooms and Human Civilization

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Mention of mushrooms has been reported in ancient literature since the inception of human civilization. Mushrooms find mention because of their wide range of properties from being poisonous to being beneficial and edible. Their poisonous nature was their most intriguing quality in early history. Throughout the centuries, poisonous fungi/mushrooms have remained a useful means of disposing of adversaries. Pliny the Elder (23–79 AD) gives details of how the Emperor Claudius was poisoned by his fourth wife Julia Agrippina. Emperor Jonan followed in 364 AD, and Pope Clement VII in 1394. In addition, the antipope Urban VI, the French King Charles VI, and the German/Spanish king Joseph Ferdinand were all poisoned with mushrooms (van Griensven, 1988).

Knowledge about fungi developed slowly. In the fourth century BC, Theophrastus gave a scientific description of fungi and considered these fungi as part of vegetable kingdom, even though they have no buds, leaves, or roots.

With the decline of classical civilization, interest in science also declined. The scholastics of the Middle Ages made no contribution to science. Scientific study made little progress in the Western World up until the late Middle Ages. Names were given, morphological descriptions were made, and mushrooms find mention as "surplus moisture from the ground and trees, from rotting wood and other things." This particularly applies to edible varieties, through the influence of thunder, lightning, and rain (van Griensven, 1988).

In China, however, as early as 1245 AD, Chen Yen-Yu had published a fungus flora, describing in detail the development, morphology, seasonal influence, growing method, harvesting, and preparation (as food) for 15 varieties of mushrooms (Wang, 1987). In 1588 Giambattista Porta published his *Phytognomoniica*. He was the first person to describe the spores of fungi. Like his contemporaries, he held the view that parasitic plants, among which he counted tree mushrooms, were unnatural and could be used against lumps and tumors on human limbs (van Griensven, 1988).

According to Theophrastus, practically everything was missing from the mushroom, and eating mushrooms was therefore harmful to human body. Clausius (1525–1609) was the first to describe the Bird's Nest (Nidularia).

The "hidden power" of earth is responsible for the occurrence of mushrooms. That is why mushrooms were known as "excrementa terrae" in the seventeenth century. It was, of course, reprehensible to eat these excretions of the earth.

In the early seventeenth century, the Italian Count Margigi describes how a white, mold-like web appears when mushrooms and truffles are carefully dug up. He calls this web, which smells

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of mushrooms and has tiny buds, "situs" (Lutjehmas, 1936). By this time all edible mushrooms including truffles were found in Europe, collected from the wild.

The Chinese and the Japanese were probably the first to cultivate mushrooms professionally, and a brief description of history published in English (Wang, 1987) refers to Shiitake mushroom cultivation by Wang Zeng in 1313 AD. The culture of the paddy straw mushroom *Volvariella volvacea* is also centuries old.

Linnaeus (1707–1778) gave the field mushroom (white button) the name *Agaricus campestris*. Finally, in his *Systema Mycologicum* (Kleiju, 1961; Poppe, 1962), Elias Fries (1707–1778) gave a methodical description of all varieties of mushrooms known at that time (van Griensven, 1988).

1.1 Domestication of Mushrooms

The mushroom is the most important horticultural cash crop grown indoors, compared to other traditional crops grown outdoors, and is the only non-green crop grown for commerce with attractive profits. Mushroom is the fruit body of a fungus, which is neither a plant nor an animal, but has a separate kingdom of its own. Fungi as a broad group either live parasitically on plants and animals or live saprophytically on dead organic matter. Fungi cause numerous diseases of plants and animals and have been reported to cause considerable crop losses with tremendous suffering to mankind from time immemorial. The role of fungi as being beneficial to humans is of recent origin, with the generation of information on existence of microorganisms and their importance to man on Earth. Today, the science of study of mycological applications for human welfare has touched greater heights with the application of molecular biological techniques to improve useful fungal cultures of yeasts and mushrooms.

The fact that certain fungi are edible has been known for many centuries, and in various European countries up to 80 distinct varieties of wild fungi are offered for sale on the market (Pinkerton, 1954). Though many edible fungi have been domesticated and are in production, the most commonly cultivated are shiitake (*Lentinula edodes*), oyster mushroom (*Pleurotus* spp.), white button mushroom (*Agaricus bisporus*), black fungus or wood-ear mushrooms (*Auricularia auricula and Auricularia polytricha*) and paddy straw mushroom *Volvariella* spp. The cultivation of shiitake by Japanese on logs dates back at least 2000 years (Ainsworth, 1976), but button mushroom cultivation is comparatively recent. Today, the button mushroom is the most widely grown in many countries, although it is the fourth mushroom most produced in quantity (see chapter 2), with most of the development of cultivation technology confined to improving this mushroom for reasons of its larger acceptability by the consumer.

The first record of (button) mushroom cultivation dates back to Abercrombie (1779), who wrote that this plant is of so very singular growth and temperature, that unless a proper idea of its nature and habit is attained, and the peculiar methods and precautions pursued in the process of its propagation and culture, little success will ensue; the whole management of it differs remarkably from that of every other species of the vegetable kingdom; and it is the most liable of any to fail without very strict observance and care in the different stages of its cultivation.

Tournefort (1707) gave a comprehensive description of the commercial production of button mushrooms. These observations recorded in earlier times bear comparison with the methods used today. At that time mushrooms were cultivated on open ground, but around 1810, Chambry (a French gardener) began to cultivate mushrooms in underground quarries in Paris, all year round. Later Callow (1831) showed that mushroom production was possible all year round in England in rooms specially heated for the purpose. Callow gave details of the design of cropping houses (crediting it to Oldacre, a garden superintendent in UK) and later successfully grew mushrooms all year round in such a structure producing a yield of 7.3 kg m^{-2} in

24 weeks of cropping, as compared to mushroom yields of 10 kg m^{-2} obtained in 1950 in the UK. It is now accepted that protected cropping of mushrooms was pioneered in caves in France, though the earliest mushroom houses were developed in England.

Large-scale mushroom production is now centered in Europe, North American (USA, Canada), Australia, South East Asia (China, Korea, Indonesia, Taiwan), and South Asia (India). The notable contributions to mushroom science in recent times were made at the beginning of the twentieth century when pure cultures of button mushrooms were grown by Duggar (1905). Other notable contributions were the preparation of mushroom compost from agro-byproducts using the short method by Sinden and Hauser (1950, 1953).

Contributions by Fritsche (1985) in breeding two new strains of white button mushroom *A. bisporus* U-1 and U-3 revolutionized commercial mushroom growing across the world. With the refinement of cultivation technology of button mushrooms on a continuing scale, it was possible to harvest more and more quantities of mushrooms per unit area/unit weight of compost. Demonstration of steam pasteurization of mushroom compost in bulk (Derks, 1973) further helped commercial mushroom growing to increase the productivity per unit area/unit weight of compost.

Finally, increased understanding of crop management techniques resulted in substantial increases in mushroom yields per unit weight of compost in a reduced cropping period, thereby giving greater profitability to the mushroom grower. Today, mushroom growers worldwide have a wide range of button mushroom cultivars available for cultivation. Computer control of cropping room environments for climate creation/simulation has made it possible to harvest mushroom yields of 30–45 kg from 100 kg compost within a cropping period of 3–4 weeks in 2–4 flushes.

With the introduction of the use of phase-I aerated bunkers for environmental protection, the composting process has become precision controlled with reduced emission of foul harmful gases without affecting mushroom yield. Use of indoor aerated bunkers has become very popular all over the world for reasons of economy in addition to being environmentally friendly. Phase-I bunkers are less space demanding and less labor oriented than traditional outdoor phase-I ricks, with the advantage of lower emission of foul gases during solid state fermentation controlled by restricted/controlled oxygen availability in the bunker.

A current science of mushrooms is presented in detail in this book, along with specific approaches in the main species of cultivated mushrooms and their technologies in different countries and continents. All steps and applications of "mushrooming" are detailed in the following 21 chapters.

References

Abercrombie J. (1779). *The Garden Mushroom, Its Nature and Cultivation*. Lockyer Davis: London, 54 pp.

Ainsworth GC. (1976). *Introduction to the History of Mycology*. Cambridge University Press: Cambridge.

Callow E. (1831). *Observations on methods now in use for the artificial growth of mushrooms, with a full explanation of an improved mode of culture*. Fellowes: London. (Reprinted, 1965, by W. S. Maney and Son Ltd: Leeds),

Derks G. (1973). 3-phase-1. *Mushroom Journal* 9:396–403.

Duggar BM. (1905). The principles of mushroom growing and mushroom spawn making. *Bulletin of US Department of Agriculture Bureau of Plant Industry*, 85:1–60.

Fritsche G. (1985). Breeding mushroom strains. *Der Champignoncultuur* 29:377–395.

Kleijn H. (1961). *Paddestoelen, hun vorm en kleu. Becht uitgevers maatschappij*, Amsterdam. (Toadstools, form and colour).

Lutjeharms WJ. (1936). Zur Geschichte der Mycologie Des XVIII, Jahrhundert. *Thesis Leiden University*, Published by v/h Koch & Knuttel, Gouda.

Pinkerton MH. (1954). *Commercial Mushroom Growing*, Benn: London.

Poppe JA. (1962). De champignonteelt en haar problem. Thesis for degree of agriculture engineer. Ghent Agricultural College. (Mushroom cultivation and its problems).

Sinden JW and Hauser E. (1950). The short method of mushroom composting. *Mushroom Science* 1:52–59.

Sinden JW and Hauser E. (1953). The nature of the composting process and its relation to short composting. *Mushroom Science* 2:123–131.

Tourneforte J de. (1707). Observations sur la naissance et sur la culture des champignons. *Memoires de l'Academie Royale des Science* 1707:58–66.

van Griensven LJLD. (1988). History and development. In: *The Cultivation of Mushrooms*, 11–28. p. 515.

Wang YC. (1987). Mycology in ancient China. *The Mycologist (Bulletin of the British Mycological Society)* 21:59–61.

2

Current Overview of Mushroom Production in the World

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Edible, medicinal, and wild mushrooms are the three major components of the global mushroom industry. Combined, the mushroom industry was valued at approximately $63 billion in 2013. Cultivated, edible mushrooms are the leading component (54%) accounting for approximately $34 billion, while medicinal mushrooms make up 38% or $24 billion and wild mushroom account for $5 billion or 8% of the total (Figure 2.1).

World production of cultivated, edible mushrooms has increased more than 30-fold since 1978 (from about 1 billion kg in 1978 to 34 billion kg in 2013). This is an extraordinary accomplishment, considering the world's population has increased only about 1.7-fold during the same period (from about 4.2 billion in 1978 to about 7.1 billion in 2013). Thus, *per capita* consumption of mushrooms has increased at a relatively rapid rate, especially since 1997, and now exceeds 4.7 kg annually (vs 1 kg in 1997; Figure 2.2).

In 2013, nearly all consumption of mushrooms in China, EU, and India was supplied from domestic sources; and nearly all consumption of mushrooms in the United States, Canada, Japan, and Australia was supplied mostly by domestic sources but also by substantial amounts of imports (USITC 2010).

China is the main producer of cultivated, edible mushrooms (Figure 2.3). Over 30 billion kg of mushrooms were produced in China in 2013 (CEFA, 2014) and this accounted for about 87% of total production. The rest of Asia produced about 1.3 billion kg, while the EU, the Americas, and other countries produced about 3.1 billion kg.

Five main genera constitute around 85% of the world's mushroom supply (Figure 2.4). *Lentinula* is the major genus, contributing about 22% of the world's cultivated mushrooms. *Pleurotus*, a close second, with five or six cultivated species, constitutes about 19% of the world's output while *Auricularia* contributes around 17%. The other two genera, *Agaricus* and *Flammulina*, are responsible for 15 and 11% of the volume, respectively.

Edible mushroom production in China by genus in 2013 is shown in Figure 2.5. *Lentinula* is the most widely grown mushroom accounting for over 7 billion kg. This represents a 106.8% increase in volume from 2010 (Figure 2.5). The second most widely grown mushroom in China is now *Auricularia*. Production of this genus (with two main species) has increased nearly 92% since 2010. *Pleurotus* is the third most widely grown genus in China 2013 accounting for nearly 6 billion kg (a 10.8% increase since 2010).

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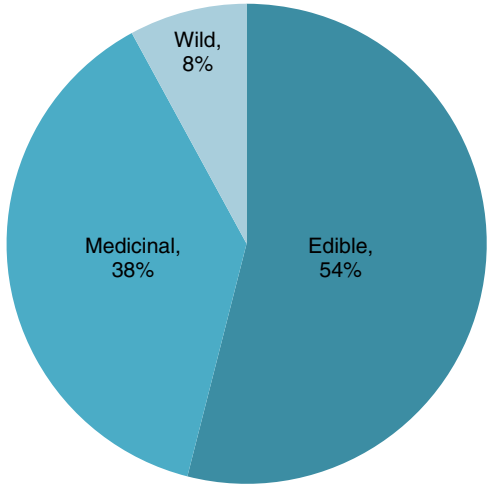


Figure 2.1 Components (edible, medicinal, and wild) of the world mushroom industry based on percentage of total value ($63 billion) (2013).

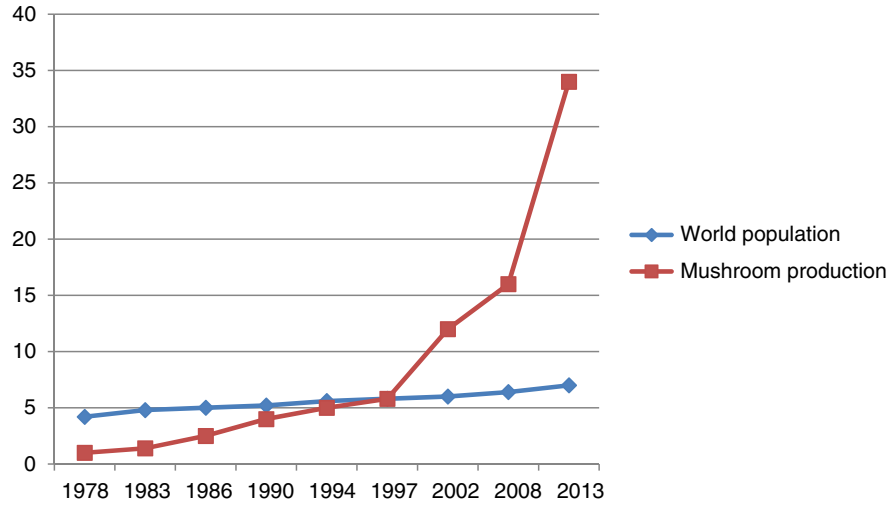


Figure 2.2 World population (billions) versus world cultivated, edible mushroom production (billion kg).

2.1 *Lentinula edodes*

Until the mid-1980s, Japan was the world's major producer of *L. edodes* (shiitake in Japanese or xianggu in Chinese) (Figure 2.6) that were grown on natural logs of the shii tree (*Castanopsis cuspidata*). However, with the development of sawdust-based techniques (Figure 2.7) that reduced crop cycle time and increased production efficiency, China soon became the major producer of xianggu by 1990 (Figure 2.6). From 1995 to 2000, Chinese farmers increased xianggu production from about 500 million kg to over 2 billion kg – a huge increase by most standards of measuring change. China now accounts for more than 95% of total output of this species. Several entire communities have been lifted from poverty because of the economic opportunities afforded to them by producing xianggu (Chang 1999, 2005).

Production of dried shiitake in Japan has been steadily declining since the early 1980s (Yamanaka 2011). During the 10-year period from 2000 to 2009, dried shiitake production

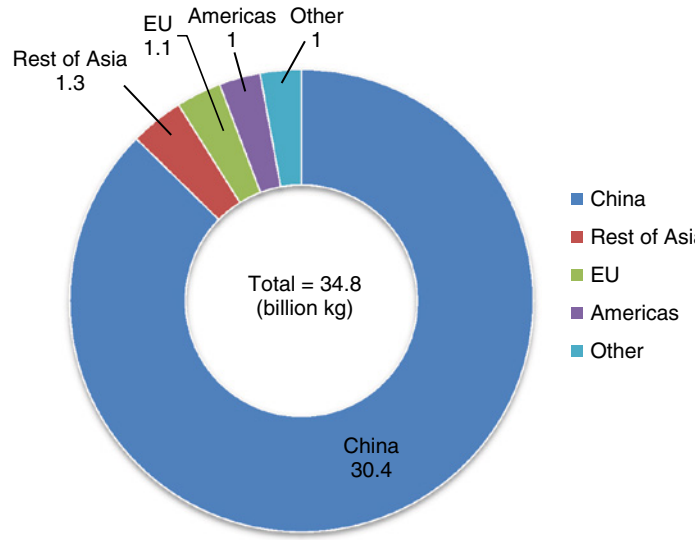


Figure 2.3 Cultivated mushroom production in China and selected regions of the world, 2013 (billion kg).

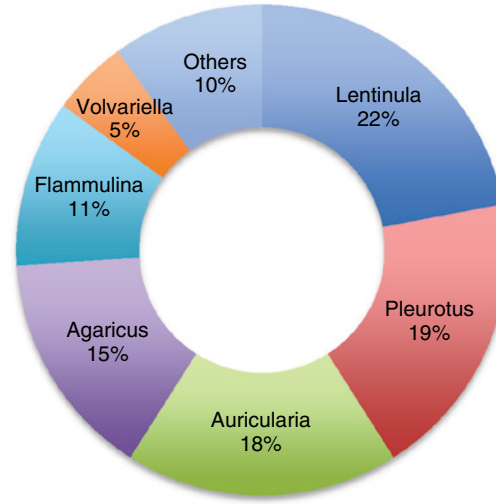


Figure 2.4 World edible mushroom production (% of total) by genus (2013).

declined by 37% while fresh shiitake production increased by 12%. Production increases for fresh shiitake were due to fulfilling the demand left by decreased imports of fresh shiitake from China. Total production of *L. edodes* (based on fresh mushrooms plus dried mushrooms converted to fresh equivalent) was slightly over 101 million kg in 2009, which ranked third with 22% of total production of edible mushrooms in Japan.

In the United States, most shiitake production is on nutrient-supplemented, sawdust-based substrates (Royse 1997, 2009, 2013, 2014). Many growers use a 16–20-day spawn run then remove the bag for browning of the exterior surface ("skin") of the "log" while other growers conduct spawn run and browning inside the bag. In general, higher rates of supplements may be used when logs are browned outside the bag resulting in higher yield potential compared to logs browned inside the bag. Over the last 10 years, shiitake production in the United States has increased by 24% (from 3.64 million kg in 2006 to 4.78 million kg in 2015) (USDA 2015). In recent years, sawdust-based logs made in China have been imported into the United States and

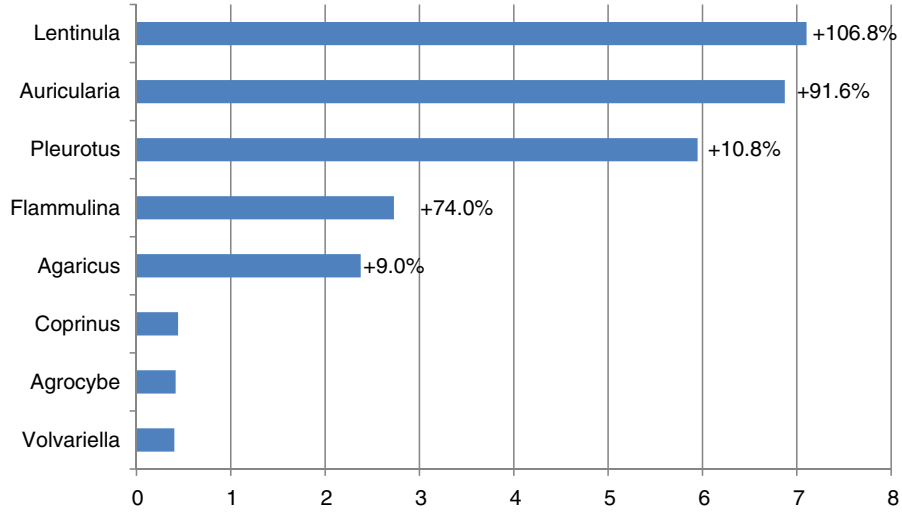


Figure 2.5 Mushroom production in China by genus (2013, CEFA 2014). Percentages following horizontal bars for each genus represent change from 2010 production levels (in billion kg).

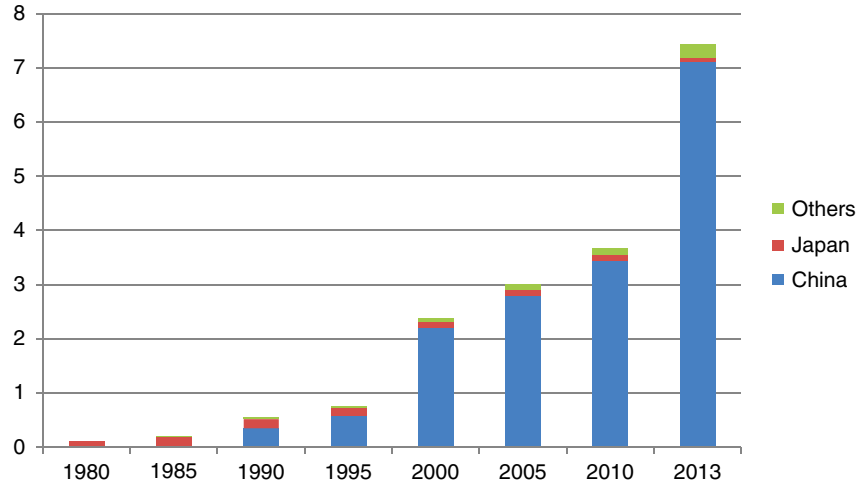


Figure 2.6 Growth in world shiitake production (1980–2013; billion kg).

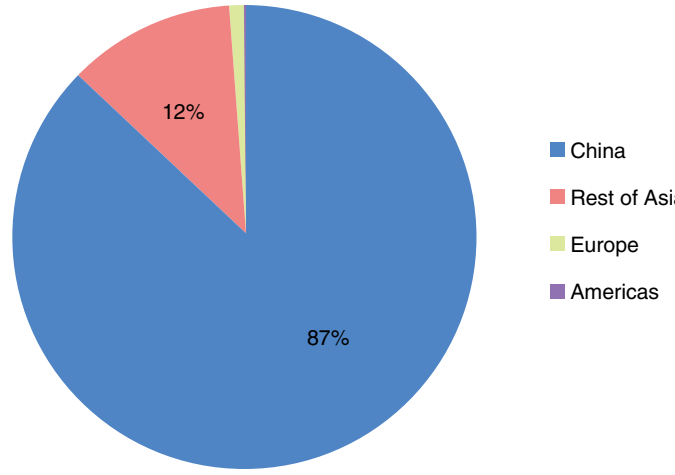
these logs have begun to gain traction with growers because of the relatively low cost and excellent mushroom quality (Figure 2.7).

2.2 *Pleurotus* spp.

Asian countries (especially China, Japan, South Korea, Taiwan, Thailand, Vietnam, and India) are the main producers and consumers of oyster mushrooms with approximately 99% of the total volume (Figure 2.8). China is the main producer with 87% of total world production of these species. Most of China's oyster mushrooms are from two species: *P. ostreatus* and

Figure 2.7 Sawdust-based "logs" of *Lentinula edodes*: (left) arranged in rows under shade cloth-covered shelters and (middle and right) with maturing mushrooms (photos: D. J. Royse and Q. Tan).

Figure 2.8 Percentage of total world *Pleurotus* spp. production in selected countries and regions.



P. cornucopiae. In the last 5 years or so, however, substantial increases in production of *P. eryngii* and *P. nebrodensis* have occurred. In China, administrative and professional agencies have developed plans to help guide growers in their initial selection of regions where production and utilization of resources may be optimized for mushroom production. The middle regions of China, especially the provinces of Henan, Hebei, and Shandong, are the major production areas for *Pleurotus* spp.

In Japan, production of *Pleurotus* spp. increased nearly 200% from 1997 (13.3 million kg) to 2010 (39.6 million kg). *Pleurotus eryngii* experienced the largest gains in production, in terms of percentage (+453%), increasing from 6.7 million kg in 2000 to over 37 million kg in 2009 (Yamanaka 2011). Most *P. eryngii* is cultivated on sawdust of Japanese cedar or ground corncobs supplemented with bran and contained in polypropylene bottles.

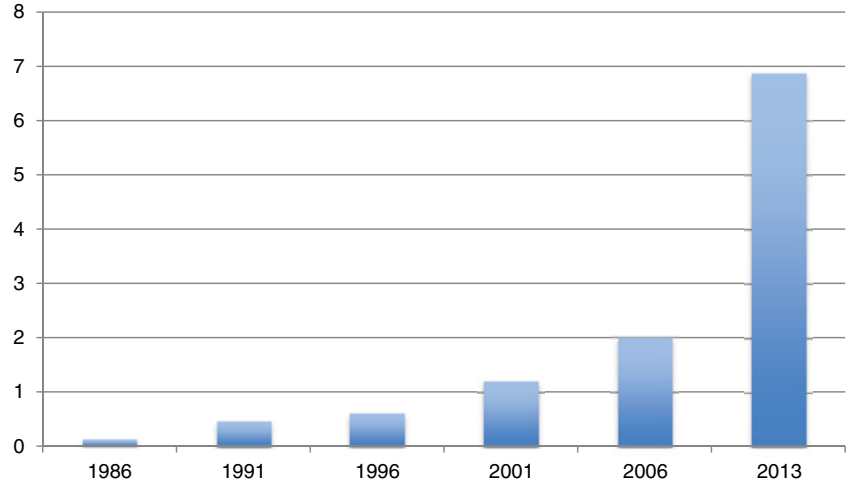


Figure 2.9 Growth in world *Auricularia* spp. production (billion kg) (1986–2013).

2.3 *Auricularia* spp.

Black fungus or wood-ear mushrooms (mostly *A. auricula* and *A. polytricha*), now widely cultivated in China, Taiwan, Thailand, Philippines, Indonesia, and Malyaysia, are considered the earliest cultivated mushrooms (Tang et al. 2010). Wood-ear production accounts for about 18% of the world's total output of mushrooms (Figure 2.4). Annual production of *Auricularia* spp. in China alone reached nearly 6.9 million kg in 2013, making them the second most widely cultivated mushrooms in that country (Figures 2.5, 2.9; CEFA 2014). Production figures for 2013 for this genus represent a 91.6% increase over 2010 figures.

Successful domestication of wild-type strains over an extended period of time by farmers in the Changbaishan and Shennongjia regions of China has led to rapid growth in production of these species. Some of the domesticated strains now have been introduced to new cultivation regions located in Northern and Southeastern regions of China.

2.4 *Agaricus bisporus*

China is the number one producer of *Agaricus bisporus* accounting for 54% (2.37 billion kg) of the world's total production of this species in 2013 (Figure 2.10). The USA produced about 9% (409 million kg) of the world's total followed by Poland (285 million kg), the Netherlands (270 million kg), and India (250 million kg).

In the last few years, production of *A. bisporus* in China has gradually moved northward as climatic conditions in the northern provinces are more conducive for mushroom production and raw materials are more readily available compared to southern provinces. This trend is expected to continue for the next few years (Li 2012). In the United States, production has increased about 11.7% over the last 10 years (from 378.9 million kg in 2006 to 423.2 million kg in 2015) (USDA 2006, 2015). Growth in production of the white variety has increased 10.1% while the brown variety (portabella and crimini) has increased 24.3% over this 10-year period.

Production of *A. bisporus* in Europe continues to move eastward (Lelley 2014; Royse 2014). Poland has become the world's third largest producer, outstripping the Netherlands by

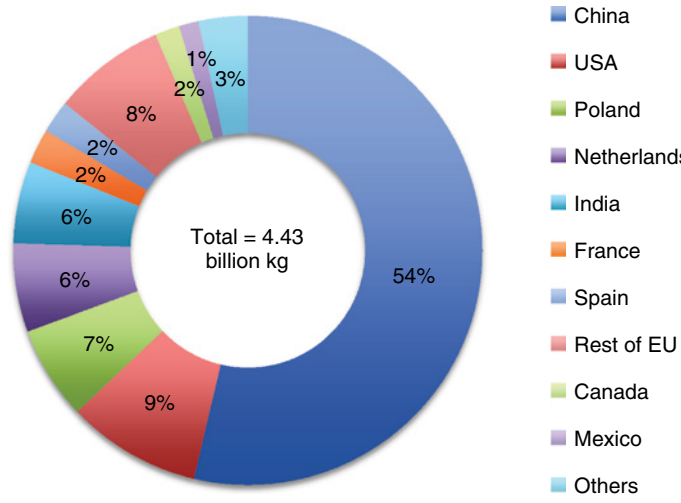


Figure 2.10 Production of *Agaricus bisporus* in selected countries and regions (2013).

approximately 9 million kg in 2013. This gap widened even further in 2014 with Poland producing 315 million kg while the Netherlands held steady at 270 million kg. Many Dutch-style farms have been constructed recently in Poland – especially in the eastern part of the country (Bieniecka and Dreve, 2012; Rozendaal 2012). Production output in Poland has recently become uncertain due to the conflict in the Ukraine and to the fact that approximately 90% of the Russian market was supplied by Poland.

In the Netherlands, the fourth largest producer of *A. bisporus*, over 90% of production is in the southeastern part of the country, that is, in the providences of Limburg, Brabant, and Gelderland (Baars 2012). Approximately 90% of the crop is exported as either frozen or canned (60%) while nearly 30% is exported as fresh mushrooms. The UK consumed about 41% of the fresh supply while France, Germany, Belgium, Norway, and Sweden bought most of the remainder of the fresh mushrooms (Baars 2012, Royse 2014).

2.5 *Flammulina velutipes*

Until the mid-1990s, Japan was the dominant producer of this species. Then, beginning in about 1997, China became the world's largest producer of *F. velutipes*. Production has increased from about 0.12 billion kg in 1995 to about 2.7 billion kg in 2013 (Figure 2.11).

In the last 10 years or so, many new enoki farms, based on bottle technology, have been constructed in China. In a description of one recent new enoki farm in China, Dreve (2014) describes the first stage of a large climate-controlled production facility covering nearly 7 ha of land and producing 60,000 kg of product per day. Expansion plans, if completed, could double this amount to 120,000 kg per day. About 80% of the farm's output is destined for the domestic market while the remainder is exported to countries in Southeast Asia and Europe.

2.6 Outlook

China is the main producer and consumer of cultivated, edible mushrooms worldwide. Growth in the mushroom industry in China, especially since 1997, is an accomplishment seldom duplicated in agriculture today. China has become an enormous producer of cultivated mushrooms, accounting for about 87% of the world's total output.

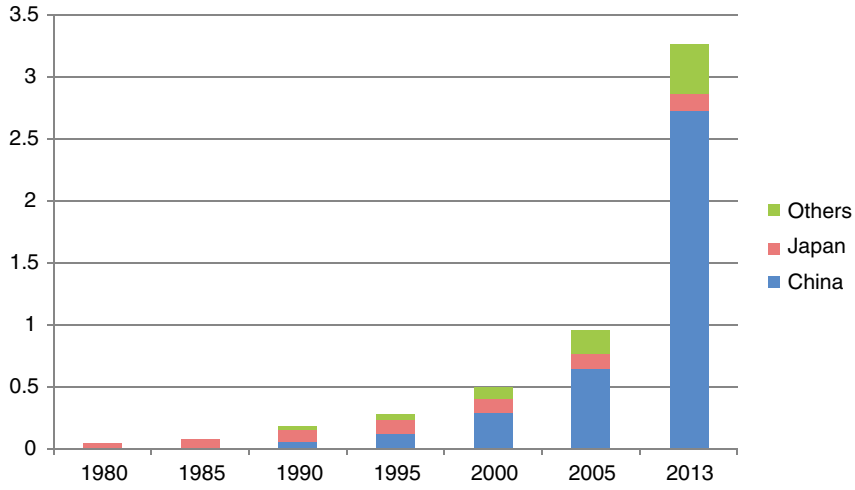


Figure 2.11 Growth in world production of *Flammulina velutipes* (billion kg) (1980–2013).

Lentinus edodes is now the world's leading cultivated edible mushroom with about 22% of the world's supply. Shiitake was traditionally cultivated on natural logs outdoors, but today most shiitake are cultivated indoors on nutrient-supplemented woodchips formed into varying shapes depending on the container in which they are grown. This allows for much faster production and leads to more crop cycles per year. *Lentinula* and four other genera (*Pleurotus*, *Auricularia*, *Agaricus*, and *Flammulina*) account for 85% of the world's total supply of cultivated edible mushrooms.

On average, consumers now enjoy about 5 kg of mushrooms per person per year. *Per capita* consumption is expected to continue to increase as consumers become more aware of the healthful benefits of incorporating mushrooms in their diet. Much more research is needed on the bioactive components in mushrooms to determine their biological responses in humans (Feeney *et al*. 2014). Promising evidence suggests that beta-glucan, vitamin D, selenium, and ergothioneine offer positive effects on immune function, intestine function, and weight management. It remains to be determined how often, how much and what species or mixtures of species should be consumed to bring about the desired biological response in humans. In the meantime, consumers can enjoy the unique culinary characteristics that mushrooms have to offer.

References

Baars J. (2012). Mushroom industry in the Netherlands – strong competitors. *World Society Mushroom Biology and Mushroom Products Bulletin* 7:1–3. http://wsmbmp.org/Bulletin_7_Content.html (accessed December 10, 2016).

Bieniecka K and R Dreve. (2012). Peiczarkalia shows Polish confidence. *Mushroom Business* 55:8–9.

Chang ST. (1999). World production of cultivated edible and medicinal mushrooms in 1997 with emphasis on *Lentinus edodes* (Berk.) Sing. in China. *International J. Med. Mush.* 1:291–300.

Chang ST. (2005). Witnessing the development of the mushroom industry in China. *Acta Edulis Fungi* 12 (Supplement):3–19.

China Edible Fungus Association (CEFA). 2014. *The survey results for the edible fungus 2013 annual analysis of China Edible Fungus Association*. www.cef.com.cn/ (accessed June 29, 2015).
Dreve R. (2014). Giant enoki farm. *Mushroom Business* 64:40–41.
Feeney MJ, Dwyer J, Hasler-Lewis CM., *et al*. (2014). Mushrooms and health summit proceedings. *J. Nutrition* (supplement) 1128S–1136S.
Lelley JI. (2014). State of the German mushroom industry. A brief summary. *WSMBMP Bulletin* 10:January 31, 2014. http://wsmbmp.org/B10Lelley.pdf (Accessed November 2, 2015).
Li Y. (2012). Present development situation and tendency of edible mushroom industry in China. *Mushroom Sci.* 18:3–9.
Royse DJ. (1997). Specialty mushrooms and their cultivation. In: J. Janick (ed.), Horticultural Reviews (Vol. 19), John Wiley & Sons, Inc.: New York, NY, pp. 59–97.
Royse DJ. (2009). Cultivation of shiitake on natural and synthetic logs. College of Agricultural Sciences, The Pennsylvania State University, University Park, PA. http://extension.psu.edu/publications/xl0083/view (Accessed February 18, 2017).
Royse DJ. (2013). Trends in mushroom production worldwide. Pages: 38–47. In: Proceedings of the 7th International Symposium on Mushrooms in Brazil, Manaus, Brazil.
Royse DJ. (2014). A global perspective on the high five: *Agaricus*, *Pleurotus*, *Lentinula*, *Auricularia* & *Flammulina*. Proceedings of the 8th International Conference on Mushroom Biology and Mushroom Products, New Delhi, India.
Rozendaal J. (2012). Poland and Ukraine. *Mushroom Business* 53:12–14.
Tang LY, Xiao L, Li L, *et al*. (2010). Analysis of genetic diversity among Chinese *Auricularia auricula* cultivars using combined ISSR and SRAP markers. *Curr. Microbiol.* 61(2):132–140.
United States Department of Agriculture (USDA) (2015). Mushrooms. National Agricultural Statistics Service, Agricultural Statistics Board. 17 p. http://usda.mannlib.cornell.edu/usda/current/Mush/Mush-08–20–2014.pdf (Accessed November 2, 2015).
United States International Trade Commission (USITC). (2010). Mushrooms Industry & Trade Summary. Office of Industries, Publication ITS-www.usitc.gov/publications/332/ITS_7.pdf (accessed December 10, 2016).
Yamanaka K. (2011). Mushroom cultivation in Japan. *World Society Mushroom Biology and Mushroom Products Bulletin* 4:1–10. http://wsmbmp.org/Bulletin_4_Content.html (accessed December 10, 2016).

3

Mushrooms: Biology and Life Cycle

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3.1 Life Cycle of Fungi

Mushrooms are macroscopic fruiting bodies produced by ascomycete and basidiomycete fungi during their sexual reproduction cycles. Most well-known species of consumed mushrooms belong to the group Basidiomycota. In addition, all cultivated species of economic importance are basidiomycetes, including such genera as *Agaricus*, *Pleurotus*, and *Lentinula*, which represent the majority of mushrooms cultivated in the world. Therefore, throughout this text, most of the information will be directed to basidiomycetes mushrooms; however, certain important ascomycetes will be addressed, including model species that do not produce mushrooms.

The life cycle of a fungus depends upon its nutritional strategy, which also defines the level of difficulty in cultivating the mushroom species for commercial purposes. Accordingly, fungi decomposers (saprophytes) are, in principle, relatively easy to cultivate. In contrast, mycorrhizal mushrooms, some species of which retain very high market value, are not able to be cultivated artificially, due to the need for direct interaction with specific tree species and complex interactions with other types of soil microorganisms, which until recently have been poorly understood. Of the many mycorrhizal mushrooms, truffle cultivation in artificial orchards has shown some success, but in general, the cultivation of certain *Tuber* species and other mycorrhizal mushrooms remains difficult.

Many species of fungi have the ability to modify their nutritional strategy to cope with environmental variation, or more often, the presence or absence of a host. Phytopathogenic fungi are a classic example of this versatility, as they switch from phytopathogenic to saprophytic modalities when their hosts die and are transformed into organic matter. This versatility enables them to survive until new living hosts with which to resume pathogenic activity become available. Mycorrhizal fungi can also exhibit saprophytic activity soon after germination of spores, until the hyphae establish a symbiotic relationship with the root tips of symbiont tree species.

In addition to varying nutritional strategies, the geographical origin of a particular species of mushroom can determine the specific change in temperature that induces its fruiting. In temperate climates, mushroom species are adapted to cycles imposed by well-defined seasons, making imperative the development of mechanisms for temperature recognition to trigger the beginning of a new cycle, as in the arrival of the autumn–winter period. It is common to refer to the lowering of temperature that induces fruiting as a stress factor. However, in nature, it is simply a mechanism that serves to define whether it is time to grow vegetatively, remain dor-

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mant, or fruit. Moreover, it is important to emphasize that the temperatures most often used for fruiting induction (12–19°C) are considered mild temperatures in species acclimated to regions with severe winters, where the temperature frequently drops below zero. Under normal fruiting conditions, a temperature of 18°C would be interpreted by the physiological system of the fungus as time to fruit before winter arrives. However, for cold climate species, the same temperature would mean the end of winter and beginning of spring, a factor that triggers mushroom fruiting following a dormant period during the adverse conditions imposed by a severe winter.

In tropical countries where seasons are not well defined and temperature is maintained in a specific range, mushrooms may be produced throughout the year without requiring temperature-sensitive development triggers. The triggering factor for fruiting in these regions is rainfall, as fungi require a high relative humidity in order to fruit. It is therefore common to produce mycorrhizal mushrooms during summer months, when high temperatures are accompanied by periods of rain. Fruiting bodies normally occur after heavy rains, and drops of temperature during such periods can also play an important role in this process.

At first, it may seem to be advantageous to grow mushrooms that are not influenced by low temperature, especially in developing countries where the maintenance of temperature-controlled mushroom houses greatly increases production costs. In industrial-scale production, however, use of mushroom species adapted to concentrate their fruiting in narrower periods yields advantages associated with shorter crop cycles. *Agaricus bisporus* is the best-known example of this type of mushroom; its crop cycle concludes 30 days following the induction of fruiting. On the other hand, *Agaricus brasiliensis* (also known as *A. subrufescens* and *A. blazei*), native to Brazil and other warmer climates, does not require a temperature decrease for fruiting induction, but necessitates a long cultivation cycle of 2–3 months. Until now, the consensus has been that *A. brasiliensis* fruiting induction is not influenced by temperature variation, in accordance with its native environment of warm climate, where mushroom fruiting occurs in longer cycles and results in weaker flushes.

For mushrooms less amenable to cultivation, particularly those for which the induction of fruiting is not temperature dependent, it is important to study other factors implicated in triggering the fruiting process. As such, in this chapter we will discuss the general aspects of the life cycle of basidiomycetes, with an emphasis on sexual reproduction.

The cultivation of mushrooms presents as a basic prerogative the process of sexual reproduction during the fungal life cycle. Many species of fungi, most of them belonging to the ascomycetes group in which asexual reproduction is predominant, have no known sexual cycle. In addition, several species phylogenetically related to the basidiomycetes are classified as "*Mycelia sterilia*" as they are not known to produce spores. However, some of these species may reproduce sexually, but only under very specific conditions, making it difficult to induce sexual reproduction in the laboratory.

Most species that utilize sexual reproduction produce macroscopic fruiting bodies, which have been known to mankind since antiquity. Some of the most appreciated species for human consumption belong to the Ascomycota phylum; such mushrooms may be collected directly from nature or produced in artificial orchards. However, most of the mushroom species consumed by man, including several other species of wild mushroom as well as numerous cultivated species, belong to the Basidiomycota phylum. As a result, a large volume of research about the mechanisms involved in sexual reproduction is focused on the basidiomycetes. While there exists a great deal of information about the sexual reproduction of ascomycetes, these studies were done mostly with pathogenic species (particularly plant pathogens), species of biotechnological interest, or model species. In this context, much of the knowledge about fungal sexual reproduction is derived from studies of the ascomycete *Saccharomyces cerevisiae*.

This yeast has been a model species for many years apart from its great biotechnological importance, due to its easy cultivation and amenability to research. During the first half of the twentieth century, the filamentous ascomycete *Neurospora crassa* was an important model species for classical genetic studies. Subsequently, given their importance and the peculiarities that distinguish them from ascomycetes, the basidiomycetes have become objects of study as well, in order to better understand their mechanisms of sexual reproduction. Two of these species in particular, *Schizophyllum commune* and *Coprinopsis cinerea*, are now models for basidiomycete genetic research.

Although not the first to study the genetics of fungi, the great awakening in the genetics of basiodiomycetes occurred as a result of the work of John and Carlene Raper, summarized in *Genetics of Sexuality in Higher Fungi* (Raper 1966). *The Mycota*, edited by Karl Esser, also dedicated a significant component to fungal genetics. These scientists both contributed to the science of fungi directly and encouraged a new generation of fungal geneticists, among them Lorna Casselton and Ursula Kües, who went on to expand upon the legacy of John and Carlene Raper, both in the generation of scientific knowledge and in the formation of subsequent generations of fungal geneticists.

This chapter is not intended as a comprehensive review of the fungal life cycle, as there are already several exceptional works of deep detail for those interested in this field of science, for example, *Sex in Fungi: Molecular Determination and Evolutionary Implications* (Heitman et al., 2007), in addition to *The Mycota I* (Kües and Fischer, 2006). The purpose of this chapter is to present an introduction to the subject for the student or mushroom grower who is interested in entering the fascinating area of Mycology, enabling access to the basic principles of the mushroom life cycle with an emphasis on sexual reproduction and its underlying mechanisms.

3.2 The Subkingdom Dykaria

Ascomycetes and basidiomycetes are unique in the kingdom Fungi for the dikaryotic phase of their life cycle. Due to this feature, these two phyla exclusively form the subkingdom Dikarya, and are known as higher fungi as a result of their greater complexity and ability to produce macroscopic structures. The two groups are distinguished from one another by their modes of sexual spore production (ascospores versus basidiospores), from which the names of the two phyla were derived. Ascomycetes and Basidiomycetes have other, minor differences, as well, such as dikaryophase time and mating type genes.

Dikaryosis is generally understood as an evolutionary stage between haploidy and diploidy; it remains intriguing how these evolutionarily arrested organisms have maintained this condition without losing their competitiveness. In fact, evidence affords a competitive advantage to the dikaryon under heterogeneous environments, as the dikaryon is more flexible, with its greater phenotypic amplitude with which to cope with environmental variations. While certain species of Basidiomycetes form a diploid rather than undergoing a dikaryophase following plasmogamy (e.g., *Armillaria mellea*), the vast majority of known species of basidiomycetes spend most of their life cycle in dikaryophase. Because of this, it is important to understand the different characteristics of the dikaryophase of ascomycetes versus basidiomycetes.

3.2.1 Dikaryosis: Concepts

Dikaryosis is defined as an association of haploid gametic nuclei in a single compartment that is not immediately followed by karyogamy. This is a unique phenomenon found only in fungi; most eukaryotes undergo plasmogamy and karyogamy in rapid succession, yielding a diploid

nucleus. For ascomycetes and basidiomycetes, however, plasmogamy and karyogamy are temporally disparate events, in particular for basidiomycetes, in which the dikaryon remains for an extended period following plasmogamy. For these organisms, the dikaryophase comprises a major period of the fungus's life.

In a typical dikaryon, two haploid nuclei are paired in the same compartment and maintain their individual haploid status rather than fusing into a single, diploid nucleus. *Schizophyllum commune*, *Coprinopsis cinerea*, and *Lentinula edodes* are examples of species that produce dikaryons. Some species of basidiomycetes have multinucleated compartments, and are not dikaryons in the strict sense of the word; nonetheless, current use of the term dikaryosis is not restricted to situations wherein only two haploid nuclei occupy the compartment. Additionally, many authors refer to an individual with two types of gametic haploid nuclei derived from different parents as a dikaryon, and the term dikaryon is also used synonymously with heterokaryon, wherein different nuclei are present in the same compartment. *Agaricus bisporus* and *A. subrufescens* are examples of basidiomycetes that produce heterokaryons with multinucleated compartments. Such nuclear behavior differences consequently result in morphological differences, which will be discussed later.

While both are dikaryotic, Ascomycota and Basidiomycota display some key differences. As mentioned previously, for basidiomycetes, the dikaryophase is a prolonged, vegetative stage, whereas for ascomycetes, the dikaryon is usually restricted to the ascogenic system within fruiting bodies, especially when the partner that acts as female produces a morphologically distinct structure to fulfill this role. In this context, it is important to emphasize that ascomycetes display morphological differences between male and female partners, whereas basidiomycete partners are usually morphologically indistinguishable. Another important morphologic difference is that the basidiomycete produces clamp connection at each apical cell division, whereas ascomycetes produce an alternate structure called a crozier. The clamp connection in basidiomycetes maintain an ordered heterokaryotic state of hyphae at every cell division. The crozier ensures dikaryosis in the ascogenic hyphae, where the ascus will be produced, in a similar function to the clamp connection, but to a more limited effect. While a clamp connection appears immediately after the dikaryon is established, a crozier is produced later in the ascogenic hyphae. Despite these morphological variations, ascomycetes and basidiomycetes share the characteristic of presenting an intrinsic combination of two haploid nuclei originating from the genotypes involved.

This leaves the question of how dikaryons are formed. In the following sections, we will discuss the basic mechanisms that control breeding between basidiomycetes and the structures and external factors involved in the process.

3.3 Homothallism, Heterothallism, and Amphithallism

First, it is important to become familiarized with the different types of hyphae, from germinating spores to the formation of a fruiting body. Germination of a uninucleate sexual spore or binucleate with identical nuclei gives rise to monokaryotic and homokaryotic hyphae, respectively.

Interaction between hyphae from different thalli leading to plasmogamy followed by nuclear migration to the tip of the hypha results in a complete dikaryotic mycelium known as dikaryon. Since cell fusion occurs between different, compatible individuals (with different nuclei), and the resulting dikaryon will also be a heterokaryon.

For homobasidiomycetes, there is a principle of differentiation during the formation of fruiting bodies that leads to the formation of a pseudotissue known as the pseudoparenchyma. In simpler language, different types of hyphae may be referred to as the primary mycelium (monokaryon), secondary mycelium (dikaryon), or tertiary mycelium (pseudoparenchyma). These terms are rarely used today, particularly in the context of genetics and molecular biology; however, they remain practical for a less specialized audience.

The monokaryon and dikaryon are less differentiated than the pseudoparenchyma (fruiting bodies), but it is possible to observe morphological differences between them. Typically, the monokaryon displays less robust mycelial growth compared to the dikaryon, and the loss of monokaryotic cultures following several subculture cycles is not uncommon, in function of its weakness of growth. The dikaryon, in turn, has more vigorous growth and is much more stable. In addition, for many species of basidiomycetes, the dikaryon produces a structure known as the clamp connection. This clamp connection allows each compartment to receive two distinct nuclei, maintaining the heterokaryotic state of the hyphae. The clamp connection is therefore an important morphological marker that distinguishes the dikaryon from the monokaryon in such species. However, this is only possible for the basidiomycetes that produce typical dikaryons. In *Lentinula edodes*, for example, the dikaryon produces frequent clamp connection that are easily observable under optical microscopy; this is not the case for *Agaricus bisporus* and *A. brasiliensis*. For these two species, a fertility test of monosporic cultures or the use of molecular markers is necessary to distinguish between homokaryosis or heterokaryosis (Nazrul and Yin Bing, 2011; Rocha de Brito et al., 2016).

3.4 Heterothallism

A fungal species is considered heterothallic when its sexual spores germinate autosterile monosporic cultures thereby requiring a cross with another culture (thallus) to generate a dikaryon, which is then able to complete the life cycle. In this case, the sexual spores have a single nucleus, or when the spores are binucleate, the nuclei are identical. *Lentinula edodes* is an example of a heterothallic species, as their monosporic cultures are unable to produce fruiting bodies unless they are crossed with another compatible monosporic culture.

3.5 Homothallism

A fungus is considered homothallic when a colony originating from a single spore is able to complete its life cycle, producing fruiting bodies via autofertilization. A homothallic species allows inbreeding among genetically identical hyphae and sharing of identical nuclei in the same compartment. However, certain homothallic species nonetheless require different genetic factors to consolidate the sexual cycle, present in different nuclei. Therefore, although specific sexual factors are required, these individuals are considered homothallic, since their monosporic cultures are self-fertile. It is important to emphasize that the concepts of homothallism and heterothallism were established in the context of whether a single thallus was able to undergo a complete life cycle without consideration of the necessity of distinct mating types genes.

To permit distinction between these species and typical homothallic species, the former is referred to as "secondary homothallic" or "pseudo homothallic," that is, they fulfill the basic requirements for homothallism; however, the presence of two distinct sex type genes in the same compartment is required for completion of the sexual cycle.

The typical species example of this system is the button mushroom *Agaricus bisporus*, known to produce predominantly binucleate spores with sexually distinct nuclei, able to produce fruiting bodies without being crossed with another culture. The categorization is imperfect, however, as the same species may differ in the number of spores per basidia. In the case of *A. bisporus*, a small percentage of basidia produce four, rather than two, basidiospores. These spores do not give rise to self-fertile cultures and are therefore considered heterothallic. Adding to this complexity, a particular *A. bisporus* strain has been found that predominantly produces four spores instead of the typical two spores per basidium (Callac et al., 1993). Therefore, even within the same species, both secondary homothallic and heterothallic strains can exist.

3.6 Amphithallism

Further complicating matters are the amphithallic species, able to produce both homokaryotic and heterokaryotic spores, such that the same strain may give rise to both secondary homothallic and heterothallic cultures. The most common (but not only) circumstances in which this occurs are when bisporic and/or trisporic basidia are present alongside tetrasporic basidia. Bisporic and trisporic basidia give rise to binucleate spores, usually with sexually distinct nuclei, while tetrasporic basidia normally give rise only to homokaryotic spores. Therefore, the same basidiocarp is able to produce spores that will follow a heterothallic life cycle (homokaryotic spores) as well as spores that follow a secondary homothallic life cycle (heterokaryotic spores), a condition defined as amphithallism. The *A. brasiliensis* species is a textbook example of this type of life cycle, displaying wide variation in its production of bisporic, trisporic, and tetrasporic basidia (Kerrigan, 2005). This feature can be influenced by environmental conditions, mainly temperature, but studies with *A. brasiliensis* also show that different strains produce different ratios of tetrasporic, trisporic, and bisporic basidia even when cultured under the same environmental conditions (Herreira et al., 2012).

While genetic determinants are somewhat responsible for these production ratios, other factors can also facilitate the transition between heterothallism and homothallism within the same species, particularly in the ascomycetes group. The historic research model yeast *S. cerevisiae*, for example, has both heterothallic and homothallic strains. Those that are homothallic have a heterothallic control mechanism; as the cells divide, a switching mechanism promotes the replacement of one mating type for the other. This mechanism allows the changing of the mother cells to the opposite mating type, thus ensuring the presence of cells of different mating types in a previously autosterile culture. According to this model, pseudo homothallism is a strategy to break down heterothallic genetic control. Besides mating type switching and the production of heterokaryotic spores, other mechanisms of homothallism include the presence of unlinked or occasionally fused mating type loci. These mechanisms produce the same results as found in heterokaryotic spores, but with the different mating types present in a single nucleus. For a better understanding of this wide range of mechanisms, it is necessary to delve into the genetic underpinnings of these processes; we recommend the book *Sex in Fungi: Molecular Determination and Evolutionary Implications* (Heitman et al., 2007) as an excellent resource.

As discussed earlier, the concepts of homothallism and heterothallism were originally defined in the context of self-fertility versus self-sterility of monosporic fungal cultures prior to knowledge of the genetic factors responsible for these different phenotypes. Research has since shown that such characteristics are controlled by an intricate genetic control interplay, the purpose of which is to trigger a series of biochemical responses via a pheromone system of

ligand–receptor interactions on the cell membrane that vary among species. However, certain main genes and their products will be discussed next.

3.7 Mating-Type Genes

For any eukaryotic organism, sexual reproduction is important to produce descendants, and for the generation of genetic variability. For fungi, in which morphological distinctions between male and female individuals are not always apparent, it is necessary to have mating-type genes to ensure that such interactions can occur. The genes involved in the process include those whose function it is to prevent self-crossing in order to promote genetic variability.

Taking, for example, a heterothallic species, the cross between two individuals (thalli) will occur only if the two parents are sexually compatible, or of distinct mating type genetic complements. Two mating-type systems can be found in these species. In the first, known as a bipolar system, one locus ensures the compatibility of the interaction, such that two individuals are compatible if they display alternate alleles at this locus, most often defined as **a** and **α**, MATa and MATα, or EHV-1 and EHV-2. The second, a tetrapolar system, involves two unlinked loci behaving as independent genes. This system generates spores of four different possible mating types, and to have compatibility between thalli, it is necessary that the two individuals be different at both loci. For example, an *A1B1* individual will be compatible with another individual *A2B2*, but will not be compatible with an *A2B1* individual, although some exceptions have been observed. It is interesting to note that a large number of alleles can be found for the two loci, so that the probability of finding a compatible individual is very high, making the outcrossing probability almost 100%.

Considering the distribution of the two systems between ascomycetes and basidiomycetes, there is a preference by ascomycetes for the bipolar system, while the tetrapolar system is somewhat more common in basidiomycetes, although a significant number of basidiomycetes favor the bipolar system. Homothallism is more common among ascomycetes, but among basidiomycetes, the Agaricomycetes, a mushroom-producing group, is mostly heterothallic. Of importance, a large number of secondary homothallic species have evolved from the heterothallic and bipolar system. A plausible cause for this is that it is easier to bypass heterothallic control and generate secondary homothallism in a system controlled by a single locus.

As mentioned previously, the yeast *S. cerevisiae* has become the primary model for detailed studies on mating type genes. Based on discoveries made with *S. cerevisiae*, these same mechanisms were found in filamentous ascomycetes and basidiomycetes, including the Agaricomycetes. It is now well established that although there are many variations, they are all derived from a single system. In brief, one locus is responsible for coding pheromones and pheromone receptors, while another locus encodes transcription factors that regulate gene expression along a sequence of events resulting in the migration of the nucleus to the apical hyphal compartment, formation of the dikaryon, and finally karyogamy and meiosis processes that generate basidiospores and ascospores.

Among the many variations, for *S. cerevisiae* and for several filamentous ascomycetes species, the pheromone–receptor interaction promotes attraction and cell fusion; however, for mushroom-forming basidiomycetes cell fusion can occur independently of this interaction. Rather, that system is utilized to facilitate other important processes, such as the formation of the clamp connection.

In *C. cinereus*, for example, the interaction between hyphae does not require pheromone–receptor contact. Only after the hyphal fusion has taken place and both nuclei are paired do the mating type genes determine the sequence of events. This explains the formation of a mycelial network by anastomosis in the absence of sexual reproduction. This does not mean that other

genetic factors are not necessary to ensure vegetative compatibility for anastomosis. In fact, such mechanisms are well described for the species of ascomycetes.

Mating type genes are located at two, unlinked loci (located at different linkage groups in the genome). These genes are known as *A* and *B*, and it is necessary that crossing partners bear different alleles at each of the loci for the sexual process to occur. In the case of basidiomycetes, these genes are multi-allelic (represented by a large number of alleles), which greatly increases the probability of outcrossing.

The *B* genes encode pheromones and pheromone receptors which are not required for cell recognition, as with *S. cerevisiae*, but rather trigger the nuclear migration process and facilitate formation of the clamp connection, when the hook must merge with the hyphal compartment that will receive the nucleus. In this aspect, the pheromone–receptor system plays a similar role to that observed in *S. cerevisiae*, but far later in the process and with a completely different purpose, a remarkable variation of the system.

In turn, the *A* genes encode transcription factors that enable the expression of genes required for synchronization between the division of the nucleus and the formation of the clamp connection. Functional transcription factors consist of two monomers, each from the different mating type partners, resulting in a heterodimer. In the same way that a receptor does not recognize a pheromone from its own mating type, a protein monomer does not form a functional transcription factor with a monomer derived from the same mating type. Therefore, the presence of different alleles for both genes (*A* and *B*) is necessary for the development of the dikaryon and the successive events that will culminate in meiosis and the production of sexual spores.

Certain rare mutations can disrupt regulation of this process. For example, a mutation that results in nonselective pheromone recognition by a receptor can override the requirement for distinct mating types. Likewise, changing one or two amino acids in a protein encoded by an *A* gene may allow the formation of heterodimer from monomers originally designated incompatible, underlining the sensitivity of the recognition mechanism, wherein small changes in the genome can result in drastic changes in pairing compatibility. Even for the basidiomycetes, small genetic changes can result in significant morphological differences among fruiting bodies. This often resulted in mushroom species misidentification, back when the taxonomic standards were restricted to the morphological characteristics of fruiting bodies and sexual spores.

3.8 *Agaricus brasiliensis* (Syn = *A. subrufescens* or *A. blazei*): An Intriguing Example of Amphithallism

Hundreds of papers have been published about this mushroom, but only a small number refer to cytology, nuclear behavior, or the fungal life cycle. The poverty of work in this area undoubtedly reflects the difficulty in studying a species that does not have the classic dikaryotic system. The first work on the cytology of *Agaricus brasiliensis* showed that the hyphae of heterokaryotic cultures were multinucleated (Labory et al., 2003). The presence of multinucleated hyphae was evidence of a more complex life cycle as compared to typical dikaryons. In a later study (Dias et al., 2008) with different strains, it was evident that the number of nuclei can range from 1 to 15, wherein the most frequent number was five per compartment, followed by six and four, respectively. An odd number of nuclei indicates that one of the nuclei has not divided and should be considered to be in a transitional phase; as such, it was concluded that *A. brasiliensis* most frequently have six nuclei per compartment. In this same study, the size of the compartments was determined as well as the diameter of the hyphae in different strains. Diameters

ranged from 3.5 to 7.0 μm with most between 4.0 and 5.0 μm, and compartment length was generally between 50 and 100 μm.

One of the most interesting aspects of the work was the fluorescence microscopy of nuclear behavior during the formation of basidiospores in a strain that produces tetrasporic basidia. The authors have chosen this strain for the study of nuclear behavior as basidiospores overwhelmingly produce the same number of nuclei. Using this approach, we observed spores containing only a single large nucleus and spores containing two smaller nuclei, suggesting that each basidiospore should present only one meiotic nucleus followed by post-meiotic mitotic division giving rise to two homokaryotic nuclei. Given these data, it was suggested that this species follows a principle of heterothallism, as homokaryotic monosporic cultures are self-sterile.

However, Herreira et al. (2012) later demonstrated that this species may vary in the number of basidiospores by basidia depending on the strain (Figure 3.1). According to Herreira, the frequency of tetrasporic basidia can be as low as 29.9% in the strain CS7, while the frequency of trisporic basidia can be 46.9% and that of bisporic basidia 23.2% in the same strain. These

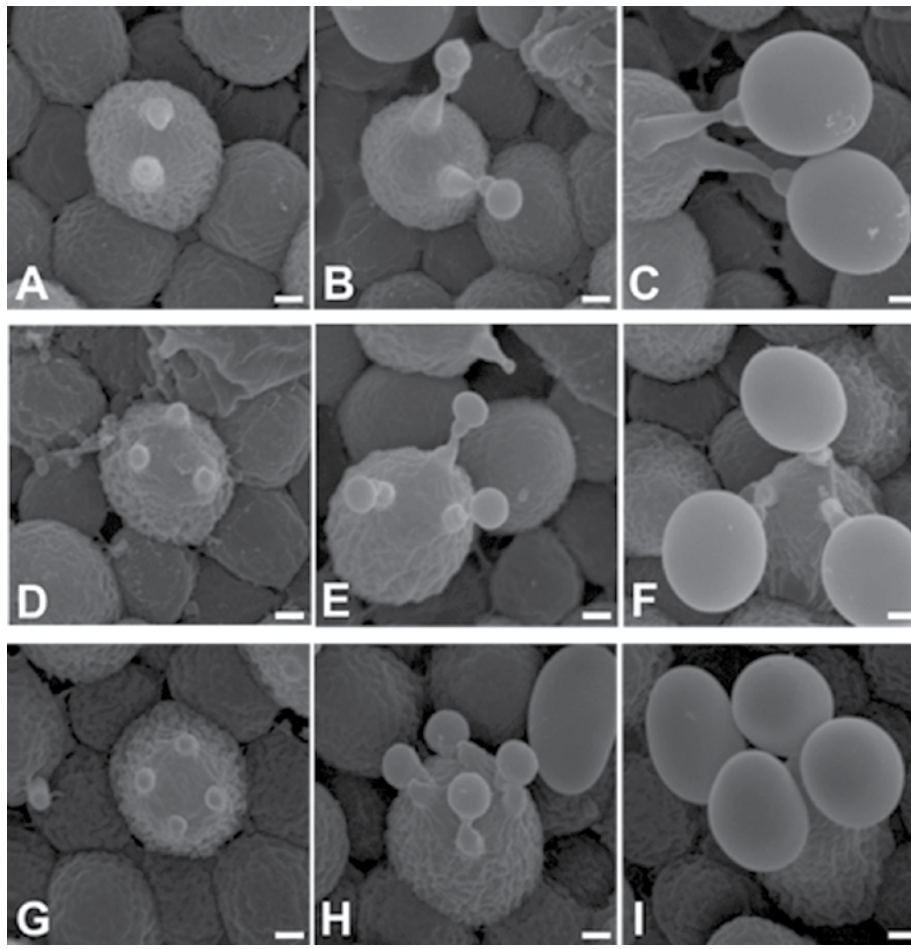

Figure 3.1 Scanning electron micrographs of *Agaricus brasiliensis* gills. A–C: Bisporic basidia. D–F: Trisporic basidia. G–1: Tetrasporic basidia. Bar = 1 mm. Herreira et al. (2012), Mycologia. With permission.

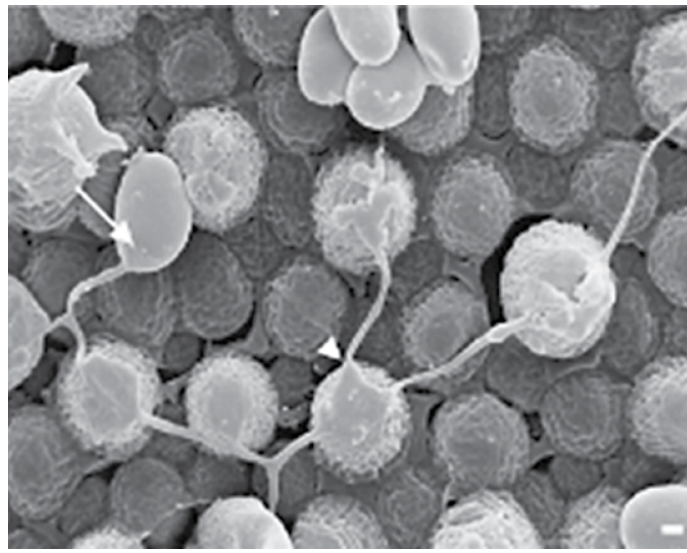

Figure 3.2 Scanning electron micrographs of *Agaricus brasiliensis* gills. The arrow indicates a basidiospore linked to a connection hyphae and two basidia. Arrowheads indicate sterigmata connected to one another by a hypha. Bar = 1 mm.

values are quite different from strain CS10, which presented frequencies of 93.9, 5.9 and 0.2% of tetrasporic, trisporic, and bisporic basidia, respectively. These results showed that large numbers of basidiospores containing two distinct nuclei will generate self-fertile, monosporic cultures, indicating a shift to secondary homothallism. These results confirm an earlier statement by Kerrigan (2005) that this species is actually amphithallic.

An intriguing finding in the work of Herreira was the production of connection hyphae between basidia of *A. brasiliensis* (Figure 3.2). At first, it was suggested that these structures allowed for the passage of nuclei from one basidia to another; however, Ribeiro (personal communication) later reported that the diameter of connection hyphae is too narrow to allow for nuclear migration. As such, the function of these structures remains a mystery.

Returning to the question of amphithallism, Thongklang et al. (2014) uncovered a high heterokaryon rate (40.43–75.46%, depending on the strain). The authors did not detect bisporic basidia, and trisporic basidia were rare (less than 1%) between basidia from the three parental strains used in the work. Considering that the basidia produced by each strain are predominantly tetrasporic, the high frequency of amphithallism in these strains can not be explained by the production of binucleate basidiospores derived from trisporic or bisporic basidia alone. These results instead suggest that heterokaryotic spores are formed due to post-meiotic mitotic division inside the basidia rather than the basidiospore. Further studies using eletron microscopy may be necessary to clarify nuclear behavior during formation of basidia and basidiospores.

3.9 Life Cycle of Uncultivated Mushrooms

Some of the most appreciated edible mushrooms in the world are produced from mycorrhizal fungi, particularly from symbiotic associations with trees from the genera *Pinus*, *Quercus*, and *Corylus*. Of all known species of mycorrhizal mushrooms, we have chosen to highlight *Tuber*

and *Morchella* (ascomycetes), and *Tricholoma*, *Boletus,* and *Cantharellus* (basidiomycetes). All of these genera have in common a much more complex life cycle as compared to the saprophytic mushrooms. This renders their cultivation far more difficult, and their consumption has been possible primarily due to their frequent natural occurrence during autumn, winter, and spring, depending upon species and geographical region. In recent decades, great efforts have been undertaken to develop cultivation techniques for these mushrooms in association with their symbiont plants (Hall et al., 2003). Some of these are discussed next.

3.10 The Truffles

The genus *Tuber* (Ascomycota) encompasses several species of which we highlight *T. melanosporum*, known as black truffle or black Périgord, and *T. magnatum*, known as Italian white truffle. For a long time, these mushrooms were predominantly European in origin, as a result of their natural occurrence and traditional continental consumption. However, due to the decline in the wild truffle harvests over the years, European countries and others with favorable climates have stepped up efforts to cultivate these mushrooms in commercial orchards. Oak (*Quercus* spp.) and hazelnut (*Corylus avellana*) are preferred species for commercial crops. However, proper handling is necessary to minimize competition from other mycorrhizal species in the establishment of the crop, since species of interest are not always robust in effecting a mycorrhization process, as has been observed with *T. magnatum*.

The life cycle of these mushrooms is not as well known as that of saprophytic mushrooms, as it is still not possible to carry out all stages of their life cycle in a laboratory setting. However, it is believed that truffles follow the same basic pattern as other species of ascomycetes, with particular differences, such as those pertaining to interactions with other soil microorganisms. The life cycle begins with the germination of spores which originate homokaryotic hyphae able to interact with the roots of the host plant. Hosts can harbor primary hyphae of different mating types, and these hyphae can extend up from the mycorrhizae growing in the soil to meet hyphae of the opposite mating type, resulting in plasmogamy and giving rise to the ascogenic hyphae, which then undergo subsequent processes of karyogamy and meiosis. As also occurs in other ascomycetes, the ascocarp is not constituted, uniformly, by heterokaryotic hyphae, since plasmogamy usually occurs within the structure which will originate the ascocarp. Following plasmogamy, the ascogenic hyphae is surrounded by homokaryotic hyphae of maternal origin, which are responsible for the formation of the majority of the truffle. Some evidence indicates that plasmogamy occurs only after the primordium is formed, such that the heterokaryotic phase of the fungus is extremely brief.

Truffles are usually harvested during autumn and winter. Depending on the region, the harvest may begin as soon as temperatures start to drop in the fall, or earlier according to the timing of rainy periods. Harvests can end before winter or shortly after it begins. In other regions, harvest may begin later in the fall and continue on through winter. In general, the sexual cycle is stimulated by a temperature drop the following summer, and spores survive through the winter and then restart the cycle in the spring.

3.11 Morels

The *Morchella* genus is responsible for the production of mushrooms known as morels, among which the species *M. esculenta* stands out. Morels were originally considered saprophytic, but now it is believed that this species can also present as mycorrhizal. Although

they are a cosmopolitan species found in various regions and continents, these mushrooms are strongly identified with North America, where a tradition of professional collectors has sprung up to meet market demand. Although they are not mycorrhizal-obligate fungi (Tedersoo et al., 2013) and can live as saprophytes, cultivation as such is extremely difficult. This species has adapted to a fire ecology, since naturally occurring, low-intensity fires are key to increasing mushroom production in the wild (Wurtz et al., 2005). Without such events, mushrooms are produced in much lower numbers. The fire effect generally manifests one or two summers following the fire. Their life cycle is marked by the production of sclerotia, which are resistant structures usually formed by the secondary mycelium. After a disturbance event, the sclerotia germinate to form an ascogenic mycelium, which then gives rise to the fruiting body (morel). Several environmental factors (extreme temperatures, heavy rainfall, lack of humidity, prolonged winter, etc.) can interrupt the dormancy of sclerotia, but as previously mentioned, fire appears to be the most crucial. Production of sclerotia is therefore regarded as an essential step in the life cycle of these fungi, without which fruiting bodies are not intensely produced.

3.12 The Chanterelles

The *Cantharellus* genus houses species of mushrooms known as chanterelles, which are also identified with North America (Pilz et al., 2003). These mushrooms also have a long tradition of consumption in Europe, but their occurrence has declined in recent decades, as has been observed with other species. *C. cibarius* produces the mushroom known as golden chanterelle, as ranges in color from yellow to orange. In North America, *C. formosus*, also known as the "Pacific Golden Chanterelle" is the best-known species. Other genera such as *Craterellus*, *Gomphus*, and *Polyozellus* also produce mushrooms referred to as chanterelles; however, only species in the *Cantharellus* genus are considered "true" chanterelles. Within this genus, there are many known species that are associated with different species of plants such as spruces (*Picea*), pine (*Pinus*), Douglas fir (*Pseudotsuga*), and oak (*Quercus*). As occurs with morels, chanterelles also support a tradition of professional collectors in North America; however, most of the mushrooms collected are exported to the European market.

Unlike other mycorrhizal mushrooms, which are typical of temperate regions in the Northern hemisphere, several species of chanterelles are also found in tropical countries, including Africa, Asia, and Latin America. This wide distribution indicates a greater versatility of this mushroom with respect to the need for low temperatures for fruiting induction. Periods of summer and hot springs, with proper humidity, favor the production of this mushroom even in the more temperate regions of North America. This adaptation to different environmental conditions seems to have enabled its wide geographic distribution.

Cantharellus has a life cycle even more complex than the other mycorrhizal mushrooms, because in addition to the mycorrhizal association, the fungus closely associates with other organisms that grow in its tissues (Garbaye, 1994), making its cultivation even more difficult. Isolation and identification of these microorganisms would vastly accelerate the development of cultivation technology. Unfortunately, attempts thus far have not been successful enough to enable cultivation on a commercial scale. Reproduction of the conditions that occur in nature, especially interactions with other microorganisms, including some uncultivable microorganisms, remains a considerable obstacle.

3.13 The Matsutake

Tricholoma matsutake or simply matsutake is a native mushroom in Japan, strongly identified with this country, and occupies the same position as truffles occupy in Europe. Also, just as for truffles in Europe, this mushroom has faced a huge decline in natural production output, and attempts to develop techniques for commercial production have yet to be successful. In North America, another species (*T. magnivelare*), known as American matsutake, can still be found in abundance, allowing for its commercial exploitation to meet the demand of both the American and Japanese markets.

The name of this mushroom originates from its host tree species ("matsu" for *Pinus* and "take" for mushroom) and can be associated with different pine species and other arboreal genera such as *Quercus*, *Castanopsis*, and *Picea*, depending upon geographical region and *Tricholoma* species. Like other species of mycorrhizal mushrooms addressed in this text, the matsutake is a typical Northern hemisphere mushroom, whose natural fruiting occurs between late summer and autumn, when temperatures drop. The production of this mushroom is associated with a structure known as the "shiro," which is defined as a dense mycelial mass that aggregates along roots and in soil particles (Guerin-Laguette et al., 2003; Vaario et al., 2011). However, while it is the dominant microorganism, *Tricholoma* hyphae are not alone in these structures, as a large variety of prokaryotes and other fungi are also present. It has been surmised that at the time of fruiting, *Tricholoma* use an alternative energy strategy involving the degradation of organic compounds, particularly hemicellulose. In this context, the different members of the Shiro microbial community likely play an important role in the production of the necessary enzymes. Such factors underline the complexity of the life cycle of this important species of mushroom as well as the difficulties in its cultivation, since it remains prohibitively challenging to reproduce the precise combination of environmental factors found in nature that culminate in a succesful sexual cycle of the fungus.

3.14 Porcini

Mushrooms known as porcini or King Boletus are a group of species from the *Boletus edulis* sensu lato. Species distinction among *B. aestivalis*, *B.aereus*, and *B. reticulatus* is only reliably performed by specialists, and for commercial purposes, all of them are referred to as belonging to the *B. edulis* complex. All these species are mycorrhizal, associating with different families of tree species, such as *Fagus*, *Pinus*, *Picea*, *Quercus*, and many others, depending on the region or species preference of the fungus itself. This group of mushrooms in also native to the Northern hemisphere, although the species *B. loyo* can be found naturally in Chile (Deschamps, 2002). *B. edulis* is now also found in certain Southern hemisphere countries such as Australia, New Zealand, and South Africa, and is believed to have been brought with its preferred tree species as they were imported from Europe (Hall et al., 1998).

As observed for morels, chanterelles, and matsutake, attempts to establish tree orchards inoculated with this fungus have been unsuccessful. Studies with ectomycorrhizae have shown that the microorganisms present in the mycorrhizal mantle, in particular the bacteria, play a vital role in the process; this has also proven to be the case with *B. edulis* (Wu et al., 2012). Other studies indicate that basidiocarp production is affected by factors outside of the mycorrhizal association. The balance of all the factors the fungus has become adapted to as it has evolved in its native environment is quite delicate and extremely difficult to reproduce. Establishment of orchards containing just one species of host tree for the fungus does not allow for the presence

of other plant species that may be essential for the establishment of the proper microbiota required to support all interactions that will culminate in the production of mushrooms.

3.15 Decreased Production of Mycorrhizal Mushrooms in the Northern Hemisphere

Strong evidence of the decline in production of mycorrhizal mushrooms in the Northern hemisphere has been well established. At first, it was speculated that this decline resulted from human intervention, since commercial interest has led to an intense exploitation of the areas of natural production of these mushrooms. Several other aspects were considered, as well, including soil compaction and environmental disruption either leading to early abortion or otherwise compromising the formation of mature fruiting bodies. These studies have shown that the answer cannot be found in any single factor; other factors besides anthropogenic action are implicated in this decline.

However, even if we wished to ascribe all of the problems observed in nature to man, we must recognize that we live in a changing world. Although anthropogenic action has contributed greatly, it alone cannot explain recent climate changes and mass extinctions of species. For example, when the Sahara region changed from a lush to an arid region, human technology was far from the power of destruction it is today. Climate change in the region nonetheless upset its existing balance through a shift in rain cycles, and only a few species were able to survive; the rest were replaced by others better adapted to the new environment.

It is undeniable that the climate is changing, no matter the cause. While the hottest regions will suffer the most obvious consequences of global warming, even in temperate regions, subtle elevations in temperature can have important ecological consequences, having a significant impact on life cycles of native species. The soil itself, with its intricate complexity of interactions between plants, animals, and microorganisms, can be transformed in response to environmental changes.

The assessment of changes in different ecosystems is relatively easy considering the animal and plant species that grow on its surface. However, drastic changes in the soil of an ecosystem have often begun long before their effects become visible. Therefore, it is important to consider the possibility that a decline in the production of mycorrhizal mushrooms is just a sign of an ongoing transformation process that has yet to reach its greatest amplitude.

It is possible that the mushroom production decline represents a fitness cost, as resources are diverted so that the organism can adapt to changes in its environment. The sexual cycle is an energetically expensive process, and the need to invest in mycelial growth may result in a negative correlation to sexual reproduction. Quantifying the production of fruiting bodies as an evaluation criterion of fungal fitness may be an important tool for the study of different species of mycorrhizal mushrooms. In this context, the study of non-edible species could yield important information, as such species are not subject to intense pressures that result from the harvest of edible species. Comparisons of fitness between edible and non-edible species could greatly enhance our understanding of the decline in the production of edible mycorrhizal mushrooms.

3.16 Fitness of Filamentous Fungi

Fitness can be defined as an organism's ability to respond to situations that necessitate increased energy expenditure, such as distributing oxygen to muscle tissues. In this report, the concept of

Darwinian fitness will be used, of which the simplest definition is "the ability of a population to maintain or increase their number in succeeding generations" (www.dictionary.com/browse/darwinian-fitness). According to Antonovics and Alexander (1989), biological fitness can be defined in the context of individual selection as "the contribution of a phenotype to a subsequent generation." According to the authors, it is more appropriate to define fitness in phenotypic rather than genotypic terms, especially when dealing with quantitative traits (those determined by several loci). One of the main reasons for this is that the measurement of a phenotype is easier and more objective than the measurement of a genotype. While we can measure changes in the copy number of a gene from one generation to the next, fitness comprises not only copy number, but also the gene expression influenced by environment. Finally, Pringle and Taylor (2002) defined fitness as a function of "survival and reproductive success of an allele, individual, or group."

For each filamentous fungus group, the fitness will be influenced by its nutritional strategy: saprophytism, simbiosis, or parasitism. For edible and/or medicinal mushrooms, the first two strategies are of primary interest, because the great majority of such mushrooms are produced by saprophytic or mycorrhizal fungi. However, most fitness studies up to this point have been conducted on species of pathogenic and saprophytic fungi that attack seeds or post-harvest fruit, in accordance with their economic importance (Pringle and Taylor, 2002). One exception was Xu's (1995) work with *Agaricus bisporus*, which is a cultivated species of basidiomycete. When evaluating different aspects of fitness of this species, Xu reported inbreeding depression in *A. bisporus* resulting in a decrease in fitness due to the loss of genetic variety. According to the author, inbreeding depression is a phenomenon already observed in other species of basidiomycetes such as *Pleurotus sajor-caju* and *Volvariella volvacea*. Some of the most important species of mycorrhizal mushrooms are basidiomycetes, such as *Tricholoma matsutake* and *Boletus edulis*. Could these species also be suffering from inbreeding depression?

The basidiomycetes, and in particular the Agaricomycetes, are characterized by promiscuous sexual behavior, with an increased number of mating type genes theoretically ensuring a high rate of outcrossing (James, 2015). However, while millions of spores are released by each basidiocarp, few of them will be dispersed over long distances. Therefore, there is a high probability of crosses between individuals resulting from the same meiotic event, as a multitude of these spores remain but a small distance from the basidiocarp. Consequences as to the fitness of these mushroom species under such conditions remain unclear. For basidiomycetes, sexual reproduction is an obligate step in the life cycle of these fungi, although studies of possible mechanisms of asexual reproduction have not been properly considered for this group. While ascomycetes, with their large numbers of sexual homothallic species, have well-characterized asexual reproduction modalities, the sexual cycle seems to occur regularly during the life cycle of mycorrhizal species. Conversely, for ascomycete and basidiomycete mycorrhizal mushrooms, the sexual cycle is not well clarified, although research is ongoing. Volk and Leonard (1990) proposed a basic life cycle for the fungus *Morchella*. Subsequently, Alvarado-Castillo et al. (2014) have expanded on the details of this cycle, however, with a number of as yet unproven possible routes (Figure 3.3). For species of the *Tuber* genus, there have also been recent entries with regards to life cycle (Paolocci et al, 2006; Kües and Martin, 2011) and much effort is being invested in the study of these mushrooms, sometimes resulting in relative success in the establishment of commercial orchards. Nevertheless, the environmental complexity remains a major challenge, and there is much remaining work to be done in delineating the set (or sets) of factors that are required for optimal fitness and cultivation of mycorrhizal mushrooms.

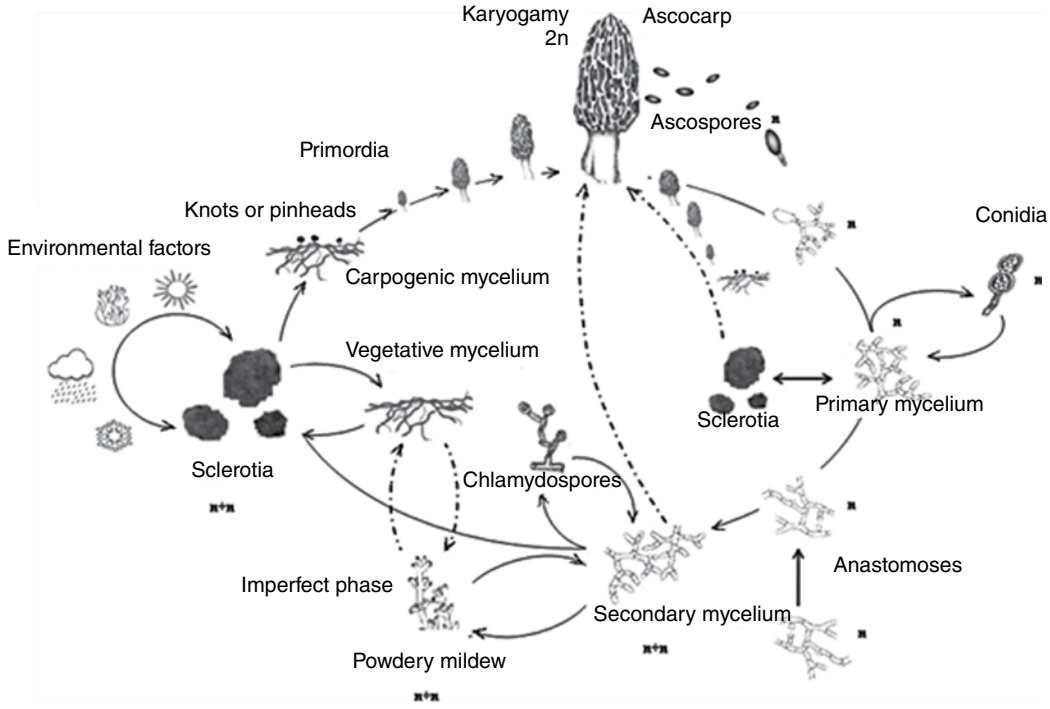

Figure 3.3 Theoretical life cycle of the genus *Morchella*. Dotted lines indicate possible routes. AlvaradoCastillo, G.; Mata, G.; Sangabriel-Conde, W. Understanding the life cycle of morels (*Morchella* spp.). Revista Mexicana de Micologia, v. 40, p. 47-50, 2014. With pennission.

3.17 Final Considerations

Mycorrhizal mushrooms are an important source of income in the areas where they occur naturally. Due to their importance, these mushrooms have been considered as an important alternative to the exploitation of forest ecosystems by logging. Public policy has allowed forest parks to be harvested in this manner, bringing benefits to the people living around these areas. Unfortunately, the mycorrhizal mushrooms species that are most prized are native to the Northern hemisphere, with its temperate-to-subtropical climate.

Nevertheless, the Southern hemisphere also features countries with climates similar to the mycorrhizal mushroom-producing regions of its Northern counterpart, including Australia, New Zealand, South Africa, Chile, Argentina, and Uruguay (Hall et al., 1998, Deschamps, 2002). In South America, Chile stands out for its wild mushroom export tradition. Chile exports *Suillus luteus* and *S. granulatus*, referred to as *Boletus luteus*, *B. edulis*, or simply *Boletus* (Deschamps, 2002). Brazil is an importer of these mushrooms from Chile, which arrive at supermarkets under the names of "Funghi Sechi" or *Boletus*. The species *S. luteus* was first classified as *B. luteus*, and only later discovered to be of another genus, leading to its reclassification as *S. luteus*. While this could explain ascribing the name *Boletus* to the exported product, certainly there also is great commercial interest in joining these mushrooms with the real *Boletus*. Morels are also produced in Chile, with *Morchella intermedia* being the most known species. The species *Phlebopus bruchii* bears a strong resemblance to the *Boletus*, which led to its initial classification as *Boletus bruchii*, and this similarity allows it to achieve twice the price

of the *Suillus* on the South American market. Important species such as *Lactarius deliciosus* and *Ramaria* spp. are also now common to Chile, as well.

In addition to Chile, Argentina and Uruguay also contain geographical regions with the potential to support the production of these mushrooms. In Uruguay, species such as *Tricholoma sulphureum*, *Lactarius deliciosus*, and *Suillus granulatus* occur naturally, while in Argentina, these last two are found in addition to *Phlebopus bruchii*, a type of mushroom highly appreciated in the Argentinian capital. As a result, Deschamps suggested that the countries of the Southern cone should invest in the exploration of *Phlebopus bruchii* species associated with the tree species *Fagara* coconut; and *Lactarius deliciosus*, *Suillus granulatus,* and *S. luteus* associated with species of *Pinus*. These species may be more amenable to cultivation in comparison to the mycorrhizal species native to the Northern hemisphere, where until recently, only a few species of *Tuber* have ever been successfully grown in orchards.

While Brazil is considered a tropical country, it exhibits comparative climatic diversity; Southern Brazil has a humid subtropical-to-temperate climate. In colder areas, the temperature drops below zero during the winter, and snow precipitation occurs in mountainous areas. As such, this region of Brazil would be an excellent location to attempt the introduction of natural production of mycorrhizal mushrooms. Sobestiansky (2005) carried out a survey of naturally occurring species in the region and reported, among many others, *Lactarius deliciosus*, *Suillus luteus*, *Boletus edulis*, and *B. brasiliensis*. In addition, several Brazilian institutions have a long tradition of studying mycorrhizal fungi associated with *Pinus* and *Eucalyptus*. Among the fungi associated with *Pinus*, the *Suillus* genus has been found to have a high occurrence of fruiting bodies in pine reforestation areas. While these areas in Brazil could be lucrative harvesting regions, the lack of wild mushroom harvest traditions among the Brazilian people presents a barrier to immediate adoption, as Brazilian people may be afraid to collect poisonous mushrooms by mistake. Investment from government agencies in cooperation with tourism agencies for the training of professionals in the identification of these species for harvesting purposes could help promote a major shift in this direction, as Brazilian consumers have shown an increasing interest in the consumption of mushrooms. In this context, wild mushrooms could have great cultural and tourist appeal, bringing additional economic benefits to mushroom-producing regions.

References

Alvarado-Castillo G, Mata G, Sangabriel-Conde W. (2014). Understanding the life cycle of morels (*Morchella* spp.). *Revista Mexicana de Micologia*, 40:47–50.

Antonovics J and Alexander HM. (1989). The concept of fitness in plant–fungal pathogen systems. In Leonard KJ and Fry WE. (Eds) *Plant Disease Epidemiology*, 2:185–201.

Callac P, Billette C, Imbernon M, Kerrigan RW. (1993). Morphological, genetic, and interfertility analyses reveal a novel, tetrasporic variety of *Agaricus bisporus* from the Sonoran desert of California. *Mycologia*, 85:835–851.

Dias ES, Labory CRG, Herrera KM, Alves AA, Torres GA, Rinker DL. (2008). Cytological studies of *Agaricus brasiliensis. World Journal of Microbiology and Biotechnology*, 24:2473–2479.

Deschamps JR. (2002). Hongos silvestres comestibles del Mercosur con valor gastronómico. Documento de Trabajo N° 86, Universidad de Belgrano. www.ub.edu.ar/investigaciones/dt_nuevos/86_deschamps.pdf (accessed December 10, 2016).

Garbaye J. (1994). Helper bacteria: a new dimension to the mycorrhizal symbiosis. *New Phytol* 128:197–210.

Guerin-Laguette A, Vaario L-M, Matsushita N, Shindo K, Suzuki K., Lapeyrie F. (2003). Growth stimulation of a Shiro-like, mycorrhiza forming, mycelium of *Tricholoma matsutake* on solid substrates by non-ionic surfactants or vegetable oil. *Mycological Progress* 2:37–44.

Hall IR, Lyon JE, Wang Y, Sinclair L. (1998). Ectomycorrhizal fungi with edible fruiting bodies. *Boletus edulis*. *Economic Botany*, 52:44–56.doi:10.1007/BF02861294.

Hall IR, Wang Y, Amicucci A. (2003). Cultivation of edible ectomycorrhizal mushrooms. *Trends Biotechnol* 21:433–438.

Heitman J; Kronstad JW, Taylor JW, Casselton LA. (Eds) (2007). *Sex in Fungi: Molecular Determination and Evolutionary Implications*. ASM Press: Washington, 1st Edn, 542 p.

Herreira KM, Alves E, Costa MD, Dias ES. (2012). Electron microscopy studies of basidiosporogenesis in *Agaricus brasiliensis*. *Mycologia*, 104:272–280.

James TY. (2015). Why mushrooms have evolved to be so promiscuous: Insights from evolutionary and ecological patterns. *Fungal Biology Reviews*, 29(3–4), December:167–178.

Kerrigan RW. (2005). *Agaricus subrufescens*, a cultivated edible and medicinal mushroom, and its synonyms. *Mycologia, New York* 97(1):12–24.

Kües U and Martin F. (2011). On the road to understanding truffles in the underground. *Fungal Genetics and Biology* 48:555–560.

Kües, U, Fischer R. (2006). *Growth, Differentiation and Sexuality*. New York: Springer, 449 p.

Labory CRG, Dias ES, Davide LC, Schwan RF, Wenzel IM. (2003). Coloração de núcleos de esporos e hifas do cogumelo *Agaricus blazei*. *Ciência e Agrotecnologia* 27:471–474,

Nazrul MI and Yin Bing B. (2011). Differentiation of homokaryons and heterokaryons of Agaricus bisporus with inter-simple sequence repeat markers. *Microbiological Research.* 166(3):226–236.

Paolocci F, Rubini A, Riccioni C, Arcioni S. (2006). Reevaluation of the life cycle of *Tuber magnatum*. *Applied and Environmental Microbiology*, 72:2390–2393.

Pilz D, Norvell L, Danell E, Molina, R. (2003). Ecology and Management of Commercially Harvested Chanterelle Mushrooms. United States Department of Agriculture, Forest Service, Pacific Northwest Research Station, General Technical Report, PNW-GTR-576, March.

Pringle A and Taylor JW (2002). The fitness of filamentous fungi. *Trends. Microbiol.* 10:474–481.

Raper J. (1966) *Genetics of Sexuality in Higher Fungi*. Ronald Press Co.: New York, 283 p.

Rocha de Brito M, Foulongne-Oriol M, Moinard M, Souza Dias E, Savoie JM, Callac P. (2016). Spore behaviors reveal a category of mating-competent infertile heterokaryons in the offspring of the medicinal fungus *Agaricus subrufescens*. *Applied Microbiology and Biotechnology*, 100:781–796.

Sobestiansky G. (2005). Contribution to a macromycete survey of the states of Rio Grande do Sul and Santa Catarina in Brazil. *Brazilian Archives of Biology and Technology* 48:37–457.

Tedersoo L, Arnold E, Hansen, K. (2013). Novel aspects in the life cycle and biotrophic interactions in Pezizomycetes (Ascomycota, Fungi). *Molecular Ecology* 22:1488–1493.

Thongklang N, Hoang E, Rodriguez Estrada AE, *et al.* (2014). Evidence for amphithallism and broad geographical hybridization potential among Agaricus subrufescens isolates from Brazil, France, and Thailand. *Fungal Biology* 118:1013–1023.

Vaario L-M., Fritze H, Spetz P, Heinonsalo J, Hanajík P, Pennanen T. (2011). *Tricholoma matsutake* dominates diverse microbial communities in different forest soils. *Applied and Environmental Microbiology* 77:8523–8531. doi:10.1128/AEM.05839-11.

Volk T. and Leonard TJ. (1990). Cytology of the life-cycle of Morchella. *Mycological Research*, 94:399–406.