Molecular Mycorrhizal Symbiosis
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EDITED BY

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WILEY Blackwell
## Contents

List of contributors vii
Foreword xi
Preface xiii

**Section 1: Structure and phylogeny of mycorrhizal symbioses, 1**

1 Origins of the mycorrhizal symbioses, 3  
*Christine Strullu-Derrien, Paul Kenrick, and Marc-André Selosse*

2 Reappraising the origin of mycorrhizas, 21  
*William R Rimington, Silvia Pressel, Katie J Field, Christine Strullu-Derrien, Jeffrey G Duckett, and Martin I Bidartondo*

3 The structure of arbuscular mycorrhizas: A cell biologist’s view, 33  
*Andrea Genre and Paola Bonfante*

4 Structure and development of ectomycorrhizal roots, 47  
*Raffaella Balestrini and Ingrid Kottke*

5 Structure and development of orchid mycorrhizas, 63  
*John Dearnaley, Silvia Perotto, and Marc-André Selosse*

**Section 2: Cellular, genetic and molecular mechanisms in the establishment of mycorrhizal symbioses, 87**

6 The evolution of the mycorrhizal lifestyles – a genomic perspective, 89  
*Annegret Kohler and Francis Martin*

7 Strigolactones and lipo-chitooligosaccharides as molecular communication signals in the arbuscular mycorrhizal symbiosis, 107  
*Clare Gough and Guillaume Bécard*

8 Calcium signaling and transcriptional regulation in arbuscular mycorrhizal symbiosis, 125  
*Leonie Lugimbuehl and Giles ED Oldroyd*

9 Signaling pathways driving the development of ectomycorrhizal symbiosis, 141  
*Yohann Daguerre, Jonathan M Plett, and Claire Veneault-Fourrey*

**Section 3: Physiology, including carbon and nutrient exchange between symbionts, 159**

10 Carbohydrate metabolism in ectomycorrhizal symbiosis, 161  
*Uwe Nehls, Arpita Das, and Dimitri Neb*

11 Nitrogen acquisition in ectomycorrhizal symbiosis, 179  
*Rodica Pena*

12 Phosphorus metabolism and transport in arbuscular mycorrhizal symbiosis, 197  
*Katsuharu Saito and Tatsuhiro Ezawa*

13 Primary metabolism in arbuscular mycorrhizal symbiosis: Carbon, nitrogen and sulfur, 217  
*Michael Bitterlich, Jan Graefe, and Philipp Franken*
14 The transportome of mycorrhizal systems, 239
    Pierre-Emmanuel Courty, Joan Doidy, Kevin Garcia, Daniel Wipf, and Sabine Dagmar Zimmermann

15 Soil organic matter decomposition mechanisms in ectomycorrhizal fungi, 257
    Anders Tunlid, Dimitrios Floudas, Roger Koide, and François Rineau

16 Homeostasis of trace elements in mycorrhizal fungi, 277
    Joske Ruytinx, Elena Martino, Piotr Rozpędek, Stefania Daghino, Katarzyna Turnau, Jan Colpaert, and Silvia Perotto

Section 4: Population and community ecology, and environmental genomics, 299

17 Molecular identification of fungi, 301
    Leho Tedersoo and R Henrik Nilsson

18 Molecular technologies applied to the ecology of ectomycorrhizal communities, 323
    Marc Buée, Erwin Sentausa, and Claude Murat

19 The biogeography of ectomycorrhizal fungi – a history of life in the subterranean, 341
    Kabir G Peay and P Brandon Matheny

20 Spatial ecology of ectomycorrhizal fungal communities, 363
    Brian J Pickles and Ian C Anderson

21 Fungal ecology in boreal forest ecosystems, 387
    Björn D Lindahl and Karina E Clemmensen

22 Ecology of ericoid mycorrhizal fungi: What insight have we gained with molecular tools and what’s missing?, 405
    Gwen Grelet, Elena Martino, Ian A Dickie, Rosnida Tajuddin, and Rebekka Artz

23 Evolutionary genomics of arbuscular mycorrhizal fungi, 421
    Rohan Riley, Philippe Charron, Timea Marton, and Nicolas Corradi

24 Mycorrhiza helper bacteria, 437
    Aurélie Deveau and Jessy Labbé

25 Mixotrophy in mycorrhizal plants: Extracting Carbon from mycorrhizal networks, 451
    Marc-André Selosse, Melissa Faust Bocayuva, Maria Catarina Megumi Kasuya, and Pierre-Emmanuel Courty

26 Second-generation molecular understanding of mycorrhizas in soil ecosystems, 473
    Ian A Dickie and Mark G St John

Index, 493
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Foreword

Hardly a day goes by without hearing something new and exciting about the “Microbiome”. Studying the community of microorganisms and their genomes in ecosystems – from cheese to animal gut to soils – is hip and trendy. It is now very strange to realize that, before the “microbiome revolution”, most plant biologists regarded mycorrhizal symbioses as being obscure and of little importance. Now, dozens of review papers in high-profile journals have been published on the plant holobiome – the host plant with its cortege of bacterial and fungal partners – and they acknowledge that mycorrhizal interactions are extremely important.

Scientists working on mycorrhizal symbioses have known for more than a century that plant-associated microbes, such as mycorrhizal fungi, take center stage in terrestrial ecosystems. A century of research has clarified the nature of what is undoubtedly the commonest and most important symbiosis in terrestrial ecosystems. Simply stated, nearly all families of plants form root symbiotic organs, termed mycorrhizas, with soil fungi. Within days of their emergence in the upper soil profiles, up to 95% of the short roots of plants are colonized by mycorrhizal fungi. The importance of this symbiosis in promoting plant nutrient status and growth is now well established, and mycorrhizas are used worldwide to develop sustainable agriculture and forestry.

Today, with the advent of molecular tools and techniques, the possibility of integration across a wide range of disciplines, from genomics to molecular ecology and field ecology, is becoming a reality. Primary research papers in the last ten years have broken the ground for new lines of research, from regulation of gene expression to the ecological relevance of mycorrhizal symbioses. As discussed in the present book, DNA barcoding methods have been routinely used to identify mycorrhizal fungi in almost every terrestrial ecosystem, and the application of these molecular methods has provided detailed insights into the complexity of mycorrhizal fungal communities and populations, offering exciting prospects for elucidation of the processes that structure their communities and biogeography. These molecular ecology studies have not only spurred work on the dynamics of mycorrhizal communities and populations, but have also generated hypotheses about their role in the changing forest ecosystems. The next challenge on the agenda is to identify the functions played by the assemblages of mycorrhizal fungi in situ.

As a prerequisite of such large-scale functional ecology studies, we now need to discover genes controlling the development and functioning of the mycorrhizal symbioses. Critical in this endeavor is the use of genomic information on the sequenced mycorrhizal fungi. The completion of the genome sequences of ectomycorrhizal, arbuscular mycorrhizal, ericoid and orchid fungal species is providing an unprecedented opportunity to identify the key components.
of interspecific and organism-environment interactions. By examining, modeling and manipulating patterns of gene expression, we can identify the genetic control points that regulate the mycorrhizal response to changing host physiology, and can better understand how these interactions control ecosystem function.

There is no doubt that massive sequencing of mycorrhizal fungi and other entities populating the plant microbiome will be fertile ground for novel hypotheses about how mycorrhizal symbioses interact with other micro-organisms and drive ecosystems. Future efforts in this area will advance our general perspective on plant and fungal ecology and evolution, and will elucidate the biological dynamics that mediate the flux of matter and energy in terrestrial ecosystems.

In planning this book, invitations for contributions were extended to leading international authorities studying mycorrhizal symbioses with molecular tools. I would like to express my deep appreciation to each author for their outstanding contribution. This book summarizes and updates both the current state of knowledge and concepts on the structure, evolution, function and ecology of mycorrhizal systems. It is hoped that the reviews, interpretations and concepts put forward by this group of leading scientists will stimulate further research, and will encourage younger scientists in our community to look to future challenges that lie ahead.

I would like to thank Wiley-Blackwell, and especially Justin Jeffryes, Bhargavi Natarajan, Metilda Shummy and Divya Narayanan for their help and active cooperation during the preparation of this book.

Francis Martin
Preface

In the preface of *Mycorrhizal Symbiosis* (1983), Harley and Smith wrote: ‘There has been so great an increase of interest in mycorrhizal symbiosis in the last ten years that is now impossible for one person or even two to keep up with all the experimental work and speculation upon it’. This is even more true in 2016. Novel high-throughput sequencing technologies have advanced our knowledge of fundamental aspects of the biology, ecology, and evolution of the major mycorrhizal symbioses. Primary research papers in the last decade have broken the ground for new lines of research, from regulation of gene expression and evolution of the mycorrhizal symbiosis to the ecological relevance of mycorrhizal symbioses in a changing environment.

The present book aims to provide the reader with a general account of what has been discovered about mutualistic mycorrhizal associations using DNA tools, and also to identify gaps in our knowledge where new information is required. The structure of the book consists of: (1) some introductory chapters on the biology, structure and evolutionary history of the major types of mycorrhizal symbioses (chapters 1–5), followed by updates on (2) the different molecular mechanisms driving the development and functioning of mycorrhizal systems (chapters 6–16) and (3) molecular analysis of mycorrhizal populations and communities at the local and continental scales (chapters 17–25). The book concludes with some form of synthesis and new avenues for future research (chapter 26).

**Harnessing mycorrhizal genomics for biological insights**

Advances in molecular tools have brought spectacular tractability to several mycorrhizal fungi, such as *Laccaria bicolor*, *Hebeloma cylindrosporum*, *Tuber melanosporum*, *Oidiodendron maius* and *Rhizophagus irregularis* (formerly *Glomus intraradices*). These flagship models were initially prized because of the ease of manipulating them in vitro and their ability to form mycorrhiza on a range of host plants. For over 15 years, the research community has harnessed them to explore a wide range of biological and ecological questions including, but not limited to: nutrient uptake and assimilation; regulation of metabolic and signaling pathways; developmental patterns; and factors structuring the populations and their adaptation to environmental cues.

The sequencing of the nuclear and mitochondrial genomes of these model species (Martin *et al.*, 2008, 2010; Tisserant *et al.*, 2013; Kohler *et al.*, 2015; Kohler and Martin, chapter 6) are important landmarks in the study of mycorrhizal symbioses, and lead to a new degree of understanding of these fascinating plant-microbe interactions, which are so important to the ecology and success of plants on this planet. It is clear from the wealth of new information gathered since the released of these genomes that having access to both the genome sequence of the mycorrhizal fungi and one of their hosts
(e.g., Populus trichocarpa, Tuskan et al., 2006; Medicago truncatula, Young et al., 2011) has provided an unprecedented opportunity to identify the fungal and plant genes and signals necessary for establishing mycorrhizal interactions (Bécard and Cough, chapter 7; Luginbuehl and Oldroyd, chapter 8; Daguerre et al., chapter 9) and the regulatory networks that allow sequestration and movement of nutrients between the mutualistic partners and the formation of a balanced symbiotic association (Nehls et al., chapter 10; Pena, chapter 11; Bitterlich et al., chapter 14; Courty et al., chapter 14; Ruytinx et al., chapter 16).

Interwoven advances in comparative genomics, RNA-Seq-based transcriptomics, and bioinformatics are providing scientists with a markedly improved repertoire of research tools that are allowing the functioning of mycorrhizal symbioses to be analyzed and comprehended at an unprecedented level of molecular detail. Our ability to explore genome function is increasing in specificity as each subsequent mycorrhizal genome is sequenced. Oligoarray technologies, and Illumina RNA-Seq, have allowed studying the expression of tens of thousands of genes in a few days in several symbiotic interactions (Kohler et al., 2015).

Comparison of genome sequences from evolutionarily and ecologically diverse fungal species has emerged as a powerful tool for identifying functionally important genomic elements in saprotrophic fungi, such as white- and brown-rotters (Floudas et al., 2012). What have we learned so far from analyzing the genomes of L. bicolor, T. melanosporum and a dozen of other mycorrhizal genomes? (Kohler and Martin, chapter 6). From these studies, we have learned that most of the sequenced mycorrhizal genomes are overloaded by a plethora of transposable elements and repeated DNA sequences (Martin et al., 2008, 2010; Kohler et al., 2015), although the impact of these repeated elements on the genome evolution and plasticity is not yet known. Mycorrhizal genomes have often undergone extensive gene family expansion, compared with other saprotrophic fungi, and these genetic innovations have often been associated with genes that encode proteins involved in symbiotic interactions (Kohler et al., 2015; Kohler and Martin, chapter 6).

Perhaps most significantly, we now know that all sequenced ectomycorrhizal, ericoid and orchid fungi possesses a battery of small secreted effector-like proteins (SSPs) (Tisserant et al., 2013; Lin et al., 2014; Kohler et al., 2015; Pellegrin et al., 2015). Some of these mycorrhiza-induced SSPs (MiSSPs) are specifically produced during symbiotic growth, and are secreted from the fungal network of hyphae colonizing the root tissues during establishment of the ectomycorrhizal and arbuscular mycorrhizal associations (Kloppholz et al., 2011; Plett et al., 2011). Several of these MiSSPs, such as the L. bicolor MiSSP7 or R. irregularis SP7, have effector functions, suppressing host defense mechanisms or communicating directly with plant cell signaling pathways to allow fungal invasion and establishment of the symbiotic interaction (Kloppholz et al., 2011; Plett et al., 2014; Daguerre et al., chapter 9).

There have been further revelations, too, such as the lack of plant cell wall-degrading enzymes (PCWDE) in both ectomycorrhizal and arbuscular mycorrhizal fungi, highlighting that these fungi are true mutualists, apparently even lacking the capacity to break down the most abundant plant polymers, lignin and crystalline cellulose (Tisserant et al., 2013; Kohler et al., 2015; Kohler and Martin, chapter 6). The absence of a gene
encoding invertase from most ectomycorrhizal and *R. irregularis* genomes is another surprise (Martin *et al.*, 2008; Tisserant *et al.*, 2013; Kohler *et al.*, 2015). It shows the dependence of the fungus on the host plant’s invertase activity within the root to supply monosaccharides to the fungus, and again underlines the mutual dependence of both partners (Nehls *et al.*, chapter 10).

The nutritional relations and interplay between fungus and plant are fascinating, and research in this area has been propelled forward dramatically by access to the genomes of mycorrhizal fungi (Courty *et al.*, chapter 14; Ruytinx *et al.*, chapter 16; Saito and Ezawa, chapter 12). The use of transcriptional profiling to study the patterns of gene expression during mycorrhiza development, which has arisen from the genome projects, is also tremendously exciting. When partnered with biochemical analysis, it provides a powerful means of determining the metabolic changes that accompany mycorrhiza formation at the whole-plant level (Bitterlich *et al.*, chapter 13).

**Harnessing mycorrhizal genomics for evolutionary insights**

By examining the similarities and differences among the genomes of living fungi, we can reconstruct features of the genomes of their long-dead ancestors. Such reconstructions provide insight into patterns of genome evolution and diversity, and how organisms evolved through the gain, loss and modification of genomic features. The greater the number of sequenced genomes from living fungi, and the broader their distribution across the tree of life, the better is our view of these ancestral genomes.

The number of mycorrhizal fungi with sequenced genomes is ever expanding, due to the efforts of many groups, such as the Mycorrhizal Genomics Initiative (MGI) (Kohler *et al.*, 2015; Kohler and Martin, chapter 6) and the 1000 Fungal Genomes project (http://1000.fungalgenomes.org/home/). The major aim of the MGI is to identify the genetic mechanisms that underpin the establishment of mycorrhizal symbioses in fungal clades covering over 200 MYA of evolution, to determine whether certain genes are selectively associated with particular symbiotic patterns, and to decipher the evolution and adaptation of ecologically important symbioses in terrestrial ecosystems (Plett and Martin, 2011).

Phylogenomic reconstruction has shown that the ectomycorrhizal symbioses in the Agaricomycotina evolved from ecologically diverse decayer precursors (white- and brown-rotters, soil and litter decayers) and radiated in parallel, following the origins of their host plant lineages (Kohler and Martin, chapter 6). Polyphyletic evolution of the ectomycorrhizal lifestyle is marked not only by convergent losses of different components of the ancestral saprotrophic apparatus (e.g., class II lignin peroxidases, GH6 and GH7 cellulohydrolases) but also by rapid genetic turnover in symbiosis-induced genes, some of which may reflect lineage-specific functional innovations, such as MiSSPs (Daguerre *et al.*, chapter 9). In contrast, ericoid and orchid fungi, such as *Oidiodendron maius* and *Tulasnella calospora*, retained an extensive arsenal of PCWDE that is probably exploited indirectly by the plant for carbohydrate supply, thus explaining their known saprotrophic ability (Dearnaley *et al.*, chapter 5; Grelet *et al.*, chapter 22).

Recently, the widely supported notion of Glomeromycota-mediated land plant
evolution was challenged by the discovery that the earliest diverging liverwort clade, the Haplomitriopsida, are symbiotic with Mucoromycotina fungi, a partially saprotrophic and ancient lineage of fungi (Bidartondo et al., 2011; Rimington et al., chapter 2). Sequencing the genome of these symbiotic Mucoromycotina, and their comparison with the Glomeromycota genomes (Tisserant et al., 2013), will provide new insight on the emergence and evolution of the symbiotic genetic blueprint in fungal symbionts belonging to the early diverging clades.

Harnessing mycorrhizal genomics for ecological insights

During the past decade, PCR-based molecular methods and DNA sequencing have been routinely used to identify mycorrhizal fungi in a wide range of ecosystems from the Arctic to the tropics (Buée et al., chapter 18; Peay and Matheny, chapter 19; Tedersoo and Nilsson, chapter 17). Also, the application of high-throughput genotyping methods, such as metabarcoding, has provided detailed insights into the complexity of mycorrhizal fungal communities and populations at the continental and local scales (Tedersoo et al., 2014; Davison et al., 2015; Peay and Matheny, chapter 19; Pickles and Anderson, chapter 20), and offers exciting prospects for elucidation of the processes that structure mycorrhizal fungal communities (Peay and Matheny, chapter 19; Grelet et al., chapter 22; Selosse et al., 25).

These tools have managed to reveal not only the high diversity of mycorrhizal fungi interacting with their host in space (Pickles and Anderson, chapter 20), but also how different environmental factors and forest land usage could alter the composition of these soil fungal communities (Buée et al., chapter 18). These molecular ecology studies will spur work on dynamics and functions of mycorrhizal communities and populations, and also generate hypotheses about their role in the changing forest ecosystems. For example, it appears that the extensive, intermingled networks of extramatrical hyphae of mycorrhizal fungi not only permeate the mineral soil horizons, but are also very abundant in litter and decaying wood debris (Lindahl and Clemmensen, chapter 21).

With improvements in molecular techniques and appropriate DNA databases (Buée et al., chapter 18; Tedersoo and Nilsson, chapter 17), identification of taxa in fungal ecology has expanded from fruit bodies, to mycorrhizal roots, to extraradical hyphae (Pickles and Anderson, chapter 20). Mycorrhizal fungi are prominent in the underlying, more decayed litter and humus, where they apparently mobilized nitrogen and made it available to their host plants, through decay mechanisms similar to those used by brown-rot fungi (Tunlid et al., chapter 15). Most importantly, mycorrhizal mutualistic associations not only shape the plant communities, but also affect the functional diversity of rhizospheric bacteria (Deveau and Labbé, chapter 24).

Initially, genomic approaches have been applied only to a restricted set of carefully chosen mycorrhizal model species adapted to the laboratory environment, such as L. bicolor and R. irregularis. The conclusions brought from the study of these model organisms, although fascinating, cannot fully embrace how the wide range of known, highly diverse mycorrhizal species adapt to their various natural environments.
However, this situation is now changing. Hundreds of ecologically and phylogenetically relevant mycorrhizal species have currently been sequenced to begin to address the genetics of adaptations and ecological interactions in natural populations (Kohler and Martin, chapter 6).

This represents a significant investment in time, manpower and money. The payoff from such large scale initiatives would be worthwhile, as it could aid establishing the needed resources for future projects in ecological genomics. For example, one should be able to measure the expression of key genes involved in soil organic matter decomposition, nutrient acquisition and symbiosis-related development processes from a diverse community of mycorrhizal symbionts in natural settings by metatranscriptomics and metaproteomics.

The newly emerging discipline of ecological genomics bridges the current gap between molecular biology studies in the laboratory – which is largely focused on understanding basic developmental and physiological processes – and systems-level analyses of genetic adaptations to environmental cues and interactions between organisms in their natural settings. It is now feasible to perform comparative sequencing of hundreds of individual genomes from a species, to obtain genome scale insights into natural variation. Using comparisons of genome-wide genotyping of single nucleotide polymorphisms (SNP) of individuals belonging to different populations, it has already been possible to identify specific genes involved in adaptive traits in *T. melanosporum* and *Suillus brevipes* (Payen et al., 2015; Branco et al., 2015). Second-generation sequencing technologies provide genomic access to almost any fungal species and its natural genetic variation, regardless of whether the species can be cultured and kept in the laboratory.

**A bright future ahead**

Thanks to the new molecular and genomic resources available, scientific topics that can be tackled in a near future will include: identification of genes and molecular processes involved in adaptation of mycorrhizal fungi to biotic and abiotic environmental cues; characterization of the genetic mechanisms of speciation; and assessing the role of epigenetic changes in the evolution and adaptation of symbionts. Successful exploration of these genetic mechanisms will form the needed basis for exploration of ecosystem-levels questions, such as: the predictability of evolutionary adaptations; the role of ectomycorrhizal communities in ecosystem stability; interaction networks among soil microbial organisms, including the microfauna (Dickie and St John, chapter 26); and nutrient fluxes in the environment (Rodica, chapter 11).

Quantitative information on what is happening in terms of transfers of carbon and nutrients is urgently needed. Measuring gene expression *in situ* is important to show the potential pathways operating, but it cannot provide the full picture of the environmental interactions without well-thought metabolomic and ecophysiological experiments, including the plant perspective.

**One book to bring them all**

As stressed above, tremendous progress has been made in recent years on genomics, molecular biology and the molecular ecology of mycorrhizal interactions, but many
questions remain unanswered. A book on this topic – the mycorrhizal symbiosis through the eyes of molecular biologists and molecular ecologists – is missing, and I hope it will be timely. It combines chapters by well-known researchers involved in a diversity of mycorrhizal systems (ectomycorrhizae, arbuscular, ericoid and orchid mycorrhizal interactions). Such a broad-ranging approach can provide a unique insight and a better understanding of the functions of the various mycorrhizal symbioses. Authors have been encouraged to discuss far-reaching extensions of their current or past work, and to propose cross-cutting research questions whenever possible. Exploring this new field of research presents great opportunities for novel discovery of key molecular mechanisms controlling plant-microbe interactions, the evolution of fungal lifestyles and ecologically relevant traits.

This book should provide a useful resource for experienced researchers as well as the new one who are moving into the field each year. The level of presentation is technically advanced, with a strong emphasis on reviewing current findings in light of the possible future directions for research. One aim of the book is to try to (re-)integrate biological and ecological knowledge into molecular mycorrhizal sciences – which I think is the next critical step, as we move beyond simply using molecular tools to describe patterns (see Dickie and St. John, chapter 26). There is no doubt that massive sequencing of soil and plant-associated entities will be fertile ground for novel hypotheses about how mycorrhizal symbioses drive ecosystems. Future efforts in this area will advance our general perspective on mycorrhizal ecology and evolution, and will hopefully elucidate the mechanisms that mediate the flux of matter and energy in terrestrial ecosystems.

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References


SECTION 1
Structure and phylogeny of mycorrhizal symbioses
1.1 Introduction

Symbiosis means an intimate and often long-term association between two or more different species. Ahmadjian and Paracer (1986) commented: “It is such a universal and important phenomenon that it should be an integral component of the education of biologists”. However, despite or because of its importance, this term has experienced much confusion, variation in usage, and controversy (Martin and Schwab, 2013 and references therein). De Bary coined the term in his monograph Die Erscheinung der Symbiose (1879) to mean “the living together of unlike organisms,” using it to describe a broad range of relationships (mutualism, commensalism, parasitism).

Our usage follows the original definition, rather than the more restrictive sense (i.e. symbiosis = mutualism) proposed by some biologists about 30–50 years ago (Martin and Schwab, 2013 and references therein). Symbioses encompass a wide variety of organismal associations in diverse environments, including: bacteria and fungi that form close alliances with the roots of plants; dinoflagellates that live within the endoderm of tropical corals; bacteria that sustain giant tube worms in the deep ocean; and so on. In addition, animals harbor many different microorganisms in their gastrointestinal tracts (Paracer and Ahmadjian, 2000; Benson et al., 2010). At the time De Bary developed his concept of symbiosis, Albert Bernhard Frank was working on plant-fungal relationships. He already published the word Symbiostismus (1877), and he was the one who introduced the term mycorrhizas to designate the type of dual organ he observed: “the entire structure is neither tree root nor fungus alone but resembles the lichen thallus, a union of two different organisms into a single, morphological organ. It can be appropriately designated as a ‘fungus-root’ or ‘mycorrhiza’” (Frank, 1885; English translation, Trappe, 2005).

The ability of fungi to form mycorrhizas with plants is one of the most remarkable and enduring adaptations to life on land. The relationship is a mutualistic one, and its occurrence is now well established in many plant species (Wang and Qiu 2006; Akhmetzhanova et al., 2012). By contrast, the number of fungal partners involved is less clear, and varies depending on mycorrhizal type (van der Heijden et al., 2015).
Molecular phylogenetics is providing insights into the evolution of different types of mycorrhizal association through time, and genomic studies of both plants and fungi are shedding light on how the complex set of interactions evolved (e.g., Floudas et al., 2012; Kohler et al., 2015). Evidence from fossils is also providing additional perspectives (e.g., Remy et al., 1994; Taylor et al., 1995; Krings et al., 2007a, 2007b, 2011; LePage et al., 1997), and recent work shows how a carefully targeted program of research can yield highly informative results (Strullu-Derrien et al., 2009, 2014a). Moreover, extinction can generate a false signal regarding the origin of evolutionary novelties in a group when only living species are taken into account (Jablonski and Shubin, 2015). As a result, the fossil record has an important role to play in establishing a chronology of when fungi and key fungal associations evolved, and in understanding their importance in ecosystems through time (Figure 1.1).

Here we present a brief review of our current knowledge of the fossil record of mycorrhizas in the context of plant evolution. In addition to providing an overview of what is known, our aim is to identify areas in which the fossil record (palaeomycology) can be of relevance to genomics, and to recommend an approach that would bridge the two disciplines.

1.2 Extant mycorrhizal diversity

Mycorrhizas are widespread, occurring in over 80% of living plant species (Strullu, 1985; Smith and Read, 2008). The fungus uses the host as a source of carbon, while
Chapter 1: Origins of the mycorrhizal symbioses

The host is supplied with mineral elements by the fungus. The two partners also protect each other against soil biotic (e.g., parasites) and abiotic (e.g., drought, toxic compounds) adversities. Some plants, such as the mosses and the angiosperm families Brassicaceae, Caryophyllaceae, Proteaceae, Cyperaceae, are generally believed to be predominantly non-mycorrhizal (Smith and Read, 2008), although mycorrhizas are rare in some other families (e.g., Nymphaeaceae – Wang and Qiu, 2006).

Today, the most common associations are the arbuscular mycorrhiza (AM) symbioses, in which fungi are all members of the phylum Glomeromycota, which form a single and ancient clade (e.g., Redecker and Raab, 2006; Blair, 2009; Berbee and Taylor, 2010). These fungi can be found in the roots of 80% of all vascular plant species, and they are obligate symbionts. With our present state of knowledge, it is impossible to grow them independently from a host plant (Fortin et al., 2005).

AM associations are characterized by branched, tree-like, intracellular fungal structures (i.e. arbuscules, hyphal coils) and, sometimes, storage organs termed vesicles (Strullu, 1985; Genre and Bonfante, 2016). Some complex and simple thalloids, liverworts (Marchantiopsida), hornworts (Anthocerophyta), lycophytes and fern gametophytes also form associations with Glomeromycota, which are structurally (e.g., Strullu, 1985; Read et al., 2000; Selosse, 2005; Ligrone et al., 2007; Pressel et al., 2010) and functionally (Strullu et al., 1981; Humphreys et al., 2010), similar to those of vascular plants.

Recently, it has been discovered that members of several early diverging clades of land plant (liverworts, hornworts, lycopsids and ferns) develop symbiotic associations with Mucoromycotina fungi, and this might also represent an ancestral land plant-fungal symbiosis (Bidartondo et al., 2011; Desirò et al., 2013; Rimington et al., 2015, 2016). Interestingly, some of these extant plants also form partnerships, sometimes simultaneously, with Glomeromycota. This symbiosis is characterized by an intracellular phase showing fine fungal coils with terminal, thin-walled swellings, and an extracellular phase with the hyphae forming semi-parenchymatous structures and thick-walled spores (Pressel et al., 2010; Rimington et al., 2016). We designate this CM symbiosis (coiled mycorrhizas) to distinguish its fine coiled intracellular phase from the arbuscular intracellular phase of AM symbiosis. Because bryophytes, lycopsids and fern gametophytes do not have roots, both AM and CM associations are best referred to as mycorrhizal-like (Smith and Read, 2008) or paramycorrhizas (Strullu-Derrien and Strullu, 2007).

Several Ascomycota, Basidiomycota and a few members of the Zygomycota form ectomycorrhizas (ECMs), mostly on shrubs and trees from temperate and Mediterranean regions, and in some parts of tropical forests. Ascomycota and Basidiomycota have been recruited more recently and on multiple occasions (van der Heijden et al., 2015 and references therein). ECM symbiosis is clearly distinguishable from all others on the basis of the absence of intracellular penetration by the fungus (Strullu, 1985; Smith and Read, 2008). The root colonization remains intercellular, and a hyphal sheath is formed around the plant root (Balestrini and Kottke, 2016). This is the type of mycorrhiza originally observed by Frank (1885).

Compared to AM, the range of plants colonized by ECM is relatively small; only a mere 3% of seed plants are ECM (Moore et al., 2011). Within the gymnosperms, ECMs are known from many Pinaceae and
from the genera *Gnetum* and *Welwitschia*. In Cupressaceae, some species in *Juniperus* and *Cupressus*, as well as the angiosperms *Poplar* and *Alnus*, can develop both AM and ECM (Smith and Read, 2008). The same fungus sometimes forms ectendomycorrhizas, where some hyphae penetrate the host cells – for example, in basal *Ericaceae* (Selosse et al., 2007).

Finally, in two plant families, namely *Orchidaceae* and *Ericaceae*, mycorrhizas involve intracellular colonization by hyphal coils. A range of Basidiomycota form orchid mycorrhizas (ORMs) while both Asco- and Basidiomycota form Ericoid mycorrhizas (ERMs) (Strullu, 1985; Selosse et al., 2007; Smith and Read, 2008). Fungi forming mycorrhizas with orchids (Dearmaley et al., 2016) typically live as saprotrophs in the soil, and likely as endophytes, or even form ECM associations with neighboring trees (Dearmaley et al., 2013; Dearmaley et al., 2016). Orchid seeds are extremely small and, in natural ecosystems, the seedlings (protocorms) of most orchids are completely dependent on colonization by fungi for carbon supply. ERM is most common under acid and infertile heathland conditions. Some ERM fungi (Helotiales, Ascomycota) are soil saprotrophs; however, recent evidence suggests that others are plant endophytes (Selosse et al., 2009). Some fungi can also form both ERM and ECM associations with different host plants (van der Heijden et al., 2015).

### 1.3 Early land plants to early forests

Land plants evolved from freshwater algae originating and diversifying through the Ordovician, Silurian and Devonian Periods (Figure 1.2). The fossil record reveals that prior to the origins of forest ecosystems (mid-Devonian; ca 387 million years ago [MYA]) early plants differed in notable ways from those of later floras, and especially from modern species (Edwards and Kenrick, 2015). Plants were small and herbaceous, with simple vascular tissues and typically leafless bifurcating axes, some of which functioned as upright stems and others as rhizoid-based rooting systems (Kenrick and Strullu-Derrien, 2014). Here, the term “axis” is preferred over stem, rhizome, and root because, in the first land plants, these organ systems differed in important aspects of structure and function from their equivalents in living plants (Tomescu et al., 2014). Another key difference from modern bryophytes or tracheophytes (vascular plants) is that life cycles showed a much greater degree of similarity between gametophytes (haploid sexual phase) and sporophytes (diploid phase; Kerp et al., 2004; Taylor et al., 2005). Similar organ and tissues systems were expressed in both phases of the life cycle.

The vascular plants, or tracheophytes, are defined by the possession of a vascular system which is composed of phloem and xylem, but it is the latter that is more commonly encountered in the fossil record, due to the resilience of its cellular components, which typically possess robust cell walls containing the polyphenolic polymer lignin (Boyce et al., 2003). Vascular tissues first appear in the fossil record in the lower part of the Devonian period (410–407 MYA), when terrestrial sediments containing fossil plants first became abundant (Kenrick et al., 2012). The evolution of lignified tissues led to arborescent plants by the mid- to late Devonian (Stein et al., 2007).

Arborescence is known to have evolved independently in many different groups,
and a variety of biomechanical strategies were employed (Spicer and Groover, 2010; Pittermann, 2010 and references therein). This dramatic increase in size was, in most groups, a consequence of the evolution of the cambium. The bifacial cambium gave rise to secondary xylem (wood) and secondary phloem, and was present in the extinct progymnosperms, which comprised two groups: the Aneurophytales and the Archaeopteridales.

Figure 1.2 Simplified phylogenetic tree showing the minimum stratigraphic ranges of selected groups based on fossils (thick bars) and their minimum implied range extensions (thin lines). Extinct and living plant groups are shown. Adapted from Kenrick and Crane (1997) and Strullu-Derrien (2010). Ord = Ordovician, Sil = Silurian, Dev = Devonian, Carb = Carboniferous, Per = Permian, Tri = Triassic, Jur = Jurassic, Cre = Cretaceous. Rhy = Rhyniophytes, Cook = Cooksonia, Zoster = Zosterophyllophytes, Psi = Psilophyton, Cladoxy = Cladoxylopsids, Aneur = Aneurophytales, Arch = Archaeopteridale, Pteri = Pteridosperms, Cord = Cordaitales. Pteridosperms or seed ferns are paraphyletic. They comprise hydrasperman Pteridosperms, Medullosales, Callistophytales Peltaspermales, Glossopteridales, Benettitales, and Caytoniales. The relationships among gymnosperms are still not well resolved. (See insert for color representation of the figure.)
Molecular mycorrhizal symbiosis (Figure 1.2). However, it was recently demonstrated that wood evolved initially (407–395 MYA) in plants of small stature that were members of Euphyllophytes, a clade that includes living Sphenophytes (horsetails), Filicophytes (ferns) and Spermatophytes (seed plants) (Figure 1.2) (Strullu-Derrien, 2010; Gerrienne et al., 2011; Hoffman and Tomescu, 2013; Strullu-Derrien et al., 2014b).

The earliest tree-sized plants developed progressively between the early mid-Devonian and early late Devonian (393 to 380 MYA) (Figures 1.2 and 1.3). Cladoxylopsid trees (an extinct group of uncertain affinity) (Stein et al., 2007, 2012) bore digitate lateral leafless branches and had long, narrow, undivided roots originating from the base of the trunk. Lycopsid trees had principally cormose bases with narrow undivided rootlets, trunks covered in microphyllous leaves, and a branched crown. Progymnosperms had conifer-type wood but reproduced with spores only; the aneurophytales had a large woody rhizome with simple narrow roots, and aerial shoots with iterative branching patterns; the Archaeopteridales had a vertical woody trunk with extensive, woody, highly-branched rooting systems, and truly leafy branchlets (or compound leaves) (Figure 1.3).

In situ fossil forests from these times are quite rare. At the fossil forest of Gilboa,

![Figure 1.3](image-url) (a) to (c) Comparative architecture of three principal arborescent strategies of the middle-upper Devonian and transverse section of the corresponding trunks (Lycopsid, Cladoxylopsid and Archaeopteridale). The color scheme is as follows: yellow, cortex; grey, primary vascular tissue; striped secondary tissue. Scheme courtesy of B. Meyer-Berthaud, modified from *Géochronique* 134, June 2015. (See insert for color representation of the figure.)