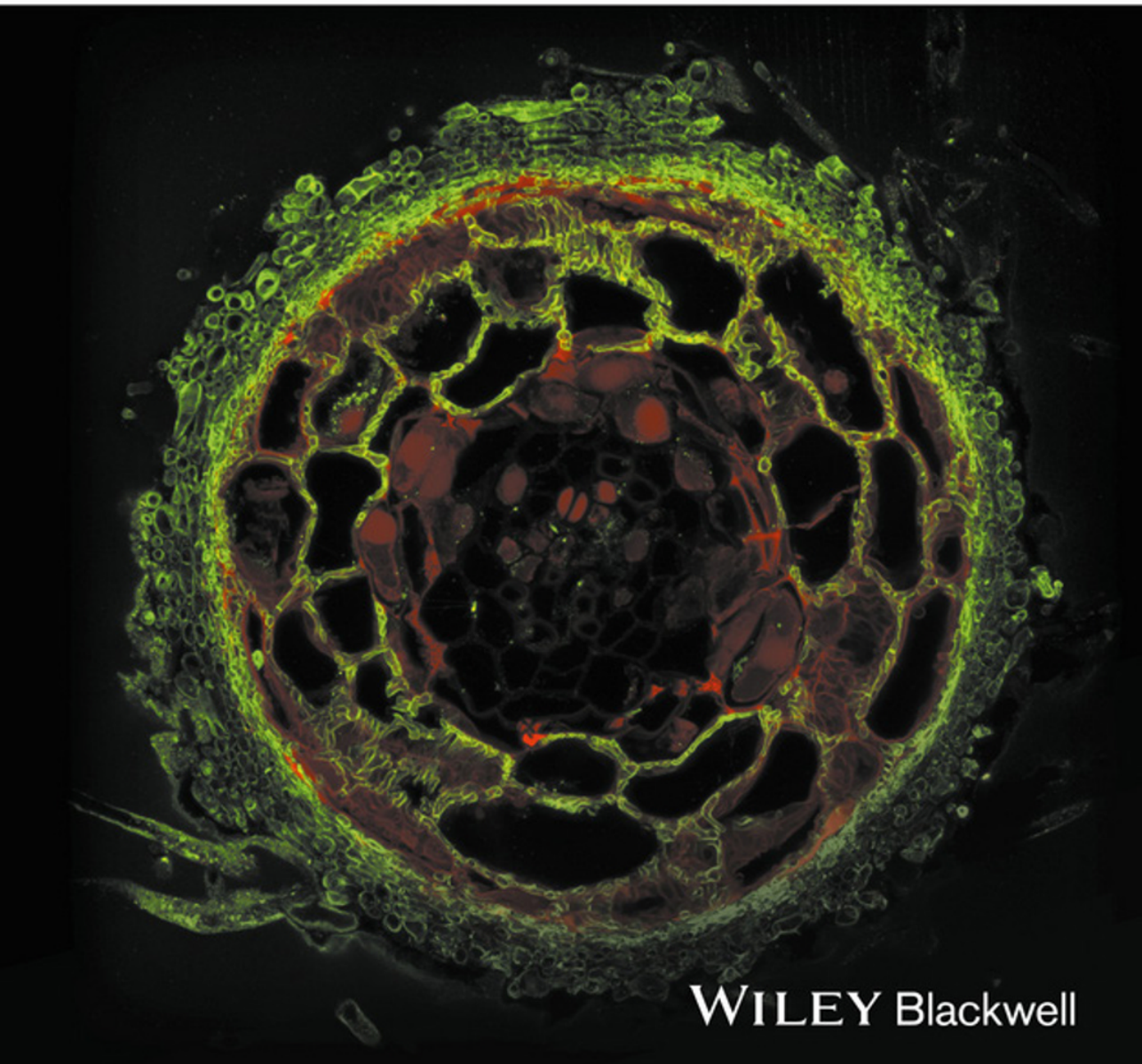
Molecular Mycorrhizal Symbiosis

Edited by **Francis Martin**



WILEY Blackwell

Molecular Mycorrhizal Symbiosis

Molecular Mycorrhizal Symbiosis

EDITED BY

Francis Martin

WILEY Blackwell

Published by John Wiley & Sons, Inc., Hoboken, New Jersey
Published simultaneously in Canada

Library of Congress Cataloging-in-Publication Data

Names: Martin, Francis, 1954– , editor.
Title: Molecular mycorrhizal symbiosis / edited by Francis Martin.
Description: Hoboken, New Jersey : John Wiley & Sons, Inc., 2016. |
Includes bibliographical references and index.
Identifiers: LCCN 2016024811 (print) | LCCN 2016041466 (ebook) | ISBN 9781118951415 (cloth) |
ISBN 9781118951439 (pdf) | ISBN 9781118951422 (epub)
Subjects: LCSH: Mycorrhizas. | Plant-fungus relationships.
Classification: LCC QK604.2.M92 M64 2016 (print) | LCC QK604.2.M92 (ebook) |
DDC 595.717/8–dc23
LC record available at https://lccn.loc.gov/2016024811

A catalogue record for this book is available from the British Library.

Cover image: © Maíra de Freitas Pereira

Set in 9.5/13pt Meridien by SPi Global, Pondicherry, India

10 9 8 7 6 5 4 3 2 1

Contents

List of contributors

Ian C Anderson
Hawkesbury Institute for the Environment,
Western Sydney University,
New South Wales,
Australia

Rebekka Artz
James Hutton Institute, Ecological Sciences Group, Craigiebuckler, Aberdeen, UK

Raffaella Balestrini
Istituto per la Protezione Sostenibile delle Piante (IPSP) del Consiglio Nazionale delle Ricerche (CNR), SS Torino, Italy

Guillaume Bécard
Laboratoire de Recherche en Sciences Végétales (LRSV), Université de Toulouse, Castanet-Tolosan, France

Martin I Bidartondo
Department of Life Sciences, Imperial College, London; Jodrell Laboratory, Royal Botanic Gardens, Kew, UK

Michael Bitterlich
Leibniz-Institute of Vegetable and Ornamental Crops and Humboldt-Universität zu Berlin, Institute of Biology, Germany

Melissa Faust Bocayuva
Department of Microbiology,
Laboratory of Mycorrhizal Association,
Viçosa Federal University, Brazil

Paola Bonfante
Department of Life Sciences and Systems Biology, University of Turin, Italy

Marc Buée
INRA, UMR 1136 INRA-Université de Lorraine, Interactions Arbres-Microorganismes, Laboratoire d'excellence ARBRE, INRA-Nancy, Champenoux, France

Philippe Charron
Canadian Institute for Advanced Research, Department of Biology, University of Ottawa, Canada

Karina E Clemmensen
Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden

Jan Colpaert
Hasselt University, Centre for Environmental Sciences, Diepenbeek, Belgium

Nicolas Corradi
Canadian Institute for Advanced Research, Department of Biology, University of Ottawa, Canada

Pierre-Emmanuel Courty
Zurich-Basel Plant Science Center,
Department of Environmental Sciences,
Botany, University of Basel, Switzerland;
Department of Biology,
University of Fribourg, Switzerland

Stefania Daghino
Department of Life Sciences and Systems Biology, University of Turin, Italy

Yohann Daguerre
INRA, UMR 1136 INRA-Université de Lorraine Interactions Arbres-Microorganismes, Laboratoire d'excellence ARBRE, INRA-Nancy, Champenoux, France

Arpita Das
University of Bremen, Faculty 2, Botany, Bremen, Germany

John Dearnaley
Faculty of Health, Engineering and Sciences, University of Southern Queensland, Australia

Aurélie Deveau
INRA, UMR 1136 INRA-Université de Lorraine Interactions Arbres-Microorganismes, Laboratoire d'excellence ARBRE, INRA-Nancy, Champenoux, France

Ian A Dickie
Bio-Protection Research Centre, Lincoln University, New Zealand

Joan Doidy
Center for Genomics and Systems Biology, Department of Biology, New York University, New York, USA

Jeffrey G Duckett
Department of Life Sciences, Plants Division, Natural History Museum, London, UK

Tatsuhiro Ezawa
Laboratory of Rhizosphere Control, Graduate School of Agriculture, Hokkaido University, Sapporo, Hokkaido, Japan

Katie J Field
Centre for Plant Sciences, Faculty of Biological Sciences, University of Leeds, UK

Dimitrios Floudas
Department of Biology, Microbial Ecology Group, Lund University, Sweden

Philipp Franken
Leibniz-Institute of Vegetable and Ornamental Crops and Humboldt-Universität zu Berlin, Institute of Biology, Germany

Kevin Garcia
Department of Bacteriology, University of Wisconsin-Madison, USA

Andrea Genre
Department of Life Sciences and Systems Biology, University of Turin, Italy

Clare Gough
Laboratoire des Interactions Plantes Micro-organismes (LIPM), Université de Toulouse, INRA, CNRS, Castanet-Tolosan, France

Jan Graefe
Leibniz-Institute of Vegetable and Ornamental Crops, Germany

Gwen Grelet
Landcare Research – Manaaki Whenua, Ecosystems and Global Change team, New Zealand

Maria Catarina Megumi Kasuya
Department of Microbiology, Laboratory of Mycorrhizal Association, Universidade Federal de Viçosa, Brazil

Paul Kenrick
Department of Earth Sciences, The Natural History Museum, London, UK

Annegret Kohler
INRA, UMR 1136 INRA-Université de Lorraine Interactions Arbres-Microorganismes, Laboratoire d'excellence ARBRE, INRA-Nancy, Champenoux, France

Roger Koide
Department of Biology, 4031 Life Sciences Building, Brigham Young University, Utah, USA

Ingrid Kottke
Eberhard-Karls-University Tübingen, Plant Evolutionary Ecology, Germany

Jessy Labbé
BioSciences Division,
Oak Ridge National Laboratory, Oak Ridge,
Tennessee, USA

Björn D Lindahl
Department of Soil and Environment,
Swedish University of Agricultural Sciences,
Uppsala, Sweden

Leonie Luginbuehl
John Innes Centre, Norwich, UK

Francis Martin
INRA, UMR 1136 INRA-Université de
Lorraine Interactions Arbres-Microorganismes,
Laboratoire d'excellence ARBRE, INRA-Nancy,
Champenoux, France

Elena Martino
Department of Life Sciences and Systems
Biology, University of Turin, Italy; INRA,
UMR 1136 INRA-Université de Lorraine
Interactions Arbres-Microorganismes,
Laboratoire d'excellence ARBRE, INRA-Nancy,
Champenoux, France

Timea Marton
Canadian Institute for Advanced Research,
Department of Biology, University of Ottawa,
Canada

P Brandon Matheny
Ecology and Evolutionary Biology,
University of Tennessee, Knoxville,
TN, USA

Claude Murat
INRA, UMR 1136 INRA-Université de
Lorraine, Interactions Arbres-Microorganismes,
Laboratoire d'excellence ARBRE,
INRA-Nancy, Champenoux,
France

Dimitri Neb
University of Bremen, Faculty 2,
Botany, Bremen,
Germany

Uwe Nehls
University of Bremen, Faculty 2,
Botany, Bremen,
Germany

R Henrik Nilsson
Department of Biological and Environmental
Sciences, University of Gothenburg,
Sweden

Giles ED Oldroyd
John Innes Centre, Norwich, UK

Kabir G Peay
Department of Biology, Stanford University,
California, USA

Rodica Pena
Forest Botany and Tree Physiology,
Buesgen-Institute, Georg-August University
Goettingen, Germany

Silvia Perotto
Department of Life Sciences and Systems
Biology, University of Turin, Italy

Brian J Pickles
Department of Forest and Conservation Sciences,
University of British Columbia, Vancouver,
Canada; School of Biological Sciences,
University of Reading, Berkshire, UK

Jonathan M Plett
Hawkesbury Institute for the Environment,
Western Sydney University, New South Wales,
Australia

Silvia Pressel
Department of Life Sciences, Plants Division,
Natural History Museum, London, UK

Rohan Riley
Canadian Institute for Advanced Research,
Department of Biology, University of Ottawa,
Canada

William R Rimington
Department of Life Sciences,
Imperial College, London, UK;

Jodrell Laboratory, Royal Botanic Gardens, Kew, UK; Department of Life Sciences, Plants Division, Natural History Museum, London, UK

François Rineau
Centre for Environmental Sciences, Hasselt University, Limburg, Belgium

Piotr Rozpądek
Institute of Environmental Sciences, Jagiellonian University, Kraków, Poland; The Franciszek Górski Institute of Plant Physiology, Polish Academy of Sciences, Kraków, Poland

Joske Ruytinx
Hasselt University, Centre for Environmental Sciences, Diepenbeek, Belgium; INRA, UMR 1136 INRA-Université de Lorraine Interactions Arbres-Microorganismes, Laboratoire d'excellence ARBRE, INRA-Nancy, Champenoux, France

Katsuharu Saito
Laboratory of Soil Biology, Faculty of Agriculture, Shinshu University, Minamiminowa, Nagano, Japan

Marc-André Selosse
Institut de Systématique, Évolution, Biodiversité (ISYEB), Muséum national d'Histoire naturelle, Paris, France; Department of Plant Taxonomy and Nature Conservation, University of Gdansk, Poland

Erwin Sentausa
INRA, UMR 1136 INRA-Université de Lorraine, Interactions Arbres-Microorganismes, Laboratoire d'excellence ARBRE, INRA-Nancy, Champenoux, France

Mark G St John
Agriculture and Agri-Food Canada, Ottawa, Canada

Christine Strullu-Derrien
INRA, UMR 1136, INRA-Université de Lorraine Interactions Arbres-Microorganismes, Laboratoire d'excellence ARBRE, INRA-Nancy, Champenoux, France; Department of Earth Sciences, The Natural History Museum, London, UK

Rosnida Tajuddin
School of Biological Sciences, Universiti Sains Malaysia, Pulau Pinang, Malaysia

Leho Tedersoo
Natural History Museum, University of Tartu, Estonia

Anders Tunlid
Department of Biology, Microbial Ecology Group, Lund University, Sweden

Katarzyna Turnau
Institute of Environmental Sciences, Jagiellonian University, Kraków, Poland; Malopolska Centre of Biotechnology, Jagiellonian University, Kraków, Poland

Claire Veneault-Fourrey
INRA, UMR 1136 INRA-Université de Lorraine Interactions Arbres-Microorganismes, Laboratoire d'excellence ARBRE, INRA-Nancy, Champenoux, France; Université de Lorraine, UMR 1136 INRA-Université de Lorraine, Interactions Arbres-Microorganismes, Laboratoire d'excellence ARBRE, Vandoeuvre-lès-Nancy, France

Daniel Wipf
URM Agroécologie, AgroSup Dijon, CNRS, INRA, Univ. Bourgogne Franche-Comté, F-21000 Dijon, France

Sabine Dagmar Zimmermann
Biochimie et Physiologie Moléculaire des Plantes, CNRS, INRA, Montpellier SupAgro, Univ. Montpellier, 34060 Montpellier, France

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 cellobiohydrolases) 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.

Acknowledgments

I am especially indebted and grateful to the authors for the high quality of their contributions. I am indebted to Annegret Kohler, Claire Fourrey, Igor Grigoriev, David Hibbett, Joey Spatafora and Ian Dickie for many helpful discussions. My research is supported by the French National Research Agency through the Laboratory of Excellence ARBRE (grant no. ANR-11-LBX-0002-01) and the Plant-Microbe Interactions Project, Genomic Science Program of the U.S. Department of Energy, Office of Science, Biological, and Environmental Research (grant no. DE-AC05-00OR22725).

References

Balestrini B and Kottke I. (2016). Structure and development of ectomycorrhizal roots. In: Martin F (ed). *Molecular Mycorrhizal Symbiosis*, pp. 47–62. Hoboken, New Jersey: John Wiley & Sons.

Bécard G and Gough C. (2016). Strigolactones and lipo-chitoligosaccharides as molecular communication signals in the AM symbiosis. In: Martin F (ed). *Molecular Mycorrhizal Symbiosis*, pp. 107–124. Hoboken, New Jersey: John Wiley & Sons.

Bidartondo MI, Read DJ, Trappe JM, Merckx V, Ligrone R and Duckett JG. (2011). The dawn of symbiosis between plants and fungi. *Biology Letters* **7**, 574–577.

Bitterlich M, Gräfe J and Franken P. (2016). Primary metabolism in arbuscular mycorrhizal symbiosis: carbon, nitrogen and sulfur. In: Martin F (ed). *Molecular Mycorrhizal Symbiosis*, pp. 217–238. Hoboken, New Jersey: John Wiley & Sons.

Branco S, Gladieux P, Ellison CE *et al.* (2015). Genetic isolation between two recently diverged populations of a symbiotic fungus. *Molecular Ecology* **24**, 2747–2758.

Buée B, Sentausa E and Murat C. (2016). Molecular technologies applied to ecology of ectomycorrhizal communities. In: Martin F (ed). *Molecular Mycorrhizal Symbiosis*, pp. 323–340. Hoboken, New Jersey: John Wiley & Sons.

Courty PE, Doidy J, Garcia K, Wipf D and Zimmermann SD. (2016). The transportome of mycorrhizal systems. In: Martin F (ed). *Molecular Mycorrhizal Symbiosis*, pp. 239–256. Hoboken, New Jersey: John Wiley & Sons.

Daguerre Y, Fourrey C and Plett JM. (2016). Signalling pathways driving the development of ectomycorrhizal symbiosis. In: Martin F (ed). *Molecular Mycorrhizal Symbiosis*, pp. 125–140. Hoboken, New Jersey: John Wiley & Sons.

Davison J, Moora M, Öpik M, Adholeya A, Ainsaar L, Bâ A, Burla S, Diedhiou AG, Hiiesalu I, Jairus T *et al*. (2015). Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism. *Science* **349**, 970–973.

Dearnaley J, Perotto S and Selosse MA. (2016). Structure and development of orchid mycorrhizas. This book.

Deveau A and Labbé J. (2016). Mycorrhiza helper bacteria. In: Martin F (ed). *Molecular Mycorrhizal Symbiosis*, pp. 437–450. Hoboken, New Jersey: John Wiley & Sons.

Dickie IA and St. John M. (2016). Second-generation molecular understanding of mycorrrhizas in soil ecosystems. In: Martin F (ed). *Molecular Mycorrhizal Symbiosis*, pp. 473–492. Hoboken, New Jersey: John Wiley & Sons.

Floudas D, Binder M, Riley R, Barry K, Blanchette Ra, Henrissat B, Martinez At, Otillar R, Spatafora JW, Yadav JS *et al*. (2012). The Paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. *Science* **336**, 1715–1719.

Genre A and Bonfante P. (2016). The structure of arbuscular mycorrhizas: a cell biologist's view. This book.

Grelet G, Martino E, Dickie IA, Tajuddin R and Artz R. (2016). Ecology of ericoid mycorrhizal fungi: what insight have we gained with molecular tools and what's missing? This book.

Harley JL and Smith SE. (1983). *Mycorrhizal Symbiosis*, pp. 483. Academic Press.

Kloppholz S, Kuhn H and Requena N. (2011). A secreted fungal effector of *Glomus intraradices* promotes symbiotic biotrophy. *Current Biology* **21**, 1204–1209.

Kohler A, Kuo A, Nagy LG, Morin E, Barry KW, Buscot F, Canbäck B, Choi C, Cichocki N, Clum A *et al*. (2015). Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. *Nature Genetics* **47**, 410–415.

Kohler A and Martin F. (2016). The evolution of the mycorrhizal lifestyles – a genomic perspective. This book.

Lin K, Limpens E, Zhang Z, Ivanov S, Saunders DGO, Mu D, Pang E, Cao H, Cha H, Lin T *et al*. (2014). Single nucleus genome sequencing reveals high similarity among nuclei of an endomycorrhizal fungus. *PLoS Genetics* **10**, e1004078.

Lindahl B and Clemmensen KE. (2016). Fungal ecology in boreal forest ecosystems. This book.

Luginbuehl L and Oldroyd GED. (2016). Calcium signaling and transcriptional regulation in AM symbiosis. In: Martin F (ed). *Molecular Mycorrhizal Symbiosis*, pp. 125–140. Hoboken, New Jersey: John Wiley & Sons.

Martin F, Aerts A, Ahrén D, Brun A, Duchaussoy F, Kohler A, Lindquist E, Salamov A, Shapiro HJ, Wuyts J *et al*. (2008). The genome sequence of the basidiomycete fungus *Laccaria bicolor* provides insights into the mycorrhizal symbiosis. *Nature* **452**, 88–92.

Martin F, Kohler A, Murat C *et al.* (2010). Périgord black truffle genome uncovers evolutionary origins and mechanisms of symbiosis. *Nature* **464**, 1033–1038.

Nehls U, Das A and Neb D. (2016). Carbohydrate metabolism in ectomycorrhizal symbiosis. In: Martin F (ed). *Molecular Mycorrhizal Symbiosis*, pp. 161–178. Hoboken, New Jersey: John Wiley & Sons.

Payen T, Murat C, Gigant A *et al.* (2015). A survey of genome-wide single nucleotide polymorphisms through genome resequencing in the Périgord black truffle (*Tuber melanosporum* Vittad.). *Molecular Ecology Resources* **15**(5), 1243–1255. doi: 10.1111/1755-0998.12391

Peay KG and Matheny PB. (2016). The biogeography of ectomycorrhizal fungi – a history of life in the subterranean. This book.

Pellegrin C, Morin E, Martin F and Veneault-Fourrey C. (2015). Comparative analysis of secretomes from ectomycorrhizal fungi with an emphasis on small secreted proteins. *Frontiers in Microbiology* **6**, 1278.

Pena R. (2016). Nitrogen acquisition in ectomycorrhizal symbiosis. This book.

Pickles BJ and Anderson IC. (2016). Spatial ecology of ectomycorrhizal fungal communities. This book.

Plett JM and Martin F. (2011). Blurred boundaries: lifestyle lessons from ectomycorrhizal fungal genomes. *Trends in Genetics* **27**, 14–22.

Plett JM, Kemppainen M, Kale Sd, Kohler A, Legué V, Brun A, Tyler B, Pardo A and Martin F. (2011). A secreted effector protein of *Laccaria bicolor* is required for symbiosis development. *Current Biology* **21**, 1197–1203.

Plett JM, Daguerre Y, Wittulsky S, Vayssieres A, Deveau A, Melton SJ, Kohler A, Morrell-Falvey J, Brun A, Veneault-Fourrey C and Martin F. (2014). The effector MiSSP7 of the mutualistic fungus *Laccaria bicolor* stabilizes the *Populus* JAZ6 protein and represses JA-responsive genes. *Proceedings of the National Academy of Sciences USA* **111**, 8299–8304.

Riley R, Charron P, Marton T and Corradi N. (2016). Evolutionary genomics of arbuscular mycorrhizal fungi. In: Martin F (ed). *Molecular Mycorrhizal Symbiosis*, pp. 421–436. Hoboken, New Jersey: John Wiley & Sons.

Rimington WR, Pressel S, Field KJ, Strullu-Derrien C, Duckett JG and Bidartondo MI. (2016). Reappraising the origin of mycorrhizas. In: Martin F (ed). *Molecular Mycorrhizal Symbiosis*, pp. 21–32. Hoboken, New Jersey: John Wiley & Sons.

Ruytinx J, Martino E, Rozpądek P, Daghino S, Turnau K, Colpaert J and Perotto S. (2016). Homeostasis of trace elements in mycorrhizal fungi. In: Martin F (ed). *Molecular Mycorrhizal Symbiosis*, pp. 217–238. Hoboken, New Jersey: John Wiley & Sons.

Saito K and Ezawa T. (2016). Phosphorus metabolism and transport in arbuscular mycorrhizal symbiosis. In: Martin F (ed). *Molecular Mycorrhizal Symbiosis*, pp. 197–216. Hoboken, New Jersey: John Wiley & Sons.

Selosse MA, Faust Bocayuva M, Kasuya MCM and Courty PE. (2016). Mixotrophy in mycorrhizal plants: extracting carbon from mycorrhizal networks. In: Martin F (ed). *Molecular Mycorrhizal Symbiosis*, pp. 451–472. Hoboken, New Jersey: John Wiley & Sons.

Strullu-Derrien C, Kenrick P and Selosse MA. (2016). Origins of the mycorrhizal symbioses. In: Martin F (ed). *Molecular Mycorrhizal Symbiosis*, pp. 3–20. Hoboken, New Jersey: John Wiley & Sons.

Tedersoo L, Bahram M, Põlme S, Kõljalg U, Yorou NS, Wijesundera R, Villarreal-Ruiz L, Vasco-Palacios A, Quang Thu P, Suija A *et al*. (2014). Global diversity and geography of soil fungi. *Science* **346**, 1078.

Tedersoo L and Nilsson RH. (2016). Molecular identification of fungi. In: Martin F (ed). *Molecular Mycorrhizal Symbiosis*, pp. 301–322. Hoboken, New Jersey: John Wiley & Sons.

Tisserant E, Malbreil M, Kuo A, Kohler A, Symeonidi A, Balestrini R, Charron P, Duensing N, Frei Dit Frey N, Gianinazzi-Pearson V *et al*. (2013). The genome of an arbuscular mycorrhizal fungus provides insights into the oldest plant symbiosis. *Proceedings of the National Academy of Sciences USA* **110**, 20117–20122.

Tunlid A, Floudas D, Koide R and Rineau F. (2016). Soil organic matter decomposition mechanisms in ectomycorrhizal fungi. In: Martin F (ed). *Molecular Mycorrhizal Symbiosis*, pp. 257–276. Hoboken, New Jersey: John Wiley & Sons.

Tuskan GA, Difazio S, Jansson S, Bohlmann J, Grigoriev I, Hellsten U, Putnam N, Ralph S, Rombauts S, Salamov A *et al*. (2006). The genome of black cottonwood, *Populus trichocarpa. Science* **313**, 1596–1604.

Young ND, Debellé F, Oldroyd GED, Geurts R, Cannon SB, Udvardi MK, Benedito VA, Mayer KFX, Gouzy J *et al*. (2011). The *Medicago* genome provides insight into the evolution of rhizobial symbioses. *Nature* **480**, 520–524.

SECTION 1

Structure and phylogeny of mycorrhizal symbioses

CHAPTER 1
Origins of the mycorrhizal symbioses

Christine Strullu-Derrien[1,2], Paul Kenrick[2], and Marc-André Selosse[3,4]

[1] *INRA, UMR 1136, INRA-Université de Lorraine Interactions Arbres-Microorganismes, Laboratoire d'excellence ARBRE, INRA-Nancy, Champenoux, France*

[2] *Department of Earth Sciences, The Natural History Museum, London, UK*

[3] *Institut de Systématique, Évolution, Biodiversité (ISYEB), Muséum national d'Histoire naturelle, Paris, France*

[4] *Department of Plant Taxonomy and Nature Conservation, University of Gdansk, Poland*

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 Mycorrhizal Symbiosis, First Edition. Edited by Francis Martin.

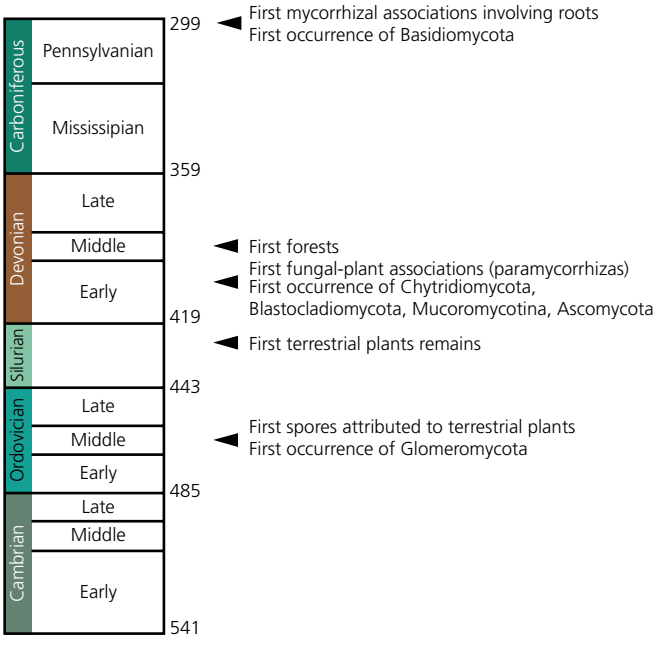


Figure 1.1 Earliest occurrences of fungi, plants and fungal-plant interactions in Palaeozoic times. Ages in millions of years are taken from the International Chronographic Chart of the International Commission on Stratigraphy, 2014. (*See insert for color representation of the figure*.)

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

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, lycopods 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, lycopods 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 (Dearnaley *et al.*, 2016) typically live as saprotrophs in the soil, and likely as endophytes, or even form ECM associations with neighboring trees (Dearnaley *et al.*, 2013; Dearnaley *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,

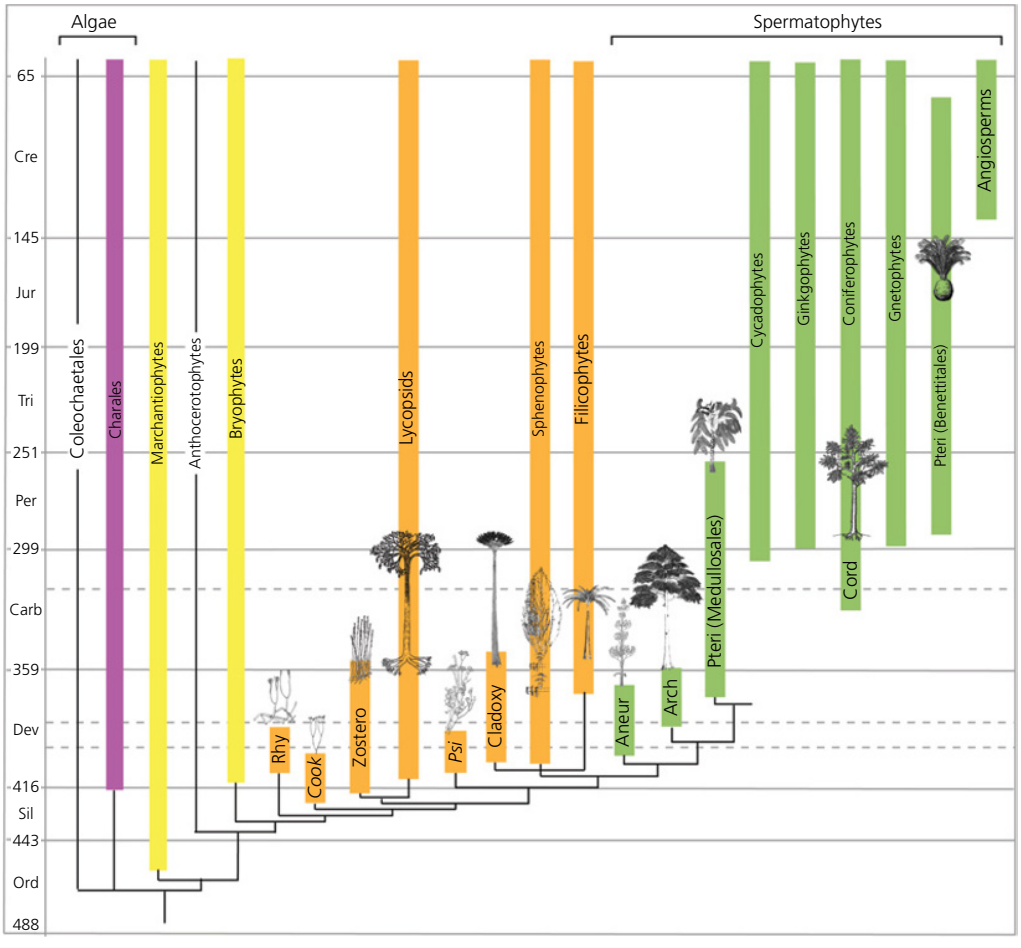


Figure 1.2 Simplified phylogenetic tree showing the minimum stratigraphic ranges of selected groups based on fossils (thick bars) and their minimum implied range extensions (thin lines). Extinct and living plant groups are shown. Adapted from Kenrick and Crane (1997) and Strullu-Derrien (2010). Ord = Ordovician, Sil = Silurian, Dev = Devonian, Carb = Carboniferous, Per = Permian, Tri = Triassic, Jur = Jurassic, Cre = Cretaceous. Rhy = Rhyniophytes, *Cook* = Cooksonia, Zostero = Zosterophyllophytes, *Psi* = Psilophyton, Cladoxy = Cladoxylopsids, Aneur = Aneurophytales, Arch = Archeopteridale, 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*.)

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). 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. Progymnopsperms 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,

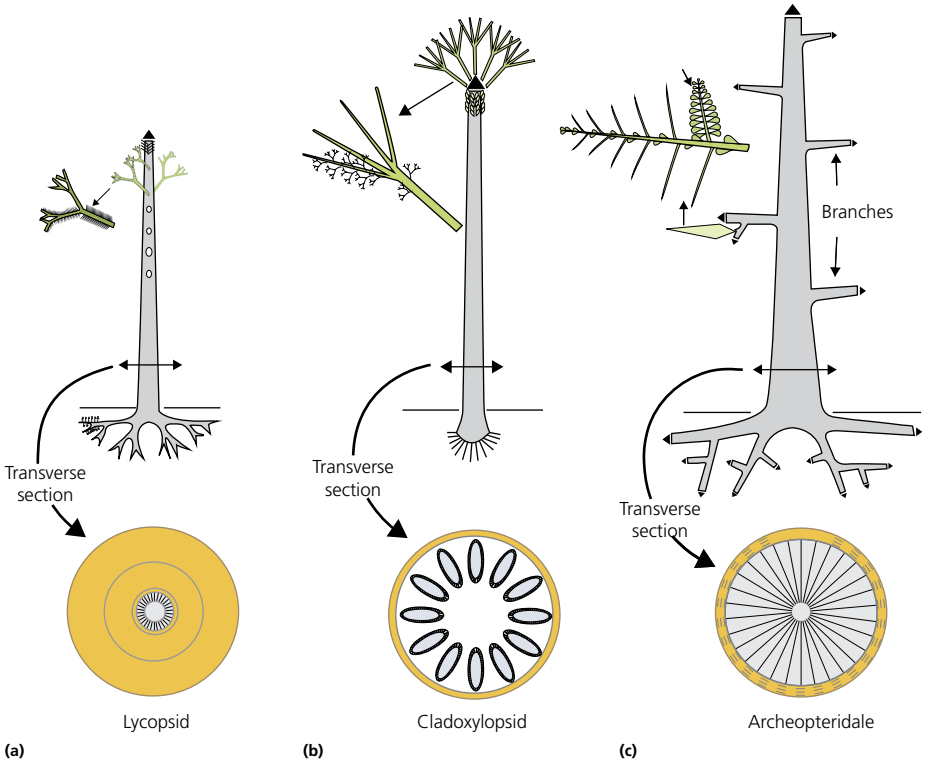


Figure 1.3 (a) to (c) Comparative architecture of three principal arborescent strategies of the middle-upper Devonian and transverse section of the corresponding trunks (Lycopsid, Cladoxylopsid and Archaeopteridale). The color scheme is as follows: yellow, cortex; grey, primary vascular tissue; striped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

New York, pseudosporochnaleans and aneurophytaleans dominate in a soil that undoubtedly was quite wet (Stein *et al.*, 2012). Nearby at Cairo, NY, a slightly older forest floor reveals archaeopteridalean and pseudosporochnalean rooting systems in a dry soil (Berry, pers. comm.). In Svalbard, separate stands of lycopsids and archaeopteridaleans are found in partially wet soils (Berry and Marshall, 2015). These forests demonstrate early spatial diversity.

By the Carboniferous Period (229–359 MYA), forests were well established in lowland coastal sites. The best known environments are also wetland communities (Greb *et al.*, 2006), comprising arborescent lycopods reaching a height of 30–40 meters. The trunks contained very little wood. Structural support was instead derived from a thick, bark-like periderm that enclosed soft pith. Ferns and horsetails were other important components of the plant communities, with arborescent forms that could reach heights of 20 m and 10–15 m, respectively. In addition, these forests also provided habitat for smaller pteridosperms (seed ferns), early conifers, and a wide range of smaller ferns, including epiphytes (Taylor *et al.*, 2009). The geological periods of the Devonian and the Carboniferous are significant because they witnessed the evolution of many of the fundamental organs and tissue systems, leading to the evolution of truly large plants and the first forest ecosystems.

1.4 AM symbioses in early (Palaeozoic) land plants

Microfossils in rocks of the mid-Ordovician period (ca 460–470 MYA) provide the earliest evidence of both plants and glomalean fungi (Rubinstein *et al.*, 2010; Redecker *et al.*, 2000), but no direct links between these organisms has been proven. The earliest direct evidence of mycorrhizal symbiosis is based on plants and fungi fosssilized *in situ* in the 407 million year old Rhynie Chert (Trewin, 2004). This site, discovered in 1912 near the village of Rhynie, about 50 km NW of Aberdeen (Scotland), is highly remarkable, both in terms of organismal diversity and the quality of preservation. The cherts formed from erupted hydrothermal fluids that periodically inundated vegetation on a low-energy alluvial plain formed by a braided river channel. Minor variations in topology across the floodplain gave rise to habitats that ranged from terrestrial to fully freshwater or brackish water. Plants, animals and fungi were petrified *in situ* or close to their sites of growth at low temperature, and fossilization is thought to have been relatively rapid, preserving remarkable details of cellular and subcellular structures (Trewin and Rice, 2004).

Between 1917 and 1921, in a series of five classic papers, Kidston and Lang described in detail four early land plants and, in the last paper, several fungi (Kidston and Lang, 1921). Observing the plants *Rhynia gwynne-vaughanii* and *Rhynia major* (now known as *Aglaophyton major*), they reported : "*The distribution and appearance of the layer of cells with very persistent dark contents immediately below the outer cortex suggests the possibility that this region might have contained a symbiotic organism…. Thus in the case of (the two species of) Rhynia also the only conclusion at present seems to be that proof of the existence of mycorrhizas is wanting, though there are grounds for further enquiry into the question*".

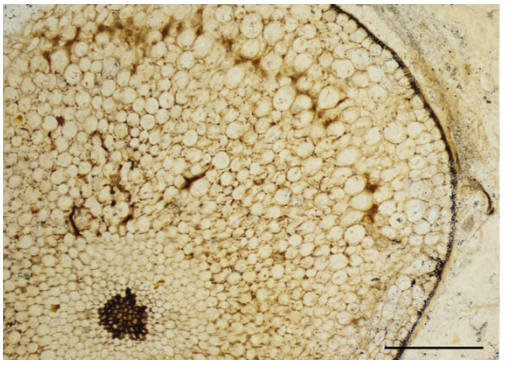
It is interesting to note that, simultaneously, Kidston and Lang discovered the plants and pioneered the concept of early symbiotic relationships. 50 years later,

Boullard and Lemoigne (1971) showed hyphae and vesicles and concluded that the same fungus was involved in a biotrophic, likely mutualistic association with both *Rhynia gwynne-vaughanii* and *Rhynia major* (= *Aglaophyton major*). However, they did not find the arbuscules characteristic of AM association. Unequivocal evidence of arbuscules was first provided by Remy *et al.* (1994) and Taylor *et al.* (1995) in the sporophyte *Aglaophyton major* (Figure 1.4a,b). This plant developed sinuous prostate axes which produced rhizoids in areas in contact with the substrate, allowing fungal colonisation to occur. Arbuscule-like structures were also recorded in *Lyonophyton rhyniensis* (the gametophyte of *A. major*) (Taylor *et al.*, 2005). Only vesicles (Karatygin *et al.*, 2006) have been described in *R. gwynne-vaughanii*, but a clear zone of fungal colonization was present in the outer cortex of the aerial axes, similar to that observed in *Aglaophyton*. Colonisation was not observed in the rhizoids. The fungus involved in the colonization of these plants has been recorded as belonging to Glomeromycota.

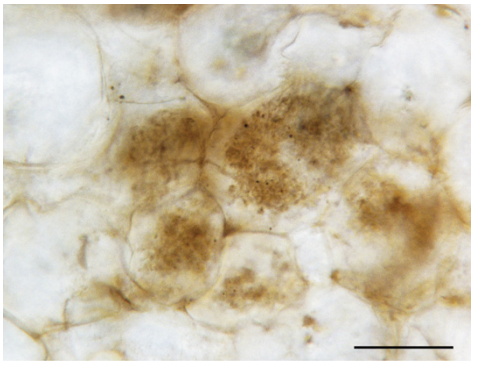
Among the three endophytes observed in *Nothia aphylla* (Krings *et al.*, 2007a, 2007b) only one closely resembles *Glomites rhyniensis* (Glomeromycota), the endomycorrhizal fungus of *Aglaophyton major*. However, a different mode of colonization was reported for *Nothia*. Intracellular fungal colonization was observed in the rhizoids and the tissues of the rhizoidal ridge, and intercellular vesicles and spores were produced in the cortex of both prostate and aerial axes, but arbuscules were not observed (Krings *et al.*, 2007a, 2007b).

Recently, two new endophytes were described colonizing the Rhynie Chert plant *Horneophyton lignieri* (Strullu-Derrien *et al.*, 2014a; Figure 1.4c,d). The rooting system of *Horneophyton* is easily distinguished from all other Rhynie plants. It comprises a corm at the base of the aerial axis, with numerous unicellular rhizoids emerging from the

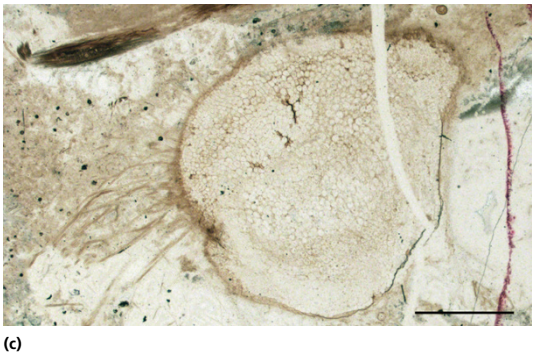
Figure 1.4 Fungal partnerships in Devonian and Carboniferous plants. (a) and (b) Fungal endophyte of the glomeromycotan type in *Aglaophyton major* from the Devonian Rhynie Chert. (a) Transverse section of an aerial axis, showing the well-defined colonized zone in the outer cortex (slide PB V15637 from the Natural History Museum, London). (b) Arbuscule-like structures in an aerial axis (slide from the University of Munster; photograph courtesy of H. Kerp). (c) and (d) Colonization of the mucoromycotean type in *Horneophyton lignieri* from the Devonian Rhynie Chert. (c) Transverse section of a corm; a zonation of fungal colonization is visible within the corm. (d) Intercellular branched thin-walled and intercellular thick-walled hyphae are present. (e) Arborescent clubmoss rootlet from the Upper Carboniferous of Great Britain (slide PB V11472 from the Natural History Museum, London). (f) AM-like fungi in stigmarian appendage. Trunk hyphae, intercalary vesicle (left), and putative arbuscule-like structures (right) are visible (slide BSPG 1964X from the Bavarian State Collection for Paleontology and Geology; photograph courtesy of M. Krings). (g) *Cordaites* rootlet from the Upper Carboniferous of Grand'Croix, France, colonized by AM fungus. The cortex comprises a reticulum of phi thickenings that are prominent in cells located close to the vascular cylinder (slide: Lignier Collection no. 194 from the University of Caen). (h) Detail of an arbuscule-like structure. The hyphal trunk of the arbuscule-like structure branches repeatedly forming a bush-like tuft within the cell (slide: Lignier Collection no. 194 from the University of Caen). Bars = 0.55 mm in A, 30 mm in B, 1.1 mm in C, 120 mm in D, 1.5 mm in E, 70 mm in F, 1.25 mm in G, and 18 mm in H. Copyright American Society of Plant Biologists (from Kenrick and Strullu-Derrien, 2014). (*See insert for color representation of the figure.*)



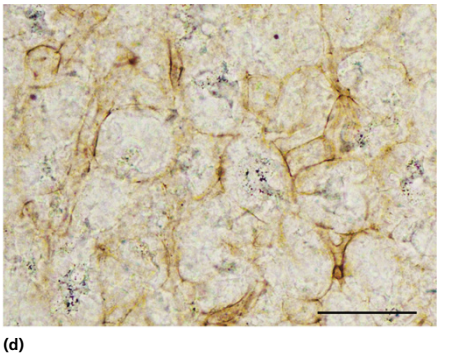
(a)



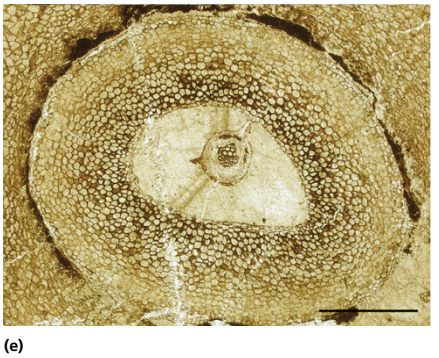
(b)



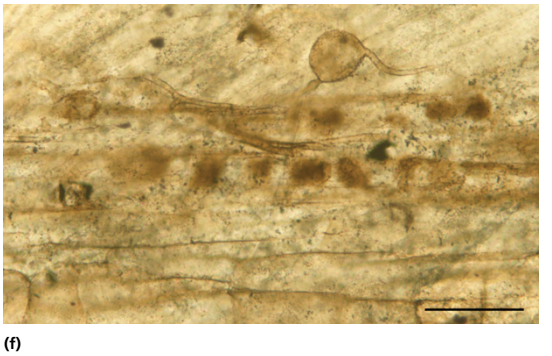
(c)



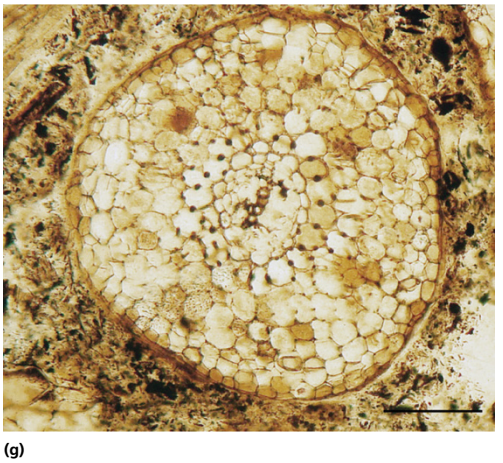
(d)



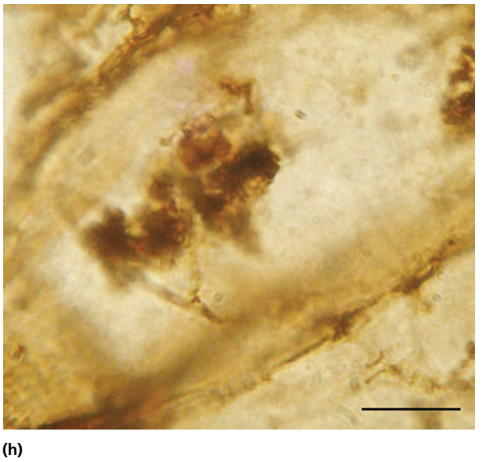
(e)



(f)



(g)



(h)

epidermis. A glomeromycotean fungus (*Palaeoglomus boullardii*) was observed in the outer cortex of the aerial axes, forming arbuscules, vesicles and spores. A fungus of the Mucoromycotina type (*Palaeoendogone gwynne-vaughaniae*) was observed in the corm of the plant, where it was present in intercellular spaces and as intracellular coils but absent from the rhizoids (Strullu-Derrien *et al.*, 2014a; Figure 1.4c,d). Krings *et al.* (2007a, 2007b) speculated that the intra- and intercellular phases of the colonization in *Nothia* might belong to different fungi. Strullu-Derrien *et al* (2014a) suggested that, as in the corm of *Horneophyton*, the intercellular hyphae in *Nothia* were most likely mucoromycotean in nature.

Colonization of the upright axes (Glomeromycota) in *Horneophyton lignieri* probably occurred through the epidermis. The mode of colonization in the corm is unclear, but fungal entry was probably not via the rhizoids. Several modes of fungal entry have been described in Rhynie Chert plants, but caution must be exercised in drawing firm conclusions, because this feature is very difficult to observe in fossils. Critical comparisons between the newly discovered *Horneophyton* endophytes, fungi previously described from the Rhynie Chert, and fungal colonization in extant lower land plants reveal several features characteristic of both Mucoromycotina and Glomeromycota. This finding indicates that early fungal symbioses were more diverse than assumed hitherto, overturning the long-held paradigm that the early endophytes were exclusively Glomeromycota (Strullu-Derrien *et al.*, 2014a). Because Devonian fossil plants are evolutionarily and structurally closer to extant bryophytes and lycophytes, comparisons with these groups, rather than the more derived vascular plants, is appropriate (Field *et al.*, 2015). These geologically early fungal-plant associations are considered to be mycorrhizal-like or paramycorrhizas (Strullu-Derrien and Strullu, 2007).

1.5 Evolution of the mycorrhizal symbioses

During the early phases of land colonization by plants, rooting systems evolved into a broad range of complex multicellular organs specializing in anchorage and nutrient acquisition (see paragraph above). However, the relationships between fungi and early trees are still not documented. Unfortunately, neither the type nor the quality of preservation allows us to observe fungal associations. The bases of the trees when found *in situ* are mostly preserved as casts, with very little anatomy remaining. To develop an understanding of mycorrhizal associations in the earliest forests, new information is needed from permineralized rooting systems or soils in the middle to latter part of the Devonian period (393–359 million years ago). Newly discovered fossils from Eurasia, on which we are currently working, may begin to provide this crucial information.

The following Carboniferous period (359–299 MYA) is famous for its extensive wetland forest communities, which gave rise to extensive coal fields in Eurasia and North America. Krings *et al.* (2011) reported an AM-like fungus in the underground organs of arborescent lycopsids from the Upper Carboniferous (ca 315 MYA). These plants had unique rooting organs (called *Stigmaria*) that developed into large, shallow bifurcating trunks that bore numerous narrow "rootlets"

(Rothwell *et al.,* 2014). The stigmarian base apparently formed by dichotomy of the shoot during embryogeny, and the "rootlets" are considered to be leaf homologues. The fungus developed near the tip of the appendages, and occupied the inner portion of the middle cortex. Hyphal threads grew along the long axis of the rootlet. Extending from these trunk hyphae were narrower hyphae that may have produced large vesicles or spores. Other branches penetrated individual cells of the cortex to form multibranched structures, interpreted as arbuscules (Krings *et al*., 2011) (Figure 1.4e,f).

The earliest fungal colonization of seed plant roots (eumycorrhizas) to date was observed in *Cordaites* (basal Coniferophytes) from the Upper Carboniferous (ca 315 MYA) (Strullu-Derrien *et al.,* 2009). AM associations developed on young rootlets exhibiting only primary growth (0.5 to 0.65 mm diameter). The fungus colonized a discontinuous zone in the central layers of the cortex. Colonization was characterized by the absence of an intercellular phase, and by the development of intraradical hyphae. While vesicles were not observed, small arbuscules did develop in some of the cortical cells (Figure 1.4g,h). Additional details of the association are difficult to resolve, owing primarily to the prominence of cortical thickenings in the rootlets. A similar masking of fine details of the mycorrhiza by cortical cell thickenings has been recorded for extant plants (cf. *Thuya occidentalis*).

Recently, mycorrhizal symbiosis was reported in the extinct gymnosperm order Glossopteridales, based on structurally preserved fossils from the Upper Permian of Antarctica (ca 260–252 MYA) (Harper *et al.,* 2013). The fungus was characterized by septate hyphae, and it was attributed to the genus *Glomites* (Taylor *et al.,* 1995), which now includes forms with aseptate to (sparsely) septate hyphae (Harper *et al.,* 2013). The fungus colonized the cortical cells of *Vertebraria* (rootlets of the seed fern *Glossopteris*) in a serpentine or helical pattern that resembles modern Paris-type mycorrhizas. Intracellular vesicles were also reported, but their occurrence was not well corroborated by the images.

Taylor *et al.* (1995) interpreted the colonization in *Aglaophyton* as symptomatic of the Arum-type, one of the two major anatomical types of colonization by AM fungi recognized in higher plants, and often associated with the fast-growing root systems of crop plants (Smith and Read, 2008). Harper *et al.* (2013) reported that the Glossopteridales specimen was the only fossil that did not have the Arum-type arbuscule morphology. However, and as also recognized by several authors (Taylor *et al*., 1995; Selosse, 2005; Strullu-Derrien *et al*., 2014a), extreme caution should be exercised when comparing fungal structures in early fossil land plants with those in modern species, especially late divergent analogues.

Root nodules (i.e. short lateral roots harboring fungal symbionts) (Russell *et al*., 2002; Dickie and Holdaway, 2011) have rarely been described in the fossil record, but recently discovered evidence suggests a lengthy geological history in gymnosperms. Schwendemann *et al.,* (2011) described root nodules in the early conifer *Notophytum* (Middle Triassic, 245–230 MYA, Antarctica) reporting probable fungal arbuscules in the cortex. This is by far the oldest known record. Cantrill and Douglas (1988) described fossil roots with nodular and abbreviated lateral roots from the Lower Cretaceous (113–100 MYA) of the Otway Basin, Victoria (Australia). A mycorrhizal

association was suggested on the basis of the general morphology of the roots, but the anatomy was not preserved and arbuscules were not observed. The roots were likely coniferous, belonging either to Taxodiaceae or Podocarpaceae.

Following a huge gap in the fossil record of mycorrhizas, material from the Middle Eocene (ca 50 MYA) has shown that both AM and ECM co-existed at that time, and that ECM occurred contemporaneously within both Gymnosperms (Pinaceae) and Angiosperms (Dipterocarpaceae). AM were described from anatomically preserved roots of the taxodiaceous conifer *Metasequoia milleri* (Stockey *et al.*, 2001). Mycorrhizal structures developed in the root cortex. Coiled hyphae were most common within cells of the inner cortical region, and these produced numerous, highly branched arbuscules.

The earliest direct fossil evidence of ECM comes from roots attributable to *Pinus* in the 50 million year old Princeton Chert. The fossils show a Hartig net that extended to the endodermis, a pseudoparenchymatous mantle, and contiguous extramatrical hyphae. The mycorrhizal rootlets lacked root hairs, and they dichotomized repeatedly, to form large, coralloid clusters (LePage *et al.,* 1997). Reproductive structures were absent. The authors suggested comparison with the extant Basidiomycota genera *Rhizopogon* and *Suillus*. Recently, ECM preserved in amber were reported from an Eocene angiosperm forest (Beimforde *et al.,* 2011). Unramified, cruciform and monopodial-pinnate ectomycorrhizas were fossilized adjacent to plant rootlets, and different developmental stages of the mycorrhizas were preserved. The mycobiont *Eomelanomyces cenococcoides* is considered to be an ascomycete, and the host was most likely a species of Dipterocarpaceae.

Currently, there is no direct fossil evidence of ectendomycorrhizas or endomycorrhizas in the orchids (ORM) and Ericaceae (ERM). A first estimate of the time of origin of these mycorrhizal forms can be derived from estimates of the age of origin of their host plant clade, derived either from fossil evidence or from calibrated molecular phylogenies of angiosperms. Direct fossil evidence of Orchidaceae is extremely rare, so one must rely on calibrated molecular phylogenies. Ramirez *et al.* (2007) suggested an origin of Orchidaceae during the late Cretaceous (76–84 MYA), coupled with a Cenozoic radiation of the most diverse epiphytic clades (Figure 1.1). In contrast, Ericaceae has an extensive fossil record (Friis *et al.*, 2011), and there are fossils assignable to the modern ERM genus *Leucothoe* from the Late Cretaceous (66–72 million years) of Central Europe (Knobloch and Mai, 1986), providing an indicative minimum age for the origin of ERM. In molecular phylogenies of Ericaceae, if one excludes the basal *Enkianthus* (AM) and the Arbutoideae and Monotropideae (further specializations in arbutoid and monotropoid mycorrhizas), the remainder of the species are basically ERM. The most recent calibrated molecular phylogenetic trees indicate a mid-Cretaceous origin for ERM (Schwery *et al.*, 2014). Despite the absence of direct fossil evidence for ORM and ERM, indirect fossil evidence of host plants, together with calibrated molecular phylogenies, imply that they evolved much later than AM and ECM, probably during the Cretaceous period.

A current hypothesis is that at the rise of ORM and ERM, fungal taxa that usually colonize the roots of other plants as endophytes were recruited as specific symbionts (see below; Selosse *et al.,* 2009; van der Heijden

et al., 2015). Thus, the ancestral AM mycorrhizas underwent replacement by other types of mycorrhizas and fungal partners in diverse plant lineages. While an adaptation to specific soil conditions (e.g., Selosse and Le Tacon, 1998; Smith and Read, 2008) is postulated to have driven this process, its timing and causes still deserves study, especially based on a closer inspection of the fossil record.

1.6 Perspectives for bridging paleomycology and genomics

Berbee and Taylor (2010) questioned how close we are to dating the phylogenetic tree of fungi. They concluded that molecular clocks calibrated by fossils are the only available tools to estimate timing of evolutionary events in fossil-poor groups. Fungi are not simply ancient and unchanging, but have evolved just as dynamically as any other group of eukaryotes, even if limited morphological criteria are available to mark this. Our brief review of the fossil record of mycorrhizal associations shows how sparse is the evidence and yet, where encountered, how informative it can be.

One problem is that discoveries of fossil mycorrhizal associations have been largely serendipitous. A second is that mycorrhizas are only preserved in a very particular and restricted set of environments of fossilization (Taylor *et al.*, 2015). Essentially, what is required is soils that are petrified, preferably in silicates, and in which original plant root cells and fungal hyphae are preserved. Such systems do occur throughout the geological record (e.g., Rhynie Chert, 407 MYA: Trewin and Rice, 2004; Central Transantarctic Mountains, Antarctica, 260–252 MYA: Harper *et al.*, 2013; Hopen, Svalbard Archipelago, 220–220 MYA: Strullu-Derrien *et al.*, 2012; Princeton chert, Columbia, 50 MYA: LePage *et al.*, 1997; Stockey *et al.*, 2001). We therefore advocate an approach that targets particular environments of preservation with specific evolutionary questions in mind.

There are two main areas in which the fossil record of mycorrhizal associations and modern genomic approaches can potentially interface and benefit from reciprocal illumination. First, fossils can help to establish the sequence in which evolutionary events occurred, and they can set minimum geological ages to the origins of taxonomic groups or organismal associations. Second, fossils fill in the gaps by extending our knowledge of the diversity of mycorrhizal associations across the plant tree of life, and by broadening our understanding of the interactions of plant and fungus at the cellular level. Furthermore, the application of high-resolution imaging techniques (e.g., Confocal Laser Scanning Microscopy) now affords a new and enhanced level of precision in documenting the details of fungal plant interactions at the cellular and subcellular levels (Strullu-Derrien *et al.*, 2015). Fossils are essential to the calibration of the tree of life of fungi and of plants, and they can provide tests of evolutionary hypotheses arising from our current understanding of the evolution of mycorrhizas, and newly formed questions emerging from the fungal tree of life and from genomic studies (Selosse *et al.*, 2015).

Ectomycorrhizal symbioses evolved from ecologically diverse decayer precursors and radiated in parallel, following the origins of their host-plant lineages (Floudas *et al.*, 2012; Kohler *et al.*, 2015). The highly polyphyletic evolution of the ECM lifestyle (Hibbett and Matheny, 2009; Tedersoo and Smith, 2013)

is marked not only by convergent losses of different components of the ancestral saprotrophic apparatus, but also by rapid genetic turnover in symbiosis-induced genes (Martin and Selosse, 2008; Eastwood *et al.*, 2011; Plett and Martin, 2011; Floudas *et al.*, 2012; Wolfe *et al.*, 2012; Kolher *et al.*, 2015). In contrast, ericoid and orchid mycorrhizal fungi retained an extensive decay apparatus that is probably exploited indirectly by the plant for carbohydrate supply, thus explaining their known saprotrophic ability (Kolher *et al.*, 2015).

Recent studies (Selosse *et al.*, 2009) provided evidence that Sebacinales (basal Hymenomycetes, Basidiomycota, with diverse mycorrhizal abilities, ranging from ECM to ERM and ORM) are endophytic in many roots systems *in natura* (Selosse *et al.*, 2009) leading to the hypothesis that many mycorrhizal lineages evolved from former root endophytes, because endophytism could act as a symbiotic "waiting room", predisposing the fungus to evolution towards a tighter mutualism with some hosts (Selosse *et al.*, 2009; van der Heijden *et al.*, 2015). There is much interest in understanding how genomes evolved in both plants and fungi to make this possible. Knowledge of the chronology of these events is also important to investigating potential environmental drivers (Selosse *et al.*, 2015).

Gymnosperms were hugely diverse during the Mesozoic era, and many important groups are now extinct. A targeted study of permineralized fossil soils would provide information on the extent to which ECM were present in gymnosperms of this time, and how they might have developed in ancient Pinaceae and in the extinct relatives of the Gnetales, such as Bennettitales. Knowledge of the early evolution of mycorrhizal associations in gymnosperms and angiosperms would also benefit from a better understanding of mycorrhizas in living species across the plant tree of life. Although ECM relations are widely reported in angiosperms, they have been documented in detail for only about 3% of living species. In particular, knowledge of their occurrence and development in basal lineages of angiosperms (e.g., *Amborella*, Austrobaileyales, Chloranthaceae, magnoliids) is lacking (Wang and Qiu 2006). The genome sequences of mycorrhizal fungi which are now available, together with those already planned and in progress, will represent foundational information for understanding the development and functioning of the mycorrhizal symbiosis (Martin and Bonito, 2013).

To understand how genomic level changes within land plants impacted on the evolution of AM it is necessary to establish the original mode of infection and host response in the earliest land plants. The early development of AM symbioses is currently best documented in the plants and fungi of the 407 million year old Rhynie Chert. Although the presence of AM has been recorded in several species, very little is understood about the details of the infection pathways and the reactions of the plants to infection. Furthermore, at least two major clades of fungi (Glomeroycota and Mucoromycotina) are now implicated in mycorrhizal symbioses in both living bryophytes and early fossils (Bidartondo *et al.*, 2011; Desirò *et al.*, 2013; Rimington *et al.*, 2015, 2016). Given that Glomeromycota and Mucoromycotina are two sister lineages (Tisserant *et al.*, 2012; Lin *et al.*, 2014), it might also be possible that their common ancestor interacted with the earliest plants. This emerging possibility deserves further analyses in both fossil and living species. A focused comparative study is needed that incorporates information

from Rhynie Chert fossils with a detailed analysis of mycorrhizal development in living groups, including liverworts, hornworts, lycopsids and ferns, to infer the original modes of infection of land plants and the basic repertoire of plant responses.

Research on the origin of the genes acting in the fungal symbiotic pathway now focuses on algal lineages related to land plants, such as charophytes. A stepwise evolution of the plant symbiotic "toolkit" in algal ancestors, with several components predating the first land plants, has been proposed recently (Delaux *et al.*, 2013). Elements of this "toolkit" may, therefore, first have facilitated the interactions between aquatic charophytes and diverse symbiotic microorganisms, later being recruited and further developed for AM evolution on land. A broader survey of the distribution and function of these genes within living green algae, especially these close to land plants, is now desirable, and the investigation of living and fossil Charophyta-fungus interactions may offer further insights.

1.7 Acknowledgments

The manuscript benefited from discussions in the 10th New Phytologist Workshop, London 9–10 September 2014, and we thank all the participants. We acknowledge Michael Krings (University of Munich), Brigitte Meyer-Berthaud (AMAP, Montpellier), as well as the journal *Plant Physiology* (www.plantphysiol.org) for permission to reuse figures. CSD thanks Francis Martin for helpful discussion concerning this manuscript. Her research stay at INRA-Nancy was funded by the Laboratory of excellence ARBRE (ANR-11-LABX-0002-01).

1.8 References

Ahmadjian V and Paracer S. (1986). *Symbiosis: an introduction to biological associations*, 1st ed. Hanover, NH: University Press of New England.

Akhmetzhanova AA, Soudzilovskaia NA, *et al*. (2012). A rediscovered treasure: mycorrhizal intensity database for 3000 vascular plant species across the former Soviet Union. *Ecology* **93**, 689–690.

Balestrini R and Kottke I. (2016). Structure and development of ectomycorrhizal roots. In: Martin F (ed). *Molecular Mycorrhizal Symbiosis*, pp. 47–62. Hoboken, New Jersey: John Wiley & Sons.

Beimforde C, Schafer N, *et al*. (2011). Ectomycorrhizas from a Lower Eocene angiosperm forest. *New Phytologist* **192**, 988–996.

Benson AK, Kelly SA, *et al*. (2010). Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 18933–18938.

Berbee ML and Taylor JW. (2010). Dating the molecular clock in fungi – how close are we? *Fungal Biology Reviews* **24**, 1–16.

Berry CM and Marshall JEA. (2015). Lycopsid forests in the early Late Devonian paleoequatorial zone of Svalbard. *Geology* **43**, 1043–1046.

Bidartondo MI, Read DJ, *et al*. (2011). The dawn of symbiosis between plants and fungi. *Biology Letters* **7**, 574–577.

Blair JE. (2009). Fungi. In: Hedges SB and Kumar S (eds). *The Timetree of life*, pp. 213–220. Oxford: University Press.

Boullard B and Lemoigne Y. (1971). Les champignons endophytes du Rhynia gwynne-vaughanii K. & L. Etude morphologique et déductions sur leur biologie. *Botaniste* **54**, 49–89.

Boyce CK, Cody GD, *et al*. (2003). Chemical evidence for cell wall lignification and the evolution of tracheids in early Devonian plants. *International Journal of Plant Sciences* **164**, 691–702.

Cantrill DJ and Douglas JG. (1988). Mycorrhizal conifer roots from the Lower Cretaceous of the Otway Basin, Victoria. *Australian Journal of Botany* **36**, 257–272.

De Bary A. (1879). *Die erscheinung der symbiose*. Strassburg, Germany: Verlag von Karl J. Trubner.

Dearnaley JDW, Martos F and Selosse MA. (2013). *Orchid mycorrhizas: molecular ecology, physiology, evolution and conservation aspects*. Berlin, Germany: Springer.

Dearnaley J, Perotto S, *et al*. (2016). Structure and development of orchid mycorrhizas. In: Martin F (ed). *Molecular Mycorrhizal Symbiosis*, pp. 63–86. Hoboken, New Jersey: John Wiley & Sons.

Delaux PM, Séjalon-Delmas N, *et al*. (2013). Evolution of the plant-microbe symbiotic "toolkit". *Trends in Plant Sciences* **18**, 298–304.

Desirò A, Duckett JG, *et al*. (2013). Fungal symbioses in hornworts: a chequered history. *Proceedings of the Royal Society B* **280**, 20130207.

Dickie IA and Holdaway RJ. (2011). Podocarp roots, mycorrhizas, and nodules. In: Turner BL and Cernusak LA (eds). *Ecology of the Podocarpaceae in tropical forests*. Smithsonian Contributions to Botany, no. 95, pp. 175–187. Washington, DC: Smithsonian Institution Scholarly Press.

Eastwood DC, Floudas D, *et al*. (2011). The plant cell wall- decomposing machinery underlies the functional diversity of forest fungi. *Science* **333**, 762–765.

Edwards D and Kenrick P. (2015). The early evolution of land plants, from fossils to genomics: a commentary on Lang (1937), "On the plant-remains from the Downtonian of England and Wales". *Philosophical Transactions of the Royal Society B* **370**, 20140343.

Field KJ, Rimington W, *et al*. (2015). First evidence of mutualism between ancient plant lineages (Haplomitriopsida liverworts) and Mucoromycotina fungi and its response to simulated Palaeozoic changes in atmospheric CO_2. *New Phytologist* **205**, 743–756.

Floudas D, Binder M, *et al*. (2012). The Paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. *Science* **336**, 1715– 1719.

Fortin JA, Declerck S and Strullu DG. (2005). *In vitro* culture of mycorrhizas. In: Declerck S, Strullu DG and Fortin JA (eds). *In Vitro Culture of Mycorrhizas*, pp. 3–14. Berlin, Heidelberg, New York: Springer.

Frank AB. (1877). Über die biologischen Verhaltnisse des Thallus einiger Krusten-Flechten. *Beiträge zur Biologie der Pflanzen* **2**, 132–200.

Frank AB. (1885). Über die auf Würzelsymbiose beruhende Ehrnährung gewisser Bäum durch unterirdische Pilze. *Berichte der Deutschen Botanischen Gesellschaft* **3**, 128–145.

Friis EM, Crane PR and Pedersen KR. (2011). *Early flowers and angiosperm evolution*. Cambridge: Cambridge University Press.

Genre A and Bonfante P. (2016). The structure of arbuscular mycorrhizas: A cell biologist's view. In: Martin F (ed). *Molecular Mycorrhizal Symbiosis*, pp. 33–46. Hoboken, New Jersey: John Wiley & Sons.

Gerrienne P, Gensel P, *et al*. (2011). A simple type of wood in two Early Devonian plants. *Science* **333**, 837.

Greb SF, DiMichele WA and Gastaldo RA. (2006). Evolution and importance of wetlands in Earth history. *Geological Society of America Special Papers* **399**, 1–40.

Harper CJ, Taylor TN, *et al*. (2013). Mycorrhizal symbiosis in the Paleozoic seed fern *Glossopteris* from Antarctica. *Review of Palaeobotany and Palynology* **192**, 22–31.

Hibbett DS and Matheny PB. (2009). Relative ages of ectomycorrhizal mushrooms and their plant hosts. *BMC Biology* **7**, 13.

Hoffman LA and Tomescu MF. (2013). An early origin of secondary growth: *Franhueberia gerriennei* gen. et sp. nov. from the Lower Devonian of Gaspé (Quebec, Canada). *American Journal of Botany* **100**, 754–763.

Humphreys CP, Franks PJ, *et al*. (2010). Mutualistic mycorrhiza-like symbiosis in the most ancient group of land plants. *Nature Communications* **1**, 103.

Jablonski D and Shubin NH. (2015). The future of the fossil record: Paleontology in the 21st century. *Proceedings of the National Academy of Sciences of the United States of America* **112**, 4852–4858.

Karatygin IV, Snigirevskaya NS and Demchenko KN. (2006). Species of the genus Glomites as plant symbionts in Early Devonian ecosystems. *Paleontological Journal* **40**, 572–579.

Kenrick P and Crane PR. (1997). The origin and early evolution of plants on land. *Nature* **389**, 33–39.

Kenrick P and Strullu-Derrien C. (2014). The origin and early evolution of roots. *Plant Physiology* **166**, 2570–2580.

Kenrick P, Wellman CH, *et al*. (2012). A timeline for terrestrialization: consequences for the carbon cycle in the Palaeozoic. *Philosophical Transactions of the Royal Society B: Biological Sciences* **367**, 519–536.

Kerp H, Trewin NH and Hass H. (2004). New gametophytes from the Early Devonian Rhynie Chert. *Transactions of the Royal Society of Edinburgh Earth Sciences* **94**, 411–428.

Kidston R and Lang WH. (1921). On old red sandstone plants showing structure, from the Rhynie chert bed, Aberdeenshire. Part V. *Transactions of the Royal Society of Edinburgh* **52**, 855–902.

Knobloch E and Mai DH. (1986). Monographie der Früchte und Samen in der Kreide von Mitteleuropa. *Rozpravy ústredního ústavu geologickénho, Praha* **47**, 1–219.

Kohler A, Kuo A, *et al*. (2015). Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. *Nature Genetics* **47**, 410–415.

Krings M, Taylor TN, *et al*. (2007a). Fungal endophytes in a 400-million-yr-old land plant: infection pathways, spatial distribution, and host responses. *New Phytologist* **174**, 648–657.

Krings M, Taylor TN, *et al*. (2007b). An alternative mode of early land plant colonization by putative endomycorrhizal fungi. *Plant Signaling & Behavior* **2**, 125–126.

Krings M, Taylor TN, *et al*. (2011). Arbuscular mycorrhizal-like fungi in Carboniferous arborescent lycopsids. *New Phytologist* **191**, 311–314.

LePage BA, Currah RS, *et al*. (1997). Fossil ectomycorrhizae from the Middle Eocene. *American Journal of Botany* **84**, 410–412.

Ligrone L, Carafa A, *et al*. (2007). Glomeromycota associations in liverworts: a molecular, cellular, and taxonomic analysis. *American Journal of Botany* **94**, 1756–1777.

Lin K, Limpens E, *et al*. (2014). Single nucleus genome sequencing reveals high similarity among nuclei of an endomycorrhizal fungus. *PLoS Genetics* **10**, e1004078.

Martin BD and Schwab E. (2013). Current Usage of Symbiosis and Associated Terminology. *International Journal of Biology* **5**, 32–45.

Martin F and Bonito GM. (2013). Ten years of Genomics for ectomycorrhizal Fungi: what have we achieved and where are we heading. In: Zambonelli A and Bonito GM (eds). *Edible Ectomycorrhizal Mushrooms*, pp. 383–401. Heidelberg, New York, Dordrecht, London: Springer.

Martin F and Selosse M-A. (2008). The *Laccaria* genome: a symbiont blueprint decoded. *New Phytologist* **180**, 296–310.

Moore D, Robson GD and Trinci APJ. (2011). *21st Century Guidebook to fungi*. Cambridge, New York: Cambridge University press.

Paracer S and Ahmadjian V. (2000). *Symbiosis, An Introduction to Biological Associations*, 2nd ed. Oxford, New York: Oxford University Press.

Pittermann J. (2010). The evolution of water transport in plants: an integrated approach. *Geobiology* **8**, 112–139.

Plett JM and Martin F. (2011). Blurred boundaries: lifestyle lessons from ectomycorrhizal fungal genomes. *Trends in Genetics* **27**, 14–22.

Pressel S, Bidartondo MI, *et al*. (2010). Fungal symbioses in bryophytes: new insights into the twenty-first century. *Phytotaxa* **9**, 238–253.

Ramirez SR, Gravendeel B, *et al*. (2007). Dating the origin of the Orchidaceae from a fossil orchid with its pollinisator. *Nature* **448**, 1042–1045.

Read DJ, Duckett JG, *et al*. (2000). Symbiotic fungal associations in 'lower' land plants. *Philosophical Transactions of the Royal Society B* **355**, 815–831.

Redecker D and Raab P. (2006). Phylogeny of the Glomeromycota (arbuscular mycorrhizal fungi): recent developments and new gene markers. *Mycologia* **98**, 885–895.

Redecker D, Kodner R and Graham LE. (2000). Glomalean fungi from the Ordovician. *Science* **289**, 1920–1921.

Remy W, Taylor TN, *et al*. (1994). Four hundred-million-year-old vesicular arbuscular mycorrhizae. *Proceedings of the National Academy of Sciences of the United States of America* **91**, 11841–11843.

Rimington WR, Pressel S, *et al*. (2015). Fungal associations of basal vascular plants: reopening a closed book? *New Phytologist* **204**, 1394–1398.

Rimington WR, Pressel P, *et al*. (2016). Reappraising the origin of mycorrhizas. In: Martin F (ed). *Molecular Mycorrhizal Symbiosis*, pp. 31–32. Hoboken, New Jersey: John Wiley & Sons.

Rothwell GW, Wyatt SE and Tomescu AMF. (2014). Plant evolution at the interface of paleontology and developmental biology: An organism-centered paradigm. *American Journal of Botany* **101**, 899–913.

Rubinstein CV, Gerrienne P, *et al*. (2010). Early Middle Ordovician evidence for land plants in Argentina (eastern Gondwana). *New Phytologist* **188**, 365–369.

Russell AJ, Bidartondo MI and Butterfield BG. (2002). The root nodules of the Podocarpaceae harbour arbuscular mycorrhizal fungi. *New Phytologist* **156**, 283–295.

Schüßler A, Schwarzott D and Walker C. (2001). A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycological Research* **105**, 1413–1421.

Schwendemann AB, Decombeix AL, *et al*. (2011). Morphological and functional stasis in mycorrhizal root. *Proceedings of the National Academy of Sciences of the United States of America* **108**, 13630–13634.

Schwery OM, Onstein RE, *et al*. (2014). As old as the mountains: the radiations of the Ericaceae. *New Phytologist*. doi:10.1111/nph.13234.

Selosse MA. (2005). Are liverworts imitating mycorrhizas? *New Phytologist* **165**, 345–349.

Selosse MA and Le Tacon F. (1998). The land flora: a phototroph–fungus partnership? *Trends in Ecology & Evolution* **13**, 15–20.

Selosse MA, Setaro S, *et al*. (2007). Sebacinales are common mycorrhizal associates of Ericaceae. *New Phytologist* **174**, 864–878.

Selosse MA, Dubois MP and Alvarez N. (2009). Do Sebacinales commonly associate with plant roots as endophytes? *Mycological Research* **113**, 1062–1069.

Selosse M-A, Strullu-Derrien C, *et al*. (2015). Plants, fungi and oomycetes: a 400-million year affair that shapes the biosphere. *New Phytologist* **206**, 501–506.

Smith SE and Read DJ. (2008). *Mycorrhizal symbiosis*. Cambridge, UK: Academic Press.

Spicer R and Groover A. (2010). Evolution of development of vascular cambia and secondary growth. *New Phytologist* **186**, 577–592.

Stein WE, Mannolini F, *et al*. (2007). Giant cladoxylopsid trees resolve the enigma of the Earth's earliest forest stumps at Gilboa. *Nature* **446**, 904–907.

Stein WE, Berry CM, *et al*. (2012). Surprisingly complex community discovered in the mid-Devonian fossil forest at Gilboa. *Nature* **483**, 78–81.

Stockey RA, Rothwell GW, *et al*. (2001). Mycorrhizal association of the extinct conifer *Metasequoia milleri*. *Mycological Research* **105**, 202–205.

Strullu DG. (1985). *Les mycorhizes, Handbuch der Pflanzenanatomie*. Berlin, Germany: Gebruder Borntraeger.

Strullu DG, Gourret JP and Garrec JP. (1981). Microanalyse des granules vacuolaires des ectomycorhizes, endomycorhizes et endomycothalles. *Physiologie Végétale* **19**, 367–378.

Strullu-Derrien C. (2010). *Recherches sur la colonisation du milieu terrestre par les plantes au cours du Dévonien infé- rieur et sur les interactions plantes/microorganismes durant les périodes Dévonien-Carbonifère*. DPhil Thesis, Angers University. Available at: http://www.sudoc.fr/157448290.

Strullu-Derrien C and Strullu DG. (2007). Mycorrhization of fossil and living plants. *Comptes Rendus Palevol, Paris*, **6–7**, 483–494.

Strullu-Derrien C, Rioult JP and Strullu DG. (2009). Mycorrhizas in upper Carboniferous Radiculites-type cordaitalean rootlets. *New Phytologist* **182**, 561–564.

Strullu-Derrien C, McLoughlin S, *et al*. (2012). Arthropod interactions with bennettitalean roots in a Triassic permineralized peat from Hopen, Svalbard Archipelago (Arctic). *Palaeogeography, Palaeoclimatology, Palaeoecology* **348–349**, 45–58.

Strullu-Derrien C, Kenrick P, *et al*. (2014a). Fungal associations in *Horneophyton ligneri* from the Rhynie Chert (ca 407 Ma) closely resemble those in extant lower land plants: novel insights into ancestral plant-fungus symbioses. *New Phytologist* **203**, 964–979.

Strullu-Derrien C, Kenrick P, *et al*. (2014b). The earliest wood and its hydraulic properties documented in ca 407 million-year-old fossils using synchrotron microtomography. *Botanical Journal of the Linnean Society* **174**, 423–437.

Strullu-Derrien C, Wawrzyniak Z, *et al*. (2015). Fungal colonization of the rooting system of an early land plant from the 407 million year old Rhynie Chert (Scotland, UK). *Botanical Journal of the Linnean Society* **179**, 201–213.

Taylor TN, Remy W, *et al*. (1995). Fossil arbuscular mycorrhizae from the Early Devonian. *Mycologia* **87**, 560–573.

Taylor TN, Kerp H and Hass H. (2005). Life history biology of early land plants: deciphering the gametophyte phase. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 5892–5897.

Taylor TN, Taylor EL and Krings M. (2009). *Paleobotany: The Biology and Evolution of Fossil Plants*. Amsterdam, Boston: Academic Press.

Taylor TN, Krings M and Taylor EL. (2015). *Fossil fungi*. Amsterdam: Elsevier.

Tedersoo L and Smith ME. (2013). Lineages of ectomycorrhizal fungi revisited: Foraging strategies and novel lineages revealed by sequences from belowground. *Fungal Biology Reviews* **27**, 83–99.

Tisserant E, Kohler A, *et al*. (2012). The transcriptome of the arbuscular mycorrhizal fungus *Glomus intraradices* (DAOM 197198) reveals functional tradeoffs in an obligate symbiont. *New Phytologist* **193**, 755–769.

Tomescu AMF, Wyatt SE, *et al*. (2014). Early evolution of the vascular plant body plan–the missing mechanisms. *Current Opinion in Plant Biology* **17**, 126–136.

Trappe JM. (2005). A.B. Frank and mycorrhizae: the challenge to evolutionary and ecologic theory. *Mycorrhiza* **15**, 277–81.

Trewin NH. (2004). History of research on the geology and palaeontology of the Rhynie area Aberdeenshire, Scotland. *Transactions of the Royal Society of Edinburgh: Earth Sciences* **94**, 285–297.

Trewin NH and Rice CM. (2004). The Rhynie hot-spring system. Geology, Biota and Mineralisation. Proceedings of the Conference held in 2003. *Transactions of the Royal Society of Edinburgh: Earth Sciences* **9**(4).

van der Heijden M, Martin FM, *et al*. (2015). Mycorrhizal ecology and evolution: the past, the present, and the future. *New Phytologist* **205**, 1406–1423.

Wang B and Qiu YL. (2006). Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza* **16**, 299–363.

Wolfe BE, Tulloss RE and Pringle A. (2012). The irreversible loss of a decomposition pathway marks the single origin of an ectomycorrhizal symbiosis. *PLoS ONE* **7**, *e39597*.

CHAPTER 2
Reappraising the origin of mycorrhizas

William R Rimington[1,2,3], Silvia Pressel[3], Katie J Field[4], Christine Strullu-Derrien[5,6], Jeffrey G Duckett[3], and Martin I Bidartondo[1,2]

[1] *Department of Life Sciences, Imperial College, London, UK*
[2] *Jodrell Laboratory, Royal Botanic Gardens, Kew, UK*
[3] *Department of Life Sciences, Plants Division, Natural History Museum, London, UK*
[4] *Centre for Plant Sciences, Faculty of Biological Sciences, University of Leeds, UK*
[5] *INRA, UMR 1136, INRA-Université de Lorraine Interactions Arbres-Microorganismes, Laboratoire d'xcellence ARBRE, INRA-Nancy, Champenoux, France*
[6] *Department of Earth Sciences, The Natural History Museum, London, UK*

2.1 Introduction

The evolution of mutually beneficial partnerships between fungi and plants some 470 million years ago (MYA) is widely considered a key event in the establishment and diversification of the land flora which transformed the biosphere and atmosphere (Pirozynski and Malloch, 1975; von Schöll *et al*., 2008). Molecular time trees show that, while some phylogenetic uncertainty exists, all major lineages of fungi likely originated long before the emergence of plants (Blair, 2009). The earliest land plants emerging from freshwater were diminutive in stature and lacked roots and vasculature; fossil evidence indicates that they were liverwort-like in appearance (Wellman *et al*., 2003; Edwards *et al*., 2014).

Symbiosis with soil fungi is likely to have provided multiple benefits, facilitating plant colonization of the poorly-developed mineral palaeosols of the Devonian (ca 400 MYA). Assuming functional analogy to modern plant-mycorrhizal symbioses, fungal partners of ancient plants are likely to have provided enhanced access to, and assimilation of, mineral nutrients through biotic weathering processes (van Breemen *et al*., 2000). Furthermore, early plants are likely to have benefitted from greater access to water from their fungal partners and other, non-nutritional, benefits such as enhanced disease resistance (Cameron *et al*., 2013) and increased tolerance to herbivory (Gehring and Whitham, 1994). Such benefits to plant partners would have been "rewarded" through provisioning photosynthetically-fixed plant carbohydrates to fungi (Selosse and Le Tacon, 1998; Selosse and Strullu-Derrien, 2015), a novel source of potentially scarce organic carbon for ancient non-saprotrophic fungi.

These mutually beneficial primeval symbioses between plants and soil-dwelling fungi – known as mycorrhizas or, more accurately, mycorrhiza-like (Smith and Read, 2008) in plants without true roots – are known to occur in the majority of extant plant species (Wang and Qiu, 2006). Notably, mycorrhiza-like associations are present within many genera of bryophytes (non-vascular plants), including many liverworts and hornworts (Read *et al*., 2000;

Molecular Mycorrhizal Symbiosis, First Edition. Edited by Francis Martin.

Wang and Qiu 2006; Desirò *et al.*, 2013), with the exception of mosses. Wang *et al.* (2010) demonstrated the presence of key plant mycorrhization genes in the rootless gametophytes of all bryophytes through to the rooted sporophytes of vascular plants. Together with the near-ubiquity of mutualistic fungal symbiosis throughout the land plant phylogeny, these findings support the hypothesis that extant plants evolved from an ancestor that engaged in mutualistic symbiosis with fungal partners long before roots evolved.

As the fungal fossil record is extremely fragmentary and can, at best, only provide circumstantial evidence of potential presence and absence of mycorrhiza-like fungal structures in a limited number of samples (Strullu-Derrien *et al.*, 2016), it is impossible to infer wider functional and evolutionary significance from fossil evidence alone. Cytological, molecular and physiological approaches to understanding the nature and functional significance of fungal symbioses in early diverging lineages of extant land plants have the potential to complement fossil evidence and to provide unique and powerful insights into the origin and evolution of mycorrhizas.

Among the earliest branching clades of plants forming mycorrhiza-like associations are the earliest divergent bryophytes (Haplomitriopsida liverworts, early diverging complex and simple thalloid liverworts, hornworts) and mycorrhizas with true roots, the lycopods (Pressel *et al.*, 2010). All of these plant groups are thought to have diverged over 400 MYA (Kenrick and Crane, 1997; Willis and McElwain 2014). A wealth of studies (Pressel *et al.*, 2010 and literature within), starting with the seminal works of 19th century botanists such as Goebel (1891,1905), have characterized in detail the cytology of fungal colonization in these plant clades.

Until recently, and rather surprisingly, molecular investigations of the fungi in early branching lineages of plants had been few (liverworts, lycophytes) or non-existent (hornworts) (Pressel *et al.*, 2010). Studies at the beginning of the 21st century (Russell and Bulman, 2005; Ligrone *et al.*, 2007; Winther and Friedman, 2008) showed that the fungal symbionts of early branching plant groups were members of the most recently evolved lineages of arbuscular mycorrhizal Glomeromycota. These findings were in line with palaeobotanical evidence of arbuscule-like structures in early Devonian plant fossils (e.g., Remy *et al.*, 1994).

Further evidence points to congruence between land plant and Glomeromycota evolution over 460 MYA. This includes both fossil (Kenrick and Crane, 1997; Redecker *et al.*, 2000) and molecular data (Simon *et al.*, 1993; Heckman *et al.*, 2001). In addition, the placement of Glomeromycota as the earliest branching mycorrhiza-forming fungi (James *et al.*, 2006), and the demonstration that the Glomeromycota associations in the complex thalloid liverworts *Marchantia paleacea* and *Preissia quadrata* are mutually beneficial and mycorrhiza-like, both in terms of plant fitness (Humphreys *et al.*, 2010) and carbon-for-nutrient exchange (Field *et al.*, 2012), lend further weight to the long-held idea of Glomeromycota as the mother of plant-fungal symbioses (Parniske, 2008).

In 2011, the widely supported notion of Glomeromycota-mediated land plant evolution was challenged by the discovery that the earliest diverging liverwort clade, the Haplomitriopsida (Crandall-Stotler *et al.*, 2009), are symbiotic with Mucoromycotina fungi, a partially saprotrophic and ancient lineage of fungi (Bidartondo *et al.*, 2011). This discovery

was made possible by the application of universal fungal primers, rather than widely used Glomeromycota-specific primers, and led to the new hypothesis that plant-Mucoromycotina symbiosis, rather than partnerships involving Glomeromycota fungi, might represent the ancestral land plant-fungal symbiosis (Bidartondo *et al.*, 2011).

In the following years, our knowledge of the diversity, distribution and nature of Mucoromycotina-plants interactions has increased considerably. Intimate Mucoromycotina symbioses are widespread across thalloid liverworts (Rimington, unpublished) and hornworts (Desirò *et al.*, 2013). They also occur in one of the earliest-diverging lineages of vascular plants, the lycopods (Rimington *et al.*, 2015).

Interestingly, many of these extant plants form partnerships also, and sometimes simultaneously (Field *et al.*, 2016), with nearly all known lineages of Glomeromycota fungi. So far, Haplomitriopsida liverwort symbioses with Mucoromycotina have been investigated physiologically, and these turn out to be mutualistic in terms of carbon-nutrient exchange (Field *et al.*, 2015a). The intertwined taxonomic histories of Glomeromycota and Mucoromycotina are explained by Stürmer (2012). This chapter discusses our recent findings, mainly focusing on the results of recent molecular analysis of living plants, with sections focusing on recent paleontological and physiological discoveries.

2.2 Fungal symbioses in non-vascular plants

Bryophytes are a monophyletic or paraphyletic group of non-vascular plants (Cox *et al.*, 2014) with three lineages: liverworts, mosses and hornworts (Qiu *et al.*, 2006). While bryophytes are firmly placed at the bottom of the land plant phylogenetic tree, in recent years the general consensus of liverworts as the earliest branching lineage of extant land plants, with hornworts sister to the vascular plants, has been challenged. For example, a recent analysis based on phylotranscriptomics places hornworts (albeit under-sampled), rather than liverworts, as sister group to all other land plants (Wickett *et al.*, 2014). Despite these recent controversies, the Haplomitriopsida are generally considered as the closest living relatives to the first land colonizers. The stem lineage of the Haplomitriopsida is estimated to have diverged from the rest of the liverworts in the Early Devonian more than 400 MYA (Heinrichs *et al.*, 2007).

2.2.1 Liverworts

Detailed cytological investigations of fungal colonisation in the Haplomitriopsida liverwort genera *Haplomitrium* and *Treubia* revealed unusual patterns, unlike any reported before in other liverworts harboring Glomeromycota (Carafa *et al.*, 2003; Duckett *et al.*, 2006). These consist of an intracellular phase, characterized by fungal coils with terminal, short-lived lumps (or swellings), and an extracellular phase associated with copious mucilage production by the host, and with the hyphae forming semi-parenchymatous structures and thick-walled spore-like structures (Carafa *et al.*, 2003; Duckett *et al.*, 2006; Pressel *et al.*, 2010). The discovery by Bidartondo *et al.* (2011) that *Treubia* and *Haplomitrium* enter in symbiosis with Mucoromycotina fungi, including *Endogone* spp., finally provided an explanation for the atypical colonization patterns reported in these plants and, given their key evolutionary position, placed Mucoromycotina partnerships as basal in liverwort evolution.

When fungal symbioses are overlaid onto a land plant phylogeny, we see that, through their 470–480 million year old history, liverworts have repeatedly gained, lost and re-acquired fungal symbionts. In line with their more recent origins (Smith and Read, 2008), the dikaryotic ascomycetes (notably the ericoid mycorrhizal fungus *Pezoloma = Rhizoscyphus ericae*) and basidiomycetes (either *Tulasnella* or Sebacinales) are restricted to derived liverwort clades, the crown thalloid group Aneuraceae and various families of leafy Jungermanniales (Bidartondo and Duckett, 2010; Pressel *et al.*, 2010). In contrast, Mucoromycotina and Glomeromycota appear to be confined to the complex and simple thalloid groups.

To date, there are only two published accounts concerning Mucoromycotina symbiosis in liverworts (Bidartondo *et al.*, 2011; Field *et al.*, 2015a). These reveal their occurrence in the Haplomitriopsida (three species each from *Haplomitrium* and *Treubia*) and, together with Glomeromycota, in a single complex thalloid liverwort genus, *Neohodgsonia,* and the two simple thalloid liverworts *Allisonia* and *Fossombronia* (Figure 2.1). However, we are currently undertaking a wide-ranging global survey of thalloid liverworts to unravel the full extent of Mucoromycotina associations in this group. Preliminary results to date, embracing about 1000 samples, show dual Mucoromycotina and Glomeromycota symbioses in at least six complex and three simple thalloid genera. Glomeromycota appear alone in at least eight and 12 complex and simple thalloid genera, respectively. Fungi appear to be absent in both early- (Blasiales and Sphaerocarpales) and late-branching liverworts (Cyathodiaceae, Oxymitraceae, Ricciaceae) (Crandall-Stotler *et al.*, 2009).

Despite conflicting views on monophyly and topology of the bryophyte clades (Cox *et al.*, 2014; Wickett *et al.*, 2014), the Haplomitriopsida remain sister to all other liverworts (Crandall-Stotler *et al.*, 2009) and, thus, their fungal associations may represent the closest homologs to the ancestral land plant fungus symbiosis.

2.2.2 Mosses

The mosses stand out as anomalous amongst early diverging land plants in lacking symbiotic fungal associations. Although there are many fungal fruiting bodies that are species-specific on mosses (Döbbeler, 2002), numerous potential fungal endophytes are reported (Davey *et al.*, 2012), and some intriguing potential interactions with basidiomycete fungi are suggested (Seitzman *et al.*, 2011), there is no evidence to date that these represent anything other than interactions with saprophytes, commensals and/or necrotrophs (Davey and Currah, 2006). There is no physiological evidence for any kind of biotrophic nutritional interdependence between mosses and fungi, or cytological evidence showing healthy fungal hyphae colonizing healthy moss cells without a host immune response (Pressel *et al.*, 2010).

These observations raise the question of why mosses do not form symbiotic associations with fungi. The answer is probably twofold (Field *et al.*, 2015c). The earliest moss lineages, *Sphagnum* and the Andreaeaopsida, obtain nutrients principally from atmospheric sources (Goffinet and Shaw, 2008). *Sphagnum* lacks rhizoids and does not form intimate relationships with underlying mineral substrates; in the Andreopsida, ill-defined filamentous extensions from the base of their stems function principally as organs of attachment to extremely nutrient-poor hard rocks.

True rhizoids appeared later in moss evolution, in the ancestors of either *Oedipodium* or the Polytrichales (Goffinet and Shaw, 2008), and they are very different from the unicellular rhizoids found in liverworts and hornworts and the unicellular root hairs of vascular plants. Moss rhizoids are multicellular, and have the same food-conducting cytology found in moss leptoids (Pressel *et al.*, 2008), which are analogous to the phloem sieve elements of vascular plants (Ligrone *et al.*, 2000). Furthermore, moss rhizoids are also highly branched structures with ultimate ramifications down to the same dimensions as the soil hyphae of fungi. Thus, mosses appear to have evolved an effective nutrient-collecting toolkit, independent of fungal associations.

2.2.3 Hornworts

The most thorough molecular study published to date of fungal associations in nonvascular land plants is for the hornworts (Desirò *et al.*, 2013). Previously, our knowledge of fungal symbioses in this group was restricted to two studies. The first is an account of the cytology of colonisation in the common species *Phaeoceros leavis*, interpreted as diagnostic of *Glomus*, albeit with atypical intercellular fungal proliferation in the mucilage-filled spaces of the hornwort thallus (Ligrone, 1988). This is somewhat reminiscent of the same in *Treubia* (Duckett *et al.*, 2006). The second is the establishment *in vitro* of an arbuscular mycorrhiza-like symbiosis in *Anthoceros punctatus*, using spores of *Glomus claroideum* (Schüßler, 2000).

The Desirò *et al.* (2013) study involved almost 200 different hornwort samples and covered approximately 10% of the global hornwort flora, including ten of the twelve hornwort genera. This analysis revealed both Glomeromycota and Mucoromycotina colonisation throughout the hornwort lineage, but absent from the early-branching genus *Leiosporoceros*, the epiphytic genus *Dendroceros*, and from *Nothoceros*, which generally grows removed from mineral soils in wet habitats. The most consistently colonized genera were *Anthoceros* and *Phaeoceros* (Figure 2.1). Surprisingly, more than a quarter of all the samples analyzed were found to contain both Glomeromycota and Mucoromycotina simultaneously.

Unlike the cyanobacterium-containing liverwort *Blasia*, which is fungus-free, the fungal hyphae within hornworts appear to be closely associated with the cyanobacterial *Nostoc* colonies that are diagnostic in these bryophytes. Through combined use of universal fungal, Mucoromycotina-specific, and Glomeromycota-specific primers, three *Endogone* spp. and ten unnamed Mucoromycotina clades were detected in hornworts. Some of the unnamed fungi include *Sphaerocreas pubescens* (Hirose *et al.*, 2014), and the rest may be *Endogonales* species not yet represented in public databases or undescribed species. These findings revealed previously unknown and unsuspected molecular phylogenetic diversity in the Mucoromycotina – comparable to that of the phylum Glomeromycota. Though closely related, most fungi from hornworts belong in Mucoromycotina clades different from those of saprotrophic species (e.g., *Endogone pisiformis*).

The discovery that hornworts are able to enter into partnership with both Glomeromycota and Mucoromycotina fungi, alone or simultaneously, or with neither, points to more versatile symbiotic options for this basal group of land plants than hitherto assumed, and suggests that the same might have been true for early land colonists (Desirò *et al.*, 2013). It must be underlined

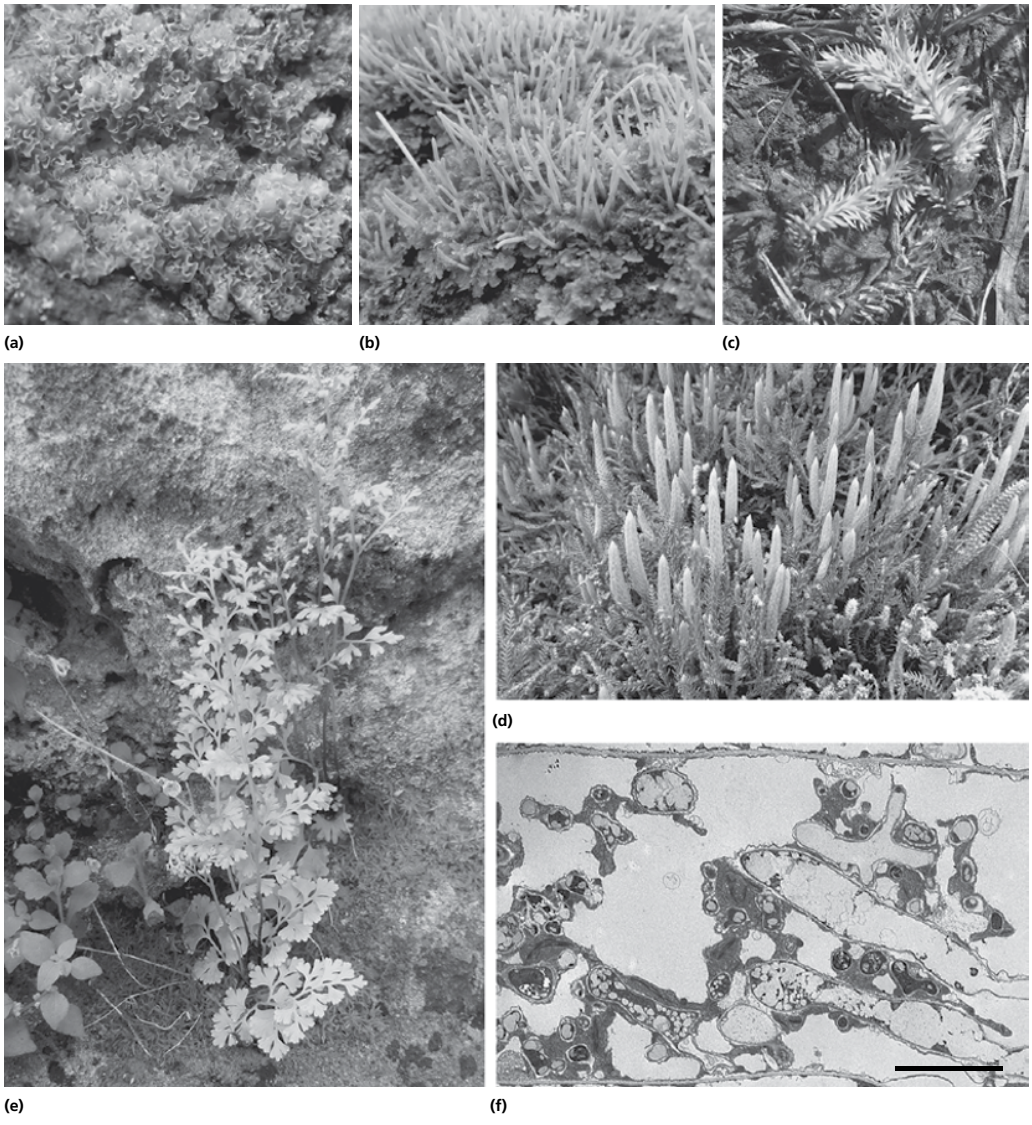


Figure 2.1 Examples of lower land plants known to harbor either Mucoromycotina or Glomeromycota fungi or both. (a) The simple thalloid liverwort *Fossombronia husnotii* Corb. (b) The hornwort *Anthoceros cristatus* Steph. (c, d) The lycopods *Lycopodiella inundata* (L.) Holub (c) and *Lycopodium fastigiatum* R. Br. (d). (e) The fern *Anogramma leptophylla* (L.) Link stands out as the only fern known to date to associate with Mucoromycotina, as well as Glomeromycota fungi. (f) Transmission electron micrograph showing a healthy host cell heavily colonized by fungal hyphae, here in the complex thalloid liverwort *Neohodgsonia mirabilis* (Perss.) Perss., known to harbor both Mucoromycotina and Glomeromycota fungi. Scale bar: 10 µm.

that, for the moment, identification of Mucoromycotina in plants relies on molecular techniques, and thus there is a pressing need to find cytological markers enabling their recognition in dual symbioses.

2.3 Fungal symbioses in vascular plants

2.3.1 Lycopods

The identity of the fungal symbionts in lycopods, the earliest diverging clade of extant vascular plants, has long puzzled scientists (Schmid and Oberwinkler 1993; Schüßler 2000). Both the sporophyte and gametophyte generations of lycopods form intimate associations with fungi thought to belong to the Glomeromycota, but with unique "lycopodioid" features (Duckett and Ligrone, 1992; Schmid and Oberwinkler, 1993). The term "lycopodioid mycothallus interactions" was coined by Schmid and Oberwinkler (1993) to describe the unique morphology of fungal colonisation in *Lycopodium clavatum* consisting of both inter- and intra-cellular fungal structures that could "not be related to any type of mycorrhizal association described to date" (Schmid and Oberwinkler, 1993).

However, in 2008, a study by Winther and Fridman seemed to dispel, once and for all, the uncertainty long surrounding the identity of the fungal symbionts in this plant group (Leake *et al.*, 2008). From a molecular analysis of seven species of lycopods from six sampling sites in Ecuador, Winther and Friedman (2008) concluded that lycopods enter in symbiosis exclusively with Glomeromycota fungi. A more recent investigation of fungal symbiosis in the lycopod *Diphasiastrum alpinum* reported an association with basidiomycete fungi (Horn *et al.*, 2013). However, the molecular and microscopical techniques used are questionable (see supplementary information for Strullu-Derrien *et al.*, 2014 for a detailed critique) and, as is also true of Winther and Friedman's study (2008), it did not include testing for the presence of Mucoromycotina.

Following the discovery of Mucoromycotina associations in liverworts and hornworts (Bidartondo *et al.*, 2011; Desirò *et al.*, 2013), in view of some striking cytological similarities between the plant-fungus interface in lycopods (Duckett and Ligrone, 1992; Schmid and Oberwinkler, 1993) and the Haplomitriopsida liverworts (Carafa *et al.*, 2003; Duckett and Ligrone, 2006), and given the limitations of previous studies, Rimington *et al.* (2015) recently reassessed the fungal symbiosis of lycopods by performing a comprehensive study of the fungal associates from 20 lycopod species from over 100 sites from every continent except Antarctica. Colonization patterns in lycopods are similar to those in hornworts (Desirò *et al.*, 2013), many being colonized by both Glomeromycota and Mucoromycotina simultaneously. Fungal colonisation and the frequency of colonisation appear to be species-specific. For example, every sample of *Lycopodiella inundata* was colonized exclusively by Mucoromycotina, whereas few *Lycopodium cernuum* samples contained fungi, and these were Glomeromycota. In addition, six new Mucoromycotina clades were discovered (Figure 2.1).

The discovery that lycopods harbor both Glomeromycota and Mucoromycotina fungi provides compelling evidence that: 1) interactions with Mucoromycotina fungi are not a peculiarity of non-vascular plants; and 2) partnerships between fungi and early branching groups of land plants are more versatile than previously envisaged.

2.3.2 Ferns

The arbuscular mycorrhizal nature of some ferns is well documented from molecular, microscopical and physiological data (Wang and Qiu, 2006; Ogura-Tsujita *et al.*, 2013; Field *et al.*, 2012, 2015b). Analyses to date of a wide range of ferns reveal that early divergent lineages, which usually have fleshy roots (e.g., Ophioglossales, Marattiales, Osmundales), invariably contain Glomeromycota fungi, whereas derived clades with thin wiry roots are most often free from fungal associates (Rimington *et al.*, 2015). The sole exception is *Anogramma leptophylla* (Polypodiales), which we now know, from sampling of numerous sites in the Mediterranean regions, contains either Mucoromycotina fungi, or both Glomeromycota and Mucoromycotina (Bidartondo *et al.*, 2011; Rimington *et al.*, 2015) (Figure 2.1). The fungi live in the perennial aestivating gametophytes (Goebel, 1905), forming fine intracellular hyphae typical of both Glomeromycota and Mucoromycotina. The presence of both fungi in *Anogramma leptophylla* is likely to be a recent acquisition, related to the unique life cycle of this derived fern.

2.4 Fungal symbioses in extinct plants

From a paleontological point of view, our current knowledge of the origins of the mycorrhizal symbiosis is based on observations of fossilized plant remains from the Rhynie Chert (Strullu-Derrien *et al.*, 2014). Fossilised Glomeromycota fungi involved in mutualisms with early plants have been known for a long time (e.g., Remy *et al.*, 1995; Taylor *et al.*, 2005; Krings *et al.*, 2007) but it is only recently that fungi with affinities to Mucoromycotina have been reported (Strullu-Derrient *et al.*, 2014). These fossils show that mycorrhiza-like associations involving both Glomeromycota and Mucoromycotina were established in early terrestrial ecosystems and that early plants likely utilized a variety of different symbioses during the colonisation of land (Field *et al.*, 2015c). For a more detailed discussion of the these recent findings of mycorrhiza-like fungi in Rhynie Chert fossils see the review of Strullu-Derrient *et al.*, 2016 (Chapter 1 of this book).

2.5 Functioning of plant-Mucoromycotina symbioses

Cytological, molecular and paleontological evidence indicating fungal presence or absence in early-branching land plant clades, though highly compelling, does not demonstrate unequivocally that plant-Mucoromycotina fungal symbioses are functionally analogous to plant-Glomeromycota symbioses. This requires quantitative experimental data. Unlike the obligately symbiotic Glomeromycota, the Mucoromycotina fungi from *Haplomitrium* and *Treubia* can be grown axenically and reintroduced into the host plants, thus fulfilling Koch's postulates (Field *et al.*, 2015a). These properties give unique tractability to experimental systems involving plants with Mucoromycotina partners, and pave the way for future studies using axenically-grown plants and fungi.

Recent experiments, using both wild-collected (with Mucoromycotina fungal partners) and axenically-grown asymbiotic plants, have shown that Mucoromycotina fungi have dramatic effects on plant morphology, unlike in plants with or without Glomeromycota (Field *et al.*, 2015a). When grown without Mucoromycotina fungi, *Treubia* failed to produce the extensive sys-

tem of mucilage-filled intercellular spaces (Field *et al.*, 2015a), normally colonized by the fungus in wild plants (Duckett *et al.*, 2006). Similarly, axenic *Haplomitrium* never developed its distinctive leafless mucilage-producing underground axes (Field *et al.*, 2015a), the site of fungal colonization in wild plants (Carafa *et al.*, 2003). The anatomy of other liverworts that harbor both fungi in the wild (e.g., *Neohodgsonia, Allisonia, Fossombronia*) remains unchanged when these are grown axenically.

The use of both stable and radio-isotope tracers (Field *et al.*, 2015a) with *Treubia lacunosa* and *Haplomitrium gibbsiae* in symbiosis with Mucoromycotina fungal partners have shown the movement of plant-produced carbohydrates to the fungi, which in return provides the plants with nitrogen and phosphorus. Consequent to these findings, plant-Mucoromycotina symbioses can now be described as both mutualistic and mycorrhizal-like. The carbon-for-nutrient exchanges in plant-Mucoromycotina symbioses are affected by atmospheric CO_2 concentrations.

Perhaps unexpectedly, the responses of plant-Mucoromycotina symbioses are opposite from those observed in plant-Glomeromycota symbioses. In liverworts paired with Glomeromycota fungal partners, reduction in atmospheric CO_2 concentration resulted in reductions of carbon allocated to fungal partners and in the amount of phosphorus returned to plant partners. This drove a reduction in functional efficiency of carbon-for-nutrient exchange between partners (Field *et al.*, 2012). These changes in resource assimilation and allocation translate into larger plant biomass and increases in reproductive effort in terms of gemmae production (Humphreys *et al.*, 2010).

However, liverwort-Mucoromycotina partnerships showed the opposite response to the same reduction in CO_2 concentration. Here, when CO_2 in the atmosphere was reduced, functional efficiency rose with plants receiving relatively greater mineral acquisition for similar plant carbon allocation (Field *et al.*, 2015a). These contrasting findings again point towards there being more dynamic and shifting fungal symbiotic scenarios throughout land plant evolution than has previously been thought.

2.6 Conclusions

The recent spate of research into plant-Mucoromycotina associations described here reveals, on the one hand, that these fungi are widespread symbionts in early-branching land plant lineages. On the other, these findings indicate that we remain a long way from knowing the full diversity, ecological importance, gentic underpinnings and evolutionary significance of the plant-Mucoromycotina symbiosis – not to mention how far it might resemble or differ, with regard to physiology, ecology, evolution and molecular signaling, from other mycorrhizal systems (Field *et al.*, 2015c). With the demonstration that symbiotic Mucoromycotina fungi can be grown axenically, the door now opens to a multitude of exciting experiments.

2.7 References

Bidartondo MI and Duckett JG. (2010). Conservative ecological and evolutionary patterns in liverwort-fungal symbioses. *Proceedings of the Royal Society B* **277**, 485–92.

Bidartondo MI, Read DJ, Trappe JM, Merckx V, Ligrone R and Duckett JG. (2011). The dawn of symbiosis between plants and fungi. *Biology Letters* **7**, 574–577.

Blair JE. (2009). Fungi. In: Hedges SB and Kumar S (eds.). *The TimeTree of Life*, pp. 215–219, Oxford: Oxford UP.

Cameron DD, Neal AL, van Wees SC and Ton J. (2013). Mycorrhiza-induced resistance: more than the sum of its parts? *Trends in Plant Science* **18**, 539–545.

Carafa A, Duckett JG and Ligrone R. (2003). Subterranean gametophytic axes in the primitive liverwort *Haplomitrium* harbour a unique type of endophytic association with aseptate fungi. *New Phytologist* **160**, 185–197.

Cox CJ, Li B, Foster PG, Embley TM and Civan P. (2014). Conflicting phylogenies for early land plants are caused by composition biases among synonymous substitutions. *Systematic Biology* **63**, 272–279.

Crandall-Stotler B, Stotler RE and Long DG. (2009). Phylogeny and classifications of the Marchantiophyta. *Edinburgh Journal of Botany* **66**, 155–198.

Davey ML and Currah RS. (2006). Interactions between mosses (Bryophyta) and fungi. *Canadian Journal of Botany* **2006**, 1509–1519.

Davey ML, Heegard E, Halvorsen R, Ohlson M and Kauserud H. (2012). Seasonal trends in the biomass and structure of bryophyte-associated fungal communities explored by 454 pyrosequencing. *New Phytologist* **195**, 844–856.

Desirò A, Duckett JG, Pressel S, Villarreal JC and Bidartondo MI. (2013). Fungal symbioses in hornworts: a chequered history. *Proceedings of the Royal Society B* **280**, 20130207.

Döbbeler P. (2002). Microniches occupied by bryophylous ascomycetes. *Nova Hedwigia* **75**, 275–306.

Duckett JG and Ligrone R. (1992). A light and electron microscope study of the fungal endophytes in the sporophyte and gametophyte of *Lycopodium cernuum* with observations on the gametophyte-sporophyte junction. *Canadian Journal of Botany* **70**, 58–72.

Duckett JG, Carafa A and Ligrone R. (2006). A highly differentiated glomeromycotean association with the mucilage-secreting, primitive antipodean liverwort *Treubia* (Treubiaceae): Clues to the origins of mycorrhizas. *American Journal of Botany* **93**, 797–813.

Edwards D, Morris JL, Richardson JB and Kenrick P. (2014). Cryptospores and cryptophytes reveal hidden diversity in early land floras. *New Phytologist* **202**, 50–78.

Field KJ, Cameron DD, Leake JR, Tille S, Bidartondo MI and Beerling DJ. (2012). Contrasting arbuscular mycorrhizal responses of vascular plants to a simulated Palaeozoic CO_2 decline. *Nature Communications* **3**, 835.

Field KJ, Rimington WR, Bidartondo MI, Allinson KE, Beerling DJ, Cameron DD, Duckett JG, Leake JR and Pressel S. (2015a). First evidence of mutualism between ancient plant lineages (Haplomitriopsida liverworts) and Mucoromycotina fungi and its response to simulated Palaeozoic changes in atmospheric CO_2. *New Phytologist* **205**, 743–756.

Field KJ, Leake JR, Tille S, Allinson KE, Rimington WR, Bidartondo MI, Beerling DJ and Cameron DD. (2015b). From mycoheterotrophy to mutualism: mycorrhizal specificity and functioning in *Ophioglossum vulgatum* sporophytes. *New Phytologist* **205**, 1492–1502.

Field KJ, Pressel S, Duckett JG, Rimington WR and Bidartondo MI. (2015c). Symbiotic options for the conquest of land. *Trends in Ecology & Evolution* **30**, 477–486.

Field KJ, Rimington WR, Bidartondo MI, Allinson KE, Beerling DJ, Cameron DD, Duckett JG, Leake JR and Pressel S. (2016). Functional analysis of liverworts in dual symbiosis with Glomeromycota and Mucoromycotina fungi under a simulated Palaeozoic CO_2 decline. *The ISME Journal* **10**, 1514–1526.

Forrest LL, Davis EC, Long DG, Crandall-Stotler BJ, Clark A and Hollingsworth ML. (2006). Unravelling the evolutionary history of the liverworts (Marchantiophyta): multiple taxa, genomes and analyses. *Bryologist* **109**, 303–334.

Gehring CA, Whitham TG. (1994). Interactions between aboveground herbivores and the mycorrhizal mutualists of plants. *Trends in Ecology and Evolution* **9**, 251–255.

Goebel K. (1891). Morphologische und Biologische Studien. IV. Uber javanische Lebermoose. 1. *Treubia*. *Annales du Jardin Botanique de Buitenzorg* **9**, 1–11.

Goebel K. (1905). *Organography of Plants Part II*. Translated by I. B. Balfour. Oxford: Clarendon Press.

Goffinet B and Shaw AJ. (2008). *Bryophyte Biology*, 2nd ed. Cambridge: Cambridge UP.

Heckman DS, Geiser DM, Eidell BR, Stauffer RL, Kardos NL and Hedges SB. (2001). Molecular evidence for the early colonization of land by fungi and plants. *Science* **293**, 1129–1133.

Heinrichs J, Hentschel J, Wilson R, Feldberg K and Schneider H. (2007). Evolution of leafy liverworts (Jungermaniidae, Marchantiophyta): estimating divergence times from chloroplast DNA sequences using penalized likelihood with integrated fossil evidence. *Taxon* **56**, 31–44.

Hirose D, Degawa Y, Yamamoto K and Yamada A. (2014). *Sphaerocreas pubescens* is a member of the Mucoromycotina closely related to fungi associated with liverworts and hornworts. *Mycoscience* **55**, 221–226.

Horn K, Franke T, Unterseher M, Schnittler M and Beenken L. (2013). Morphological and molecular analyses of fungal endophytes of achlorophyllous gametophytes of *Diphasiastrum alpinum* (Lycopodiaceae). *American Journal of Botany* **100**, 2158–2174.

Humphreys CP, Franks PJ, Rees M, Bidartondo MI, Leake JR and Beerling DJ. (2010). Mutualistic mycorrhiza-like symbiosis in the most ancient group of land plants. *Nature Communications* **103**, 2041–1723.

James TY, Kauff F, Schoch CL, Matheny PB, Hofstetter V, Cox CJ, Celio G, Gueidan C, Fraker E, Miadlikowska J, *et al*. (2006). Reconstructing the early evolution of fungi using a six-gene phylogeny. *Nature* **443**, 818–822.

Kenrick P and Crane PR. (1997). The origin and early evolution of plants on land. *Nature* **389**, 33–39.

Krings M, Taylor TN, Hass H, Kerp H, Dotzler N and Hermsen EJ. (2007). Fungal endophytes in a 400-million-yr-old land plant: infection pathways, spatial distribution, and host responses. *New Phytologist* **174**, 648–657.

Leake JR, Cameron DD and Beerling DJ. (2008). Fungal fidelity in the myco-heterotroph-to-autotroph lifecycle of Lycopodiaceae: a case of parental nurture? *New Phytologist* **177**, 572–576.

Ligrone R. (1988). Ultrastructure of a fungal endophyte in *Phaeoceros laevis* (L.) Prosk. (Anthocerotophyta). *Botanical Gazette* **149**, 92–100.

Ligrone R, Duckett JG and Renzaglia KS. (2000). Conducting tissues and phyletic relationships of bryophytes. *Philosophical Transactions of the Royal Society B* **355**, 795–814.

Ligrone R, Carafa A, Lumini E, Bianciotto V, Bonfante P and Duckett JG. (2007). Glomeromycotean associations in liverworts: A molecular cellular and taxonomic analysis. *American Journal of Botany* **94**, 1756–1777.

Mark DF, Rice CM and Trewin NH. (2013). Discussion on "A high-precision U-Pb age constraint on the Rhynie chert Konservat-Lagerstätte: time scale and other implications". *Journal of the Geological Society* **170**, 701–703.

Ogura-Tsujita Y, Sakoda A, Ebihara A, Yukawa T and Imaichi R. (2013). Arbuscular mycorrhiza formation in cordate gametophytes of two ferns, *Angiopteris lygodiifolia* and *Osmunda japonica*. *Journal of Plant Research* **126**, 41–50.

Parniske M. (2008). Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nature Reviews Microbiology* **6**, 763–775.

Pirozynski KA and Malloch DW. (1975). The origin of land plants: A matter of mycotrophism. *Biosystems* **6**, 153–164.

Pressel S, Ligrone R and Duckett JG. (2008). Cellular differentiation in moss protonemata; a morphological and experimental study. *Annals of Botany* **102**, 227–245.

Pressel S, Bidartondo MI, Ligrone R and Duckett JG. (2010). Fungal symbioses in bryophytes: New insights in the Twenty First Century. *Phytotaxa* **9**, 238–253.

Qiu YL, Li LB, Wang B, Chen ZD, Knoop V, Groth-Malonek M, Dombrovska O, Lee J, Kent L, Rest J, *et al*. (2006). The deepest divergences in land plants inferred from phylogenomic evidence. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 15511–15516.

Read DJ, Duckett JG, Francis R, Ligrone R and Russell A. (2000). Symbiotic fungal associations in 'lower' land plants. *Philosophical Transactions of the Royal Society of London Series B* **355**, 815–830.

Redecker D, Kodner R and Graham LE. (2000). Glomalean fungi from the Ordovician. *Science* **289**, 1920–1921.

Remy W, Taylor TN, Hass H and Kerp H. (1994). Four hundred-million-year-old vesicular-arbuscular mycorrhizae. *Proceedings of the National Academy of Sciences of the United States of America* **91**, 11841–11843.

Rimington WR, Pressel S, Duckett JG and Bidartondo MI. (2015). Fungal associations of basal vascular plants: reopening a closed book? *New Phytologist* **204**, 1394–1398.

Russell J and Bulman S. (2005). The liverwort *Marchantia foliacea* forms a specialized association with mycorrhizal fungi in the genus *Glomus*. *New Phytologist* **165**, 567–579.

Schmid E and Oberwinkler F. (1993). Mycorrhiza-like interaction between the achlorophyllous gametophyte of *Lycopodium clavatum* L. and its fungal endophyte studied by light and electron microscopy. *New Phytologist* **124**, 69–81.

Schüßler A. (2000). *Glomus claroideum* forms an arbuscular mycorrhiza-like symbiosis with the hornwort *Anthoceros punctatus*. *Mycorrhiza* **10**, 15–21.

Schüßler A, Schwarzott D and Walker C. (2001). A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycological Research* **105**, 1413–1421.

Seitzman BH, Ouimette A, Mixon RL, Hobbie EA and Hibbett DS. (2011). Conservation of biotrophy in Hygrophoraceae inferred from cobined stable isotope and phylogenetic analysis. *Mycologia* **103**, 280–290.

Selosse MA and Le Tacon F. (1998). The land flora: a phototroph-fungus partnership? *Trends in Ecology and Evolution* **13**, 15–20.

Selosse MA and Strullu-Derrien C. (2015). *Origins of the terrestrial flora: a symbiosis with fungi?* In: Maurel MC, Grandcolas P (eds.). Origins. Les Ullis, France, EDP Sciences (in press).

Simon L, Bousquet J, Levesque RC and Lalonde M. (1993). Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. *Nature* **363**, 67–69.

Smith SE and Read DJ. (2008). *Mycorrhizal symbiosis, third edition*. Cambridge, UK: Academic Press.

Strullu-Derrien C and Strullu DG. (2007). Mycorrhization of fossil and living plants. *Comptes Rendus Palevol* **6**, 483–494.

Strullu-Derrien C, Kenrick P, Pressel S, Duckett JG, Rioult J-P and Strullu D-G. (2014). Fungal associations in *Horneophyton ligneri* from the Rhynie Chert (c. 407 million year old) closely resemble those in extant lower land plants: novel insights into ancestral plant–fungus symbioses. *New Phytologist* **203**, 964–979.

Strullu-Derrien C, Kenrick P and Selosse MA. (2016). Origins of the mycorrhizal symbioses. In: Martin F (ed). *Molecular Mycorrhizal Symbiosis*, pp. 3–20. Hoboken, New Jersey: John Wiley & Sons.

Stürmer SL. (2012). A history of taxonomy and systematics of arbuscular mycorrhizal fungi belonging to the phylum Glomeromycota. *Mycorrhiza* **22**, 247–258.

Taylor TN, Kerp H and Hass H. (2005). Life history biology of early land plants: deciphering the gametophyte phase. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 5892–5897.

van Breemen N, Finlay R, Lundström U, Jongmans AG, Giesler R and Olsson M. (2000). Mycorrhizal weathering: a true case of mineral plant nutrition? *Biogeochemistry* **49**, 53–67.

van Schöll L, Kuyper TW, Smits MM, Landeweert R, Hoffland E and van Breemen N. (2008). Rock-eating mycorrhizas: their role in plant nutrition and biogeochemical cycles. *Plant and Soil* **303**, 35–47.

Wang B and Qiu YL. (2006). Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza* **16**, 299–363.

Wang B, Yeun LH, Xue JY, Liu Y, Ane JM and Qiu YL. (2010). Presence of three mycorrhizal genes in the common ancestor of land plants suggests a key role of mycorrhizas in the colonization of land by plants. *New Phytologist* **186**, 514–525.

Wellman CH, Osterloff PL and Mohiuddin U. (2003). Fragments of the earliest land plants. *Nature* **425**, 282–285.

Wickett NJ, Mirarab S, Nguyen N, Warnow T, *et al*. (2014). Phylotranscriptomic analysis of the origin and early diversification of land plants. *Proceedings of the National Academy of Sciences* **111**, E4859–E4868.

Willis KJ and McElwain JC. (2014). *The Evolution of Plants*, 2nd ed. Oxford: Oxford UP.

Winther JL and Friedman WE. (2008). Arbuscular mycorrhizal associations in Lycopodiaceae. *New Phytologist* **177**, 790–801.

CHAPTER 3

The structure of arbuscular mycorrhizas: A cell biologist's view

Andrea Genre and Paola Bonfante
Department of Life Sciences and Systems Biology, University of Turin, Italy

3.1 Introduction

Arbuscular mycorrhizal fungi form a homogeneous group of soil fungi that are found in most terrestrial ecosystems. They all belong to Glomeromycota, a basal fungal taxon which is currently considered phylogenetically related to Mucoromycotina, on the basis of genome sequence data from *Rhizophagus irregularis* (Tisserant *et al*., 2013; Lin *et al*., 2014). Glomeromycota are estimated to form symbiotic associations with about 80% of plants, from liverworts and ferns to gymnosperms and angiosperms (Bonfante and Genre, 2008). This ecological success is the result of the major selective advantages that arbuscular mycorrhizas (AM) interactions provide to both the plant and fungus. When lab experiments have compared symbiotic individuals with plants that were grown in the absence of glomeromycetes, striking differences have been observed: AM fungi boost plant growth, improve their capacity to absorb water and mineral nutrients (in particular, phosphate and nitrogen) and, through both this enhancement of plant health and a basal triggering of defense responses, protect plants from pathogens (Smith and Read, 2008).

Besides improving plant overall fitness, AM play a central role in nutrient cycles, soil stability and – last but not least – the survival and diffusion of AM fungi. Similarly to ectomycorrhizal fungi, in fact, glomeromycetes only accomplish their life cycle when growing in association with their plant hosts (although AM fungal reproduction is currently considered strictly asexual). Unlike ectomycorrhizal fungi, they cannot be grown for more than a few weeks in the absence of the host, a feature that characterizes AM fungi as obligate biotrophs (Bonfante and Genre, 2010).

The wide diffusion of AM and the remarkably low host specificity of most glomeromycete species appears to be related to the ancient origin of the AM interaction; AM-like structures have been repeatedly identified in 400–450 million year old fossils (Remy *et al*., 1994; Redecker *et al*., 2000; Strullu-Derrien *et al*., 2014, 2016). Furthermore, symbiosis-specific genes are found throughout the plant kingdom, including the most basal clades, strongly supporting the hypothesis that AM symbiosis played a role during the plant conquest of dry lands, and has since undergone minimal modifications (Brachmann and Parniske, 2006).

Molecular Mycorrhizal Symbiosis, First Edition. Edited by Francis Martin.