Genetic and genomic glimpses of the elusive arbuscular mycorrhizal fungi

Genetic and genomic glimpses of the elusive arbuscular mycorrhizal fungi

Luisa Lanfranco , J Peter W Young

Arbuscular mycorrhizal fungi (AMF), which form an ancient and widespread mutualistic symbiosis with plants, are a crucial but still enigmatic component of the plant microbiome. Nowadays, their obligate biotrophy is no longer an obstacle to deciphering the role played by AMF in this fascinating symbiosis. The first genome-wide transcriptomic analysis of an AMF showed a metabolic complexity with no sign of massive gene loss, and the presence of genes for meiotic recombination suggests that AMF are not simple clonal organisms, as originally thought. New findings on suppression of host defenses and nutrient exchange processes have shed light on the mechanisms that contribute to such an intimate and long-lasting integration between living plant and fungal cells.

Arbuscular mycorrhizal fungi (AMF) are among the most abundant organisms on Earth: on a very conservative estimate, they represent 5–10% of soil microbial biomass [1]. They establish an intimate association with the roots of most land plants in which the fungi supply mineral nutrients from the soil while acquiring C compounds from the photosynthetic host [2]. AMF are obligate biotrophs: they have adopted plant tissues as a preferential, perhaps a mandatory, niche for the successful completion of their life cycle [3]. Despite their importance in ecology and agriculture, AMF have been slow to reveal their secrets. However, our knowledge of AMF genes has increased markedly recently, leading to significant progress on various fronts. Many of these advances have depended on studies at the level of genes.

The diversity and classification of AMF

All known AMF belong to a single fungal clade, the phylum Glomeromycota, which appears to be a sister group of Ascomycota and Basidiomycota on the basis of nuclear ribosomal RNA phylogeny [4 and 5]. However, this taxonomic position has been called into question. Phylogenetic reconstructions based on mitochondrial [6·,7 and 8] or protein-coding nuclear [9 and 10] sequences suggest a closer relationship with Mucorales or Mortierellales (Zygomycetes). This is intriguing because a coenocytic mycelium, which is a morphological hallmark of Zygomycetes, is also characteristic of AMF.

The classification of AMF has been hampered by the paucity of morphological features, but it has long been clear that the names needed revision. The genus name *Glomus*, in particular, has been used for fungi that are so diverse that they should be in several different families. A major new molecular phylogenetic study, based on ribosomal RNA genes of almost all available AMF cultures, provides the support for a radical rationalization of AMF names [5]. For example, the frequently studied *Glomus intraradices*DAOM197198, which was recently reidentified as *G. irregulare* [11] has now become *Rhizophagus irregularis*. Meanwhile, *G. mosseae* becomes *Funneliformis*, and *G. claroideum* and *G. etunicatum*become *Claroideoglomus*. In this review, we will keep the 'old' names. In the short term, these name changes will cause confusion, but the important message is that these fungi are very different from each other, and we can expect that their functional properties will be equally diverse. In the long run, it will not be sufficient just to study one model AMF.

For more than a decade, much of our information about the diversity of field communities of AMF has come from studies of ribosomal genes amplified from roots or, less frequently, soil. They have shown that natural communities of AMF are complex with an average of 20–30 taxa in a single habitat sample, but numbers exceeding 50 have been recorded [12, 13 and 14]. AMF researchers were therefore well placed when the wave of next-generation sequencing hit environmental microbiology, and recent papers have

used massive sequencing to reveal yet more diversity [15, 16 and 17]. In the light of these data, the 230 morphospecies described so far in the Glomeromycota [5] appear as a sensational underestimate.

The mysterious genetic processes of AMF

AMF are multinucleate throughout their life cycle, with many nuclei sharing a common cytoplasm in the spores as well as the hyphae [18•]. No sexual cycle has ever been seen, but nuclei can be transferred from one mycelium to a genetically different one of the same species via anastomoses (hyphal fusions), at least in laboratory controlled conditions, with a frequency inversely related to the genetic distance [19]. Genetic markers from both parents are found in the resulting mycelium, which is presumably a heterokaryon with more than one coexisting type of nucleus. Such a process could explain the frequent observation of multiple 'allelic' forms of many genes within a single isolate, and also the apparent recombination of variants at different loci seen when multiple isolates are compared [20, 21 and 22]. In a very interesting recent study, Angelard *et al.* [23] created hybrids of *G. intraradices* by anastomosis and observed subsequent segregation of genetic markers and substantial changes in symbiotic effects on host plant (growth increase and enhanced mRNA abundance of specific genes) compared to the parental isolates. In related papers, the same group also documented changes in fungal phenotype [24] and in plant gene expression [25]. None of these phenomena provide direct evidence of genetic exchange between nuclei within the heterokaryon, but the finding of a conserved catalog of genes known to be required for correct meiotic recombination within a genome survey of 4 *Glomus* species [10] suggests that AMF are, in principle, able to undergo a conventional meiosis. Interestingly, many corresponding transcripts were found within a collection of expressed sequences [26••]. It is clear that AMF are not simple clonal organisms, which has implications both for the stability of inoculant strains and for the eventual possibility of deliberate strain improvement.

The colonization process: a fungal perspective

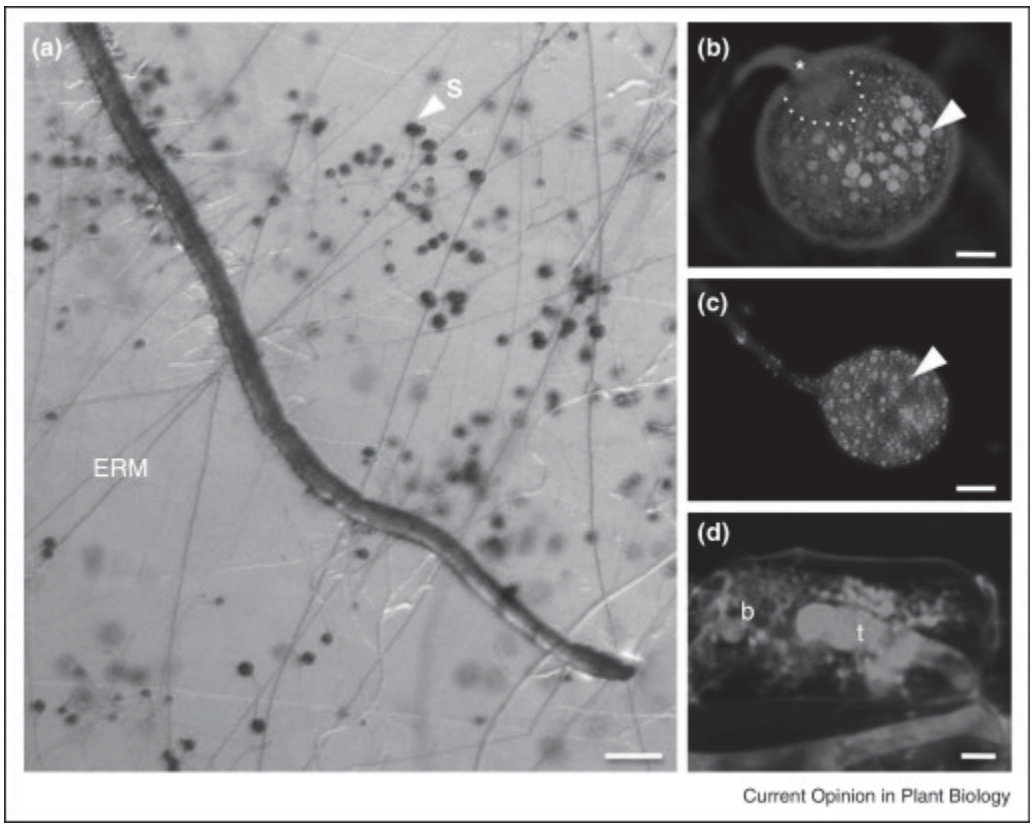
A major discovery of 2011 was the identification of the 'Myc factors', the signal molecules released by AMF and detected by the host plant. Maillet *et al.* [27] demonstrated that the signals in *G. intraradices* exudates were sulfated and nonsulfated lipochitooligosaccharides, very similar to the Nod factors produced by nitrogen-fixing rhizobia. This has been a central topic in mycorrhizal research and the subject of recent authoritative reviews [28 and 29].

The invasion of plant tissues is still a black box, although an emerging model suggests that AM and pathogenic fungi might use some conserved pathways [30 and 31]. Suppression of host defences, which guarantees the long-lasting interactions between living plant and fungal cells, is considered the first component of obligate biotrophism. Small secreted proteins (SSPs) from many plant-interacting microorganisms act as effectors, that is, proteins delivered to the plant cells to subvert host metabolism and modulate defence responses [32]. Indeed, the first SSP characterized from an AMF, named SP7, was shown to enter the plant cell nucleus and to interact with the *Medicago truncatula* transcription factor MtERF19 [33••]. This interaction leads to inhibition of MtERF19-mediated defence gene upregulation. The crucial role of SP7 is witnessed by the observation that roots constitutively expressing SP7 showed an accelerated mycorrhizal colonization. Interestingly, a fungal control over *MtERF19* transcription is also likely to occur: *G. intraradices* crude extract, in analogy to crude extract from pathogens, strongly induces MtERF19 expression while the presence of AMF hyphae only led to a mild *MtERF19* activation.

Given the huge number of putative SSPs implied by the transcriptome of arbuscules [26••], exploring the repertoire of effectors and their mechanism of action seems a crucial step towards understanding plant–AMF compatibility.

The transcriptome opens a window on the genome

Much of our knowledge of AMF genetics, molecular biology and physiology is restricted to the species *G. intraradices*. The isolate DAOM197198 (Figure 1) was chosen for the first genome sequencing project on AMF [34]. The production of an assembled genome has proven to be an arduous challenge due to a gene space larger than expected, abundant transposons and a high level of polymorphism [34], as well as the difficulty of preparing sufficient pure genomic DNA. Initial sequence data [34] provided an indirect estimate of genome size of about 150 Mb, a value that has recently been confirmed experimentally [35]. Nevertheless, the determination of the mitochondrial genome sequence of a *G. intraradices* isolate based on whole-genome shotgun sequencing [6•], and the recent publication of the mitochondrial genome of two*Gigaspora* isolates [7 and 8], demonstrate that there is no longer any technical obstacle to obtaining sequence data from AMF.



(A)*In vitro* culture of *Agrobacterium rhizogenes*-transformed roots of *Cichorium intybus* colonized by the AM fungus *Glomus* sp. DAOM 197198. ERM: extraradical mycelium; S: spores. **(B)** Confocal microscopy image showing a germinated spore stained with Nile Red, highlighting lipid bodies in bright yellow (arrowhead). An area where the cytoplasm is depleted of lipid bodies is evident (dotted line) around the emergence of the germination hypha (asterisk). **(C)** Nile Red-stained spore developing from extraradical hyphae, containing smaller lipid bodies (arrowhead). **(D)** An arbuscule stained by acid fuchsin inside a root cortical cell. The fine branches (b) surround the arbuscule trunk (t) and fill most of the host cell lumen. Bars = 300 μ m in A, 10 μ m in **B–D**.

The *G. intraradices* isolates that have been examined have no intracellular bacteria. While this simplifies the genome project somewhat, it is actually unusual for AMF. Fungi in most other genera of the Glomeromycota appear to have bacteria as permanent intracellular residents [36 and 37]. The genome sequence of one such bacterium, *Candidatus* Glomeribacter gigasporarum has recently been published [38•]: it reveals its metabolic dependence on the fungal host for both energy and nutrition. Interestingly, while phylogenetic analyses placed *Ca.* G. gigasporarum in the Burkholderiaceae, metabolic network analyses clustered it with insect endobacteria, indicating convergent evolution towards an intracellular lifestyle.

In the absence of a complete sequence, our knowledge of the *G. intraradices* DAOM197198 genome has recently been dramatically expanded by the publication of genome-wide transcriptomic data [26••] (Table 1). A rather large and highly diversified gene repertoire was inferred. The uniqueness of *Glomus*, and most probably of the AMF lineage, is reflected in the fact that 58.2% of the transcripts have no match in public nucleotide databases. A striking feature of the *Glomus* transcript data set is an abundance of sequence polymorphisms (Table 1). This was not unexpected, since sequence variants have been described for a number of genes [18•], but the fact that these variants appear as expressed sequences implies the possibility of divergent functional roles.

Table 1

***Glomus* transcriptome at a glance. A summary of the findings of Tisserant *et al.* (2012)**

Number of NRVTs (non-redundant expressed transcripts)	25 906 (~18 500 estimated genes)	
with match in public databases	10 823 (41.8%)	
match with *Rhizopus oryzae* proteins	9035	
match with *Mucor circinelloides* proteins	9265	
Representation of core eukaryotic genes	245 out of 248 (98.7%) Missing genes: metal-binding protein, spindle assembly checkpoint protein, monooxygenase involved in coenzyme Q (ubiquinone) biosynthesis	
SNPs (single nucleotide polymorphisms)	43 872 in 3963 NRVTs (15.3% of total NRVTs) Most polymorphic NRVTs contain <5 SNPs Highest number of SNPs found in a 60S ribosomal protein L17 (15 SNPs) and in a Ras-related Rab (11 SNPs)	
Traits shared with biotrophs	Examples	
Reduced arsenal of plant cell wall degrading enzymes	*Laccaria bicolor*[a] (M), *Tuber melanosporum*[b] (M), *Blumeria graminis*[c] (P), *Ustilago maydis*[d] (P)	
Abundant repertoire of secreted proteins with potential effector function	*L. bicolor* (M), *B. graminis* (P), *U. maydis* (P)	
Absence of invertase	*L. bicolor* (M), *B. graminis* (P)	
Gene regulation based on oligoarray data (>5 fold; *P*-value < 0.05)	upregulated	downregulated
Intraradical mycelium vs spores	395 (2.1%)	569 (3.0%)
Extraradical mycelium vs spores	202 (1.1%)	74 (0.4%)

•According to Parra *et al.* (2009) [58].
M: mycorrhizal.
P: pathogen.
[a] Martin *et al.* (2008) [59].
[b] Martin *et al.* (2010) [41].
[c] Spanu *et al.* (2010) [39].
[d] Kämper *et al.* (2006) [60].

Although certainly not exhaustive, these transcriptomic data are a valuable tool to infer *Glomus* metabolic complexity. Given that most metabolic pathways are represented, the obligate biotrophy of AMF cannot be ascribed to massive gene loss. This view is also emerging from the study of obligate biotrophic pathogens such as the fungus *Blumeria graminis* [39] and the oomycetes *Albugo laibachii* and *Hyaloperonospora arabodopsis* [40]. The loss of certain pathways (i.e. thiamine biosynthesis, which is also, curiously, missing in *Glomus* transcriptome) has been interpreted as a secondary phase, while the evolution of ways to circumvent host defences and to develop a sophisticated exchange interface with the host is considered the primary step towards obligate biotrophy [40]. Indeed, recurrent themes in biotrophs (and *Glomus* fits perfectly) include a decrease of the enzymatic arsenal against plant cell wall polymers and the presence of SSPs mainly expressed *in planta*, all changes that are presumably aimed to elude plant immunity.

The abundance of transposable elements (TE) seems also common to some biotrophs and possibly the main explanation for the expansion of genome size, at least in the ectomycorrhizal fungus *Tuber melanosporum* and the pathogen *B. graminis* [39, 41 and 42]. Based on the partial genome data, *Glomus*also fits this pattern (J Lee and JPWY, unpublished data). In pathogens such as *Phytophthora*, TE may also contribute to the diversification of effectors to overcome host resistance [43]. Can we envisage a comparable relationship between TE and mutualism?

Stable genetic transformation of AMF has not yet been achieved [44], but Helber *et al.* [45••] recently used HIGS (host-induced gene silencing) as a tool to silence AMF genes expressed *in planta* (discussed below). Since the RNA silencing is based on the movement of RNA molecules, the success of HIGS with AMF opens the fascinating question of whether horizontal gene transfer events (HGT) have occurred at the plant‐fungus interface, where there has been intimate contact between the partners for more than 400 million years. The parasitic plant *Striga hermonthica*, which forms an invasive organ called haustorium to allow transfer of nutrients form the host plant, has recently provided an example of eukaryotic‐eukaryotic HGT [46].

New insights into nutrient exchange in the AMF symbiosis

Nutrient exchange has probably been at the heart of the plant‐glomeromycotan interaction since the earliest days of plants on land. Evidence comes from a recent study showing that AMF colonization of a thalloid liverwort, a member of the most ancient extant clade of land plants, significantly promoted plant fitness through fungal-enhanced acquisition of phosphorus and nitrogen [47].

Perhaps surprisingly, oligoarrays based on the *Glomus* EST sequences did not show a drastic transcriptional reprogramming in the different stages of the fungal life cycle, as changes in abundance were only observed for a relatively small percentage of transcripts [26••] (Table 1). Unfortunately, these are mainly ascribed to orphan genes of unknown function, but they surely deserve investigation, with priority given to those expressed in the IRM. Here, at least on the basis of genes with known function, the activation of metabolism of sterol/lipid, chitin and transporters was observed (Figure 2). Investigations of the ERM are also desirable since, unlike most obligate biotrophs that live completely embedded within the host, a large portion of AMF mycelium develops in the soil. *Glomus* is equipped with tools to exploit soil resources and assimilate major nutrients: nitrogen and sulfur acquisition pathways are not lost (as in other obligate biotrophs [39 and 40]) and, most importantly, these are active in both the IRM and the ERM. On paper, *Glomus* has astonishing saprotrophic potentials [48 and 49].

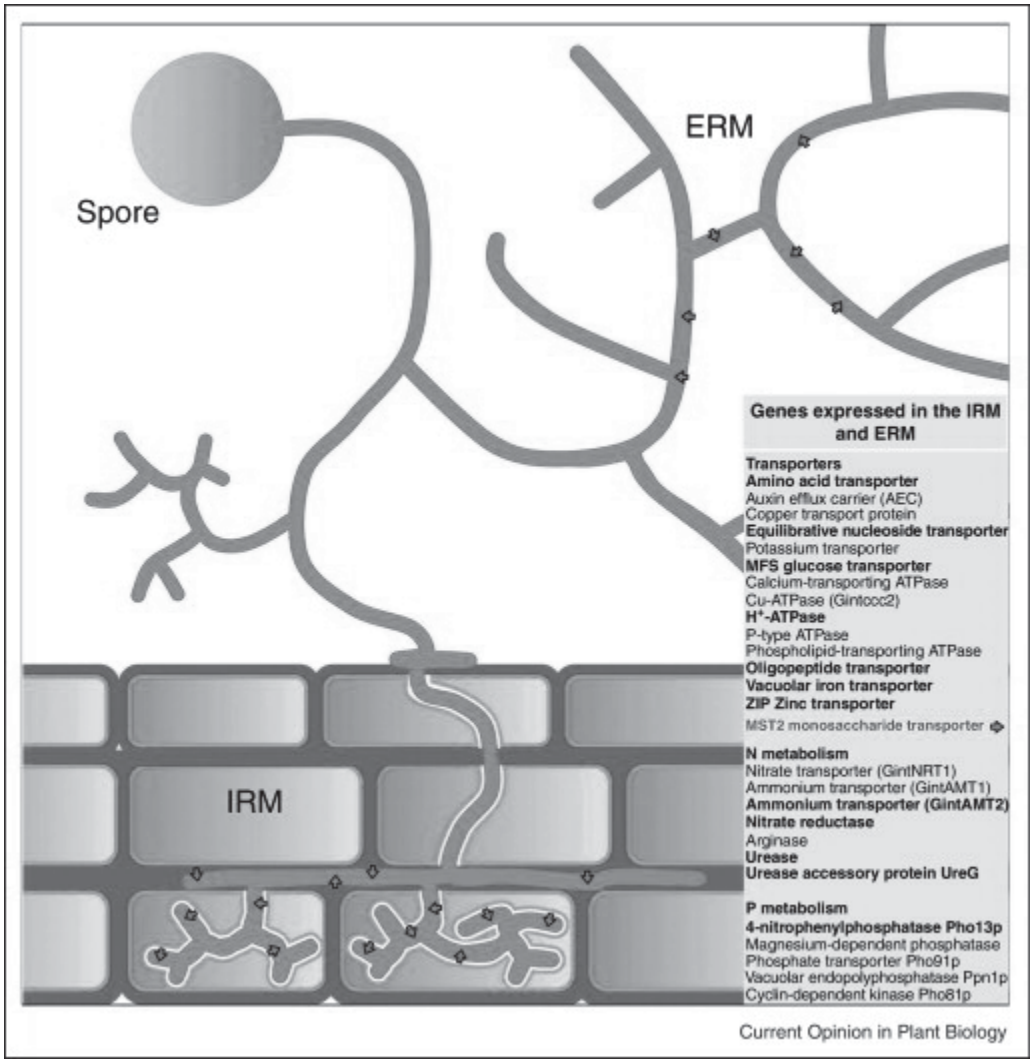


Figure 2.

Gene expression during different life stages of an AMF. Hyphae germinated from a spore branch extensively in the proximity of the roots upon perception of root exudates; the fungus then differentiates a hyphopodium on the root surface, before epidermal cell penetration. In the inner cortical layers highly branched intracellular structures (arbuscules) are formed. In addition to the intraradical mycelium (IRM), the symbiotic phase includes an extraradical mycelium (ERM) that is highly efficient in the exploration and exploitation of soil resources. Based on data from Tisserant *et al.* (2012) [26¨], a selection of genes related to transport processes and P and N metabolism expressed in the IRM and the ERM are shown (those in bold are upregulated in IRM *versus*ERM). Note that an intense metabolism occurs not only in the *in planta* phase but also in the ERM. A remarkable example of this pattern is the monosaccharide transporter MST2 [45¨]. MST2 is likely to support xylose/glucose uptake at the plant‑fungal interface in the IRM but also from the soil matrix in the ERM.

Based on the preliminary genome data, Helber *et al.* [45¨] identified the first monosaccharide transporter in AMF, probably responsible for the uptake of C compounds at the symbiotic interface. The gene MST2 is highly expressed in the *in planta* phase (arbuscules and intercellular hyphae). Several findings make this study of particular interest. First, the high affinity and transport capability for xylose residues suggest the use of derivatives from plant cell wall polymers as an additional or alternative carbon source, which is consistent with the apparent absence of an invertase gene within the *Glomus* transcriptome [26¨]. Secondly, MST2 expression in the ERM, which is indeed able to take up glucose and xylose, again underlines a certain degree of metabolic independence from the host plant.

To establish a mutualistic symbiosis, a functional linkage between C and P exchange under a fine control of both partners is likely to occur. The P availability is clearly a key factor in the control of nutrient exchanges

[50 and 51]. Indeed, MST2 expression correlates with, and even anticipates, the expression of *M. truncatula* MtPT4, a well known plant marker of a functional symbiosis responsible for the uptake of P delivered by the AMF [52]. Moreover, *in planta MST2* expression is downregulated by high P, as is the plant mycorrhiza-specific *MtPT4* [45¨]. On the contrary, an active control by the fungus of the transfer of nutrients at the interface seems also to occur [53, 54 and 55]. This view has been further enlarged with the work by Kiers *et al.* [56¨], who elegantly demonstrated that both the plant and the fungus can perceive changes in the resources supplied by the reciprocal partner and then adjust the allocation of their own resources accordingly. Using the *in vitro* culture system, they showed that more C was supplied to the more cooperative fungus, defined as the one that was more efficient in terms of plant growth response and costs of C per unit of P transferred. This cooperative species responded to C rewards with a reciprocal P transfer increase, whereas the less-cooperative species stored P in the host-inaccessible form of long-chained polyphosphates. Remarkably, signalling concerning resource allocation may even precede the *in planta*phase, since changes in plant C metabolism already occurred upon perception of AMF diffusible molecules [57].

Conclusions

Our understanding of AMF at every level from cell to ecosystem is increasingly benefiting from gene-based studies. Progress will accelerate immeasurably once we have complete sequences of some AMF genomes. With preliminary data, our improved expertise in sample preparation, and the rapid developments in DNA sequencing technology, this is an eminently achievable goal for the next two years.

Meanwhile, DNA sequencing will provide tools to investigate the forces that are shaping AMF communities and the impact these have on ecosystem functioning. At a more intimate scale, there is a clear urgency to characterize the repertoire of AMF putative effectors and compare them with the evolutionary strategies that distantly related plant-interacting organisms (bacteria, fungi, oomycetes, nematodes) have evolved. The molecular basis of the intimate and stable integration in AM symbiosis, based on reciprocal rewards through resource allocation, is another challenge in mycorrhizal research. Finally, we cannot ignore the enigmatic role of the AMF endobacteria, which are a third component of the complex interphylum network of the AM symbiosis.

Acknowledgements

The authors would like to thank Andrea Genre for help in preparing Figure 1 and Figure 2. Research has been supported by the BIOBIT-CIPE Regional Project (LL).

References

1. A.H. Fitter, T. Helgason, A. Hodge **Nutritional exchanges in the arbuscular mycorrhizal symbiosis: implications for sustainable agriculture** Fungal Biol Rev, 25 (2011), pp. 68‑72
2. S.E. Smith, F.A. Smith **Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales** Annu Rev Plant Biol, 62 (2011), pp. 227‑250
3. P. Bonfante, A. Genre **Mechanisms underlying beneficial plant fungus interactions in mycorrhizal symbiosis** Nat Commun, 1 (2010), p. 48
4. Schüßler, D. Schwarzott, C. Walker **A new fungal phylum, the Glomeromycota: phylogeny and evolution**Mycol Res, 105 (2001), pp. 1413‑1421

5. M. Krüger, C. Krüger, C. Walker, H. Stockinger, A. Schüßler **Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level** New Phytol, 193 (2012), pp. 970‐984
6. J. Lee, J.P. Young **The mitochondrial genome sequence of the arbuscular mycorrhizal fungus *Glomus intraradices* isolate 494 and implications for the phylogenetic placement of *Glomus*** New Phytol, 183 (2009), pp. 200‐211 The first complete mitochondrial sequence from the phylum Glomeromycota shows typical fungal features and indicates a phylogenetic relatedness to Mucormycotina. This study also demonstrates the feasibility of obtaining high-quality sequence (nuclear as well as mitochondrial) after whole-genome amplification of DNA from AMF spores.
7. Pelin A, Pombert JF, Salvioli A, Bonen L, Bonfante P, Corradi N: **The mitochondrial genome of the arbuscular mycorrhizal fungus *Gigaspora margarita* reveals two unsuspected *trans*-splicing events of group I introns**. *New Phytol*, in press.
8. M. Nadimi, D. Beaudet, L. Forget, M. Hijri, B.F. Lang **Group I intron‐mediated trans-splicing in mitochondria of Gigaspora rosea and a robust phylogenetic affiliation of arbuscular mycorrhizal fungi with Mortierellales**
9. Y. Liu, E.T. Steenkamp, H. Brinkmann, L. Forget, H. Philippe, B.F. Lang **Phylogenomic analyses predict sistergroup relationship of nucleariids and fungi and paraphyly of zygomycetes with significant support** BMC Evol Biol, 9 (2009), p. 272
10. S. Halary, S.B. Malik, L. Lildhar, C.H. Slamovits, M. Hijri, N. Corradi **Conserved meiotic machinery in *Glomus* spp., a putatively ancient asexual fungal lineage** Genome Biol Evol, 3 (2011), pp. 950‐958
11. H. Stockinger, C. Walker, A. Schüßler **'*Glomus intraradices* DAOM197198', a model fungus in arbuscular mycorrhiza research, is not *Glomus intraradices*** New Phytol, 183 (2009), pp. 1176‐1187
12. M. Öpik, M. Moora, J. Liira, M. Zobel **Composition of root-colonizing arbuscular mycorrhizal fungal communities in different ecosystems around the globe** J Ecol, 94 (2006), pp. 778‐790
13. M. Öpik, M. Moora, M. Zobel, U. Saks, R. Wheatley, F. Wright, T. Daniell **High diversity of arbuscular mycorrhizal fungi in a boreal herb-rich coniferous forest** New Phytol, 179 (2008), pp. 867‐876
14. A.J. Dumbrell, M. Nelson, T. Helgason, C. Dytham, A.H. Fitter **Idiosyncrasy and overdominance in the structure of natural communities of arbuscular mycorrhizal fungi: is there a role for stochastic processes?** J Ecol, 98 (2010), pp. 419‐428
15. E. Lumini, A. Orgiazzi, R. Borriello, P. Bonfante, V. Bianciotto **Disclosing arbuscular mycorrhizal fungal biodiversity in soil through a land-use gradient using a pyrosequencing approach** Environ Microbiol, 12 (2011), pp. 2165‐2179
16. A.J. Dumbrell, P.D. Ashton, N. Aziz, G. Feng, M. Nelson, C. Dytham, A.H. Fitter, T. Helgason **Distinct seasonal assemblages of arbuscular mycorrhizal fungi revealed by massively parallel pyrosequencing** New Phytol, 190 (2011), pp. 794‐804
17. M. Dai, C. Hamel, M. St. Arnaud, Y. He, C. Grant, N. Lupwayi, H. Janzen, S.S. Malhi, X. Yang, Z. Zhou **Arbuscular mycorrhizal fungi assemblages in Chernozem great groups revealed by massively parallel pyrosequencing** Can J Microbiol, 58 (2012), pp. 81‐92I
18. R. Sanders, D. Croll **Arbuscular mycorrhiza: the challenge to understand the genetics of the fungal partner** Annu Rev Genet, 44 (2010), pp. 271‐292 A comprehensive and critical overview of the most relevant work on genetic exchange, recombination and segregation events in AM fungi.

19. D. Croll, M. Giovannetti, A.M. Koch, C. Sbrana, M. Ehinger, P.J. Lammers, I.R. Sanders **Nonself vegetative fusion and genetic exchange in the arbuscular mycorrhizal fungus *Glomus intraradices*** New Phytol, 181 (2009), pp. 924‑937
20. P. Vandenkoornhuyse, C. Leyval, I. Bonnin **High genetic diversity in arbuscular mycorrhizal fungi: evidence for recombination events** Heredity, 87 (2001), pp. 243‑253
21. D. Croll, I.R. Sanders **Recombination in *Glomus intraradices*, a supposed ancient asexual arbuscular mycorrhizal fungus** BMC Evol Biol, 9 (2009), p. 13
22. H.C. den Bakker, N.W. Vankuren, J.B. Morton, T.E. Pawlowska **Clonality and recombination in the life history of an asexual arbuscular mycorrhizal fungus** Mol Biol Evol, 27 (2010), pp. 2474‑2486
23. C. Angelard, A. Colard, H. Niculita-Hirzel, D. Croll, I.R. Sanders **Segregation in a mycorrhizal fungus alters rice growth and symbiosis-specific gene transcription** Curr Biol, 20 (2010), pp. 1216‑1221
24. C. Angelard, I.R. Sanders **Effect of segregation and genetic exchange on arbuscular mycorrhizal fungi in colonization of roots** New Phytol, 189 (2011), pp. 652‑657
25. Colard, C. Angelard, I.R. Sanders **Genetic exchange in an arbuscular mycorrhizal fungus results in increased rice growth and altered mycorrhiza-specific gene transcription** Appl Environ Microbiol, 77 (2011), p. 6510
26. E. Tisserant, A. Kohler, P. Dozolme-Seddas, R. Balestrini, K.A. Benabdellah, A.D. Colard, D. Croll, C. Da Silva, S.K. Gomez, R. Koul, *et al.* **The transcriptome of the arbuscular mycorrhizal fungus *Glomus intraradices* (DAOM 197198) reveals functional tradeoffs in an obligate symbiont** New Phytol, 193 (2012), pp. 755‑769 The paper shows the first genome-wide analysis of the transcriptome from the AM model fungus*Glomus intraradices* DAOM 197198 with a transcript profiling on symbiotic and asymbiotic fungal structures based on oligoarray and 454 sequencing data. It provides the best indication of AMF genome content so far.
27. F. Maillet, V. Poinsot, O. André, V. Puech-Pagès, A. Haouy, M. Gueunier, L. Cromer, D. Giraudet, D. Formey, A. Niebel, *et al.* **Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza** Nature, 469 (2011), pp. 58‑63
28. P. Bonfante, N. Requena **Dating in the dark: how roots respond to fungal signals to establish arbuscular mycorrhizal symbiosis** Curr Opin Plant Biol, 14 (2011), pp. 1‑7
29. C. Gough, J. Cullimore **Lipo-chitooligosaccharide signaling in endosymbiotic plant-microbe interactions** Mol Plant Microb Interact, 24 (2011), pp. 867‑878
30. S. Heupel, B. Roser, H. Kuhn, M.H. Lebrun, F. Villalba, N. Requena **Erl1, a novel Era-like GTPase from *Magnaporthe oryzae*, is required for full root virulence and is conserved in the mutualistic symbiont *Glomus intraradices*** Mol Plant Microb Interact, 23 (2010), pp. 67‑81
31. M. Tollot, J.W. Sak Hoi, D. van Tuinen, C. Christine Arnould, O. Chatagnier, B. Dumas, V.Gianinazzi-Pearson, P.M.A. Seddas **An STE12 gene identified in the mycorrhizal fungus *Glomus intraradices* restores infectivity of a hemibiotrophic plant pathogen** New Phytol, 181 (2009), pp. 693‑707
32. R.H.Y. Jiang **Dynamics of effectors in host-pathogen interactions** Mycology, 2 (2011), pp. 210‑217
33. S. Kloppholz, H. Kuhn, N. Requena **A secreted fungal effector of *Glomus intraradices* promotes symbiotic biotrophy** Curr Biol, 21 (2011), pp. 1‑6 An outstanding paper showing that an AMF exploits a secreted protein to attenuate plant defence responses and sustain its biotrophic lifestyle.
34. F. Martin, V. Gianinazzi-Pearson, M. Hijri, P. Lammers, N. Requena, I.R. Sanders, Y. Shachar Hill, H. Shapiro, G.A. Tuskan, J.P.W. Young **The long hard road to a completed *Glomus intraradices* genome** New Phytol, 180 (2008), pp. 747‑750

35. K.A. Sędzielewska, J. Fuchs, E.M. Temsch, K. Keith Baronian, R. Watzke, G. Kunze **Estimation of the *Glomus intraradices* nuclear DNA content** New Phytol, 192 (2011), pp. 794‐797
36. P. Bonfante, I.A. Anca **Plants, mycorrhizal fungi, and bacteria: a network of interactions** Annu Rev Microbiol, 63 (2009), pp. 363‐383
37. M. Naumann, A. Schüßler, P. Bonfante **The obligate endobacteria of arbuscular mycorrhizal fungi are ancient heritable components related to the *Mollicutes*** ISME J, 4 (2010), pp. 862‐871
38. S. Ghignone, A. Salvioli, I. Anca, E. Lumini, G. Ortu, L. Petiti, S. Cruveiller, V. Bianciotto, P.Piffanelli, L. Lanfranco, *et al.* **The genome of the obligate endobacterium of an AM fungus reveals an interphylum network of nutritional interactions** ISME J, 6 (2012), pp. 136‐145 The paper describes the first in-depth genome characterization of an endobacterium, *Candidatus*Glomeribacter gigasporarum, symbiont of the AM fungus *Gigaspora margarita.* A mosaic of genetic determinants reminiscent of symbiotic, saprotrophic and pathogenic species are integrated in a reduced genome of 1.72 Mb.
39. P.D. Spanu, J.C. Abbott, J. Amselem, T.A. Burgis, D.M. Soanes, K. Stüber, E.V.L. van Themaat, J.K.M. Brown, S.A. Butcher, S.J. Gurr, *et al.* **Genome expansion and gene loss in powdery mildew fungi reveal tradeoffs in extreme parasitism** Science, 330 (2010), pp. 1543‐1546
40. E. Kemen, A. Gardiner, T. Schultz-Larsen, A.C. Kemen, A.L. Balmuth, A. Robert-Seilaniantz, K. Bailey, E. Holub, D.J. Studholme, D. Maclean, *et al.* **Gene gain and loss during evolution of obligate parasitism in the white rust pathogen of *Arabidopsis thaliana*** PLoS Biol, 9 (2011), p. e1001094
41. F. Martin, A. Kohler, C. Murat, R. Balestrini, P.M. Coutinho, O. Jaillon, B. Montanini, E. Morin, B. Noel, R. Percudani, *et al.* **Périgord black truffle genome uncovers evolutionary origins and mechanisms of symbiosis** Nature, 464 (2010), pp. 1033‐1038
42. P. Spanu **The genomics of obligate (and nonobligate) biotrophs** Annu Rev Phytopathol, 50 (2012), pp. 5.1‐5.19
43. R.H. Jiang, S. Tripathy, F. Govers, B.M. Tyler **RXLR effector reservoir in two *Phytophthora* species is dominated by a single rapidly evolving superfamily with more than 700 members** Proc Natl Acad Sci USA, 105 (2008), pp. 4874‐4879
44. N. Helber, N. Requena **Expression of the fluorescence markers DsRed and GFP fused to a nuclear localization signal in the arbuscular mycorrhizal fungus *Glomus intraradices*** New Phytol, 177 (2008), pp. 537‐548
45. N. Helber, K. Wippel, N. Sauer, S. Schaarschmidt, B. Hause, N. Requena **A versatile monosaccharide transporter that operates in the arbuscular mycorrhizal fungus*Glomus* sp is crucial for the symbiotic relationship with plants** Plant Cell, 23 (2011), pp. 3812‐3823 The detailed characterization of a *Glomus* high-affinity monosaccharide transporter with a broad substrate spectrum and active at multiple symbiotic interfaces is reported. The paper also provides the first demonstration of successful host-induced gene silencing (HIGS) in AM fungi.
46. S. Yoshida, S. Maruyama, H. Nozaki, K. Shirasu **Horizontal gene transfer by the parasitic plant *Striga hermonthica*** Science, 328 (2010), p. 1128
47. C.P. Humphreys, P.J. Franks, M. Rees, M.I. Bidartondo, K.R. Leake, D.J. Beerling **Mutualistic mycorrhiza-like symbiosis in the most ancient group of land plants**
48. A. Hodge, C.D. Campbell, A.H. Fitter **An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material** Nature, 413 (2001), pp. 297‐299
49. Hodge, A.H. Fitter **Substantial nitrogen acquisition by arbuscular mycorrhizal fungi from organic material has implications for N cycling** Proc Natl Acad Sci USA, 107 (2010), pp. 13754‐13759

50. P.A. Olsson, J. Rahm, N. Aliasgharzad **Carbon dynamics in mycorrhizal symbioses is linked to carbon costs and phosphorus benefits** FEMS Microbiol Ecol, 72 (2010), pp. 123‑131
51. F. Breuillin, J. Schramm, M. Hajirezaei, A. Ahkami, P. Favre, U. Druege, B. Hause, M. Bucher, T. Kretzschmar, E. Bossolini, *et al.* **Phosphate systemically inhibits development of arbuscular mycorrhiza in *Petunia hybrida* and represses genes involved in mycorrhizal functioning** Plant J, 64 (2010), pp. 1002‑1017
52. H. Javot, R.V. Penmetsa, N. Terzaghi, D.R. Cooke, M.J. Harrison **A *Medicago truncatula* phosphate transporter indispensable for the arbuscular mycorrhizal symbiosis** Proc Natl Acad Sci USA, 104 (2007), pp. 1720‑1725
53. R. Balestrini, J. Gomez-Ariza, L. Lanfranco, P. Bonfante **Laser microdissection reveals that transcripts for five plant and one fungal phosphate transporter genes are contemporaneously present in arbusculated cells** Mol Plant Microbe Interact, 20 (2007), pp. 1055‑1062
54. E.C. Hammer, J. Pallon, H. Wallander, P.A. Olsson **Tit for tat? A mycorrhizal fungus accumulates phosphorus under low carbon availability** FEMS Microbiol Ecol, 76 (2011), pp. 236‑244
55. J. Pérez-Tienda, P.S. Testillano, R. Balestrini, V. Fiorilli, C. Azcón-Aguilar, N. Ferrol **GintAMT2, a new member of the ammonium transporter family in the arbuscular mycorrhizal fungus *Glomus intraradices*** Fungal Genet Biol, 48 (2011), pp. 1044‑1055
56. E.T. Kiers, M. Duhamel, Y. Beesetty, J.A. Mensah, O. Franken, E. Verbruggen, C.R. Fellbaum, G.A. Kowalchuk, M.M. Hart, A. Bago, *et al.* **Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis** Science, 333 (2011), pp. 880‑882 By tracking resources exchange between the partners in the an *in vitro* root organ culture, the authors show that AM fungi provide more phosphorus (P) in direct response to a greater host carbon (C) supply. One fungal species was more cooperative than the others, providing more P per unit C, and the plant rewarded this partner with more C. The authors describe this as a ‘market economy’.
57. C. Gutjahr, M. Novero, M. Guether, O. Montanari, M. Udvardi, P. Bonfante **Pre-symbiotic factors released by the arbuscular mycorrhizal fungus Gigaspora margarita induce starch accumulation in Lotus japonicus roots** New Phytol, 183 (2009), pp. 53‑61
58. G. Parra, K. Bradnam, Z. Ning, T. Keane, I. Korf **Assessing the gene space in draft genomes** Nucl Acids Res, 37 (2009), pp. 289‑297
59. F. Martin, A. Aerts, D. Ahrén, A. Brun, E.G.J. Danchin, F. Duchaussoy, J.A. Gibon, A. Kohler, E. Lindquist, V. Pereda, *et al.* **The genome of *Laccaria bicolor* provides insights into mycorrhizal symbiosis** Nature, 452 (2008), pp. 88‑92
60. J. Kämper, R. Kahmann, M. Bolker, L.J. Ma, T. Brefort, J. Barry, B.J. Saville, F. Banuett, J.W. Kronstad, S.E. Gold, *et al.* **Insights from the genome of the biotrophic fungal plant pathogen *Ustilago maydis*** Nature, 444 (2006), pp. 97‑101