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Plant evolution driven by symbiotic and pathogenic interactions

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Abstract

During 450 million years of diversification on land, plants and microbes have evolved together. This is reflected in today's continuum of associations ranging from parasitism to mutualism. Through phylogenetics, cell biology and reverse genetics extending beyond flowering plants into Bryophytes, scientists have started to unravel the genetic basis and evolutionary trajectories of plant-microbe associations. Protection against pathogens and support of beneficial, symbiotic, microorganisms are sustained by a blend of conserved and clade-specific plant mechanisms evolving at different speeds. We propose that symbiosis consistently emerges from the co-option of protection mechanisms and general cell biology principles. Exploring and harnessing the diversity of molecular mechanisms employed in non-flowering plant-microbe interactions may extend the possibilities for engineering symbiosis-competent and pathogen resilient crops.

Main text:

Eighty percent of our planet's total organic matter is from plants (*1*) and plants are essential to most extant aquatic and terrestrial ecosystems, providing fixed carbon, shade and shelter for a myriad of organisms. Beyond their current ecological roles, the evolution of plants over the past billion years is linked with the evolution of other organisms and biotic interactions with plants have shaped plant diversity (*2*). Mutually beneficial interactions, defined as symbiosis in its narrow sense, facilitated major transitions, such as the migration from exclusively aquatic environments to land (*3*) and the arms race between plants and pathogens drives diversification of plant immunity (*4*).

The plant lineage encompasses three major groups, the recently described algal group of the Prasinodermophyta (*5*), the chlorophytes such as the model alga *Chlamydomonas reinhardtii*, and the Streptophytes. Streptophytes derive their name from the Greek strepto, for twisted, referring to the sperm of some members, and harbour a group of paraphyletic green algae and all land plants, also known as embryophytes. Among the paraphyletic streptophyte algae, phylogenomic analyses support the Zygnematophyceae (conjugating algae) as the sister clade to the embryophytes (Fig. 1). Phylogenomic analyses also revisited the embryophyte phylogenies, resolving two main monophyletic clades: the Bryophytes (or non-vascular plants) and the Tracheophytes (or vascular plants) that diverged more than 400 million years ago (*6, 7*). Both the Tracheophytes and the Bryophytes diversified in hundreds of thousands of species on land, giving rise to mosses, such as *Physcomitrium patens* (formerly *Physcomitrella patens*), liverworts, such as *Marchantia polymorpha*, grouped within the Bryophytes and Tracheophyte species as diverse as ferns, gymnosperms or angiosperms (flowering plants) (Fig. 1)

Understanding ancient plants helps us to unravel how extant plant life diversity evolved and to harness it in our efforts to sustainably improve agricultural germplasm. Yet, defining the

morphology and physiology of ancient plants based on the fossil record alone is challenging and trying to determine the underlying molecular mechanisms is impossible. The fact that Bryophytes and Tracheophytes both form monophyletic clades allows inferring these ancestral traits and states. Following the parsimony principle, any trait shared by both clades was present in their most recent common ancestor. It is thus possible to infer traits of ancestral plants based on observations made on extant Bryophytes and Tracheophytes, opening a window into the past. Such an inference follows a conserved rationale, involving phenotyping of diverse extant species, phylogenetics, and reverse genetics (*8*).

By now, genome and transcriptome assemblies have been generated for most of the main plant clades, including genomes representing five of the six orders of streptophyte algae, each of the three Bryophyte clades, as well as lycophytes and ferns (Table 1). Bryophyte genome information and genome-editing tools were first developed for the moss *P. patens* and later for the liverwort *Marchantia polymorpha*. Establishment of these two models unlocked the study of biological processes ranging from hormone biology to sexual reproduction and development of specific cell types (*9*). Contrasting the knowledge from angiosperms with these recent studies in Bryophytes proved to be insightful to reconstruct the principles behind the evolution of their morphological diversity (*8*).

Profiling of microbial communities found in the plant environment, on the plant surface and within plant tissues (referred to as endophytes) has revealed marked differences in their composition (*10*, *11*). In both the Tracheophytes and Bryophytes endophytic communities represent a subset of the microorganisms found in the environment. Endophytic communities are shaped by active microbial mechanisms such as the diverse pathogen infection structures and strategies to penetrate plant tissues. On the plant side, genetic and cell biology studies in

multiple model angiosperms have documented genetic programs to either support colonization, or defend against intruders.

Here, we provide an update on the evolution of plant-microbe interactions at multiple scales with a focus on plant processes. We integrate current knowledge into a conceptual model of plant microbe evolution to highlight the single emergence of a symbiosis support mechanism and the repeated emergence of alternative strategies in those lineages that had eliminated it. Finally, we propose future directions to test this model and further expand our understanding of the evolution of the interactions between plants and their microbiome. For comprehensive reviews summarising the state of the art in plant-microbe interactions, we recommend detailed reviews on arbuscular mycorrhizal symbiosis (*12*), nitrogen fixing root-nodule symbiosis (*13*) and the plant immune system (*14*).

Diversity of protection mechanisms

The dynamics of plants defending against microbes, and pathogens manipulating plants to achieve infection, impose strong selective pressures resulting in rapid adaptive evolutionary changes. Plants have evolved conserved and intertwined strategies to form barriers, perceive microbes, activate defense responses and deploy antimicrobials. This is supplemented with plant lineage-specific but often conceptually convergent strategies.

Extra- and intracellular immunity.

A two-tiered, cell autonomous, innate immunity utilizes pattern recognition receptors (PRRs) residing at the cell periphery which perceive broadly conserved microbe (or pathogen) -associated molecular patterns (MAMPs) and initiate defense responses. In addition, intracellular resistance proteins, often members of the Nucleotide-binding Leucine-Rich-

repeat (NLR) family of proteins, survey within the plant cell for microbe-triggered alterations, or the presence of microbial proteins termed effectors.

Extracellular immunity is primarily achieved through Receptor-like kinases (RLK) and receptor-like proteins (RLPs) harbouring extracellular protein domains, such as Leucine-Rich Repeats (LRR), Lysin-motif (LysM), EGF or Lectin domains that are anchored to the plasma membrane via a single transmembrane domain. RLKs furthermore carry intracellular kinase domains (*15*). Extracellular domains mediate the recognition of ubiquitous and conserved MAMPs such as peptides derived from bacterial flagellin, chito-oligosaccharides, that are constituents of the cell wall or lipo-chitooligosaccharides. The latter two had formerly been associated with symbiosis (*16*) but their presence extends beyond symbiotic fungi (*17*). Changes in receptor complexes, often involving conserved co-receptors such as BAK1 or SOBIR1 can then result in elevated, or oscillating, concentrations of intracellular calcium and the activation of downstream kinase cascades to activate defense gene networks. A full breadth of receptor types, specificities and responses are covered elsewhere (*15*, *18*).

Phylogenetic analyses have identified RLKs and RLPs in both Bryophytes and Tracheophytes (*4*). Given the widespread presence of microbial flagellin and chitin, it is expected that the corresponding plant recognition systems are conserved. Furthermore, RLKs are not only involved in immune processes, they also regulate plant symbiosis alongside growth and development. In support, the majority of LRR-RLK genes underwent purifying selection over long time scales (*19*). However, perception of specific MAMPs may also be restricted within smaller clades, such as the Brassicaceae-specific perception of elf18, a peptide of the bacterial elongation factor EF-Tu, by the Elongation Factor Receptor (EFR) (*20*) and lineage-specific receptor repertoires (*14*).

Chitin perception and signalling are conserved and were likely present in the common ancestor of Bryophytes and Tracheophytes (*21*). In the moss *P. patens* a PpCERK1 ortholog mediates MPK4a/b activation and calcium oscillations can be triggered by chitin application (*22*).

By contrast to a possibly conserved capability of perceiving chitin, the Bryophytes *M. polymorpha* and *P. patens* appear to be insensitive to the bacterial flagellin epitope flg22 and *P. patens* does not encode a cognate FLAGELLIN SENSING 2 receptor (*22–24*), which otherwise is widespread in Angiosperms. Future work needs to establish a non-vascular plant *FLS2* phylogeny and test whether other immunogenic epitopes of flagellin are perceived in Bryophytes and whether plant secreted glycosidases such as BGAL1 fulfil conserved functions to release immunogenic peptides from glycosylated flagellin (*25*).

Proteins fulfilling essential cellular and immune processes are evolutionarily conserved and frequently targeted by microbial host-translocated virulence proteins (*26*). The receptor-like kinase BAK1 is involved in integrating multiple signalling inputs in Angiosperms, from hormonal signals to immunity, and perfectly illustrates this concept. BAK1 orthologs from lineages that diverged hundreds of million years ago can be targeted by the same effector, facilitating infection (*24*). Intracellular immunity is one strategy to protect such evolutionary constrained key proteins.

Intracellular immunity is mainly relying on a family of disease resistance proteins, the NLR proteins, which carry a central nucleotide-binding adaptor shared by APAF-1, certain R gene products and a CED-4 (NB-ARC) domain followed by LRRs. An evolutionary arms race with pathogen effectors results in NLRs being among the most polymorphic protein families in plants. Together they ensure recognition of the diverse and overlapping activities of microbes within plant cells to elicit an immune response. An emerging theme is that

several NLRs form genetic and functional networks (*27*). NLRs may act as sensors and often carry integrated additional domains which represent cellular components targeted by microbes. Others are executors that trigger a defense response when a cognate sensor NLR has detected a microbe, through diverse mechanisms including the formation of wheel-like resistosome complexes that may ensure a coordinated functionality of NLR N-termini (*28*, *29*). Helper NLRs participate in the formation of lineage specific networks of immune receptors (*30*, *31*). Based on phylogenies built using the NB-ARC domain and their N-terminal domains, NLRs are subclassified into three major monophyletic clades: CNL, RNL and TNL with either a Rx-type Coiled coil (CNL), RPW8-type Coiled coil (RNL) or Toll/Interleukin1 receptor (TIR) domain (TNL) (*32*). CNLs, RNLs, but no TNLs have been identified in the genomes of Bryophytes (*32*). Instead, additional types of N-terminal domains such as protein kinase, DUF676 and a/b hydrolase domains may have independently replaced the TIR domains in a lineage-specific manner in mosses and liverworts (*33*).

Defense hormone signalling.

Almost all plant hormones have been linked to immunity. Prominent hormones distributing information across organs and tissues in Angiosperms are salicylic acid (SA), jasmonic acid (JA) and ethylene. The salicylic acid (SA) pathway predominantly mediates resistance against biotrophic pathogens which rely on living host tissues, while JA signalling is more prominently contributing to resistance against tissue-killing necrotrophic pathogens and herbivorous insect pests (*34*). A comparative genomics approach led Bowles *et al.* (*35*) to conclude that the fundamental backbone for the biosynthesis and signalling pathways of Ethylene, JA and SA either predated or accompanied land plant transition (Fig. 1).

COI1 is a co-receptor of the active form of JA in tracheophytes, JA-Isoleucine (JA-Ile), that mediates defenses against pathogens in *Arabidopsis thaliana*. While many aspects of

JA-Ile signalling seem conserved in Bryophytes, researchers uncovered that the *M. polymorpha* COI1 homolog, MpCOI1, is a co-receptor for dinor-12-oxo-phytodienoic acid (dn-OPDA) but not JA-Ile (*36*). The difference observed between Tracheophytes and Bryophytes is here linked to a single substitution (a neo-functionalization in Tracheophytes) in COI1 and to the concomitant evolution of JA-Ile biosynthesis. Contrary to what has been established in *A. thaliana*, dn-OPDA may be prioritized over SA in *M. polymorpha* as the SA mediated promotion of a necrotrophic fungus infection could be stopped when dn-OPDA was applied simultaneously (*37*).

Much less is known on the molecular components of SA signalling pathways in Bryophytes. The ability to produce SA is conserved in Angiosperms and Bryophytes (*37*, *38*), but the degree of conservation of its function during interactions with microbes requires further clarification (*39*).

Pathogenicity-related genes.

Defense responses that include the local or systemic upregulation of pathogenicity-related (PR) genes with antimicrobial activity seem to be conserved in Tracheophytes and Bryophytes (*40*). Similar to Angiosperms, ferns and *P. patens* upregulate PR genes upon application of salicylic acid or pathogen challenge (*39*). *M. polymorpha* also induces all major PR gene families during infection with an oomycete, with the exception of defensin (PR12) and thionin (PR13) proteins which seem absent from the liverwort genome (*41*).

Metabolic defense strategies.

Phenylpropanoid-mediated biochemical defences are shared between divergent land plant lineages. Oomycete infection of *M. polymorpha* as well as *P. patens* (*42*) results in the upregulation of phenylpropanoid metabolism enzymes including those involved in flavonoid

biosynthesis. These genes show a similar induction pattern in the liverwort *M. polymorpha* and in the Angiosperm *Nicotiana benthamiana* (*41*). Pigmented flavonoids such as anthocyanins are widely distributed in Angiosperms, contributing to the diversity of flower colors and often protect against abiotic stress by acting as antioxidants. When stressed, liverworts from the Marchantia genus accumulate a different red-colored pigment, riccionidin A, a member of the newly defined group of auronidins (*43*). Auronidins, a pigment class distinct from anthocyanins, seem to functionally 'replace' them in bryophytes and it is likely that anthocyanins were not present in the last common ancestor of Bryophytes and Tracheophytes (*43*). In *M. polymorpha*, *MpMYB14*, is the key regulator of auronidin production in response to stress. *MpMYB14* is activated during infection stress responses and plants carrying a loss-of-function *myb14* mutation display increased susceptibility to oomycete infection (*41*). Conversely, overexpression of *MYB14* in sectors of the *M. polymorpha* thallus resulted in increased resistance to infection.

Another recurring principle involving metabolites is the formation of idioblasts with specialised secretory compartments and both Angiosperms and liverworts have evolved lineage specific strategies to achieve this goal (Figure 2). Specialised myrosin cells lining the leaf veins of cruciferous plants store large amounts of myrosinase enzyme. Myrosinases cleave the bond between sulfur and glucose in glucosinolates to produce toxic isothiocyanates when plants are damaged by pests. Similarly, specialised oil body cells of *M. polymorpha* accumulate terpenoid compounds and attenuated oil body development makes *M. polymorpha* thalli much more vulnerable to herbivory by pill bugs (*44*). While myrosin cells possibly evolved from guard cell myrosinase-based defenses and share overlapping transcriptional regulatory networks with them (*45*), oil body cells likely represent a liverwort-specific innovation (*44*).

Plant support for microbial colonization

Both pathogenic and symbiotic microbes rely on general plant cell processes to enter and establish structures inside plant tissues and cells. This is well documented through mutations in susceptibility genes which lead to increased colonisation resistance (*46*, *47*). In addition, Intracellular symbiosis relies on the provision of dedicated accommodation mechanisms via evolutionarily-conserved genetic modules, which may either be hijacked by pathogens or were recruited into immune processes (**Box 1**).

Plant symbiosis in the narrow sense refers to mutualistic interactions with benefits for both the host plants and their micro-symbionts. These have been classified in two categories: the intracellular and the intercellular symbiosis. In both types host plants actively deploy accommodation mechanisms. The diverse intracellular types of symbiotic associations mostly rely on a conserved genetic module supplemented with lineage-specific adjustments. By contrast, intercellular symbiosis seems to repeatedly evolve through convergent mechanisms.

A unique and conserved principle for symbiotic intracellular accommodation.

With the exception of nitrogen-fixing bacteria in a few families of Angiosperms (*48*), intracellular symbioses in plants involve fungal symbionts, collectively called mycorrhizae. Mycorrhizae were originally described in Tracheophytes and are formed between fungal (*myco*) symbionts and the roots (*rhiza*). Despite their name, they are also present in plant species, or even specific alternating generations, that do not form multicellular roots, such as the thallus of Bryophytes and the gametophyte of Lycophytes and ferns. Among examples of mycorrhizal symbiosis, arguably the most ancient is formed with arbuscular mycorrhizal (AM) fungi. AM symbiosis is found in 70% of the extant land plants, including Tracheophytes and Bryophytes, suggesting an origin before the divergence of these two clades 430 million years ago (*49*). Supporting this inference, the oldest plant macrofossils

display intracellular arbuscules similar to those found in extant plant species (*50*, *51*). Although it cannot be ruled out that these structures originated from a different type of association, the consensus in the community is that AM symbiosis evolved soon after, or concomitantly with, the first land plants and has been conserved in most plant lineages since then, including crop plants such as rice (**Fig. 3**) (*49*).

At the molecular level, it has been demonstrated that the activation of a symbiotic program in host plants is triggered by the perception of AM fungi-produced chitin-based molecules via a *signalling module* that includes cell periphery LysM-receptor-like kinases and a downstream signalling cascade composed of a receptor-like kinase (SYMRK), nuclear envelope-localized ion channels, a kinase (CCaMK) and its target transcription factors CYCLOPS (*12*). Subsequent transcriptional reprogramming and cellular rearrangements such as nuclear migration towards the entry site culminate in the generation of a passage through the cell wall, via host-mediated cell wall loosening, and the formation of an infection conduit. A number of proteins, collectively referred to as the *infection module*, contribute to the formation or expansion of this structure, including VAPYRIN and SYP132A (*12*).

Comprehensive phylogenetic analyses of the genes involved in the signalling and infection modules have been conducted on genomic and transcriptomic datasets covering most of the plant diversity, including chlorophyte and streptophyte green algae, Bryophytes and Tracheophytes (*52–56*). These analyses revealed conserved principles for the establishment of AM symbiosis in embryophytes. Indeed, signalling module genes such as the LysM-RLK *CERK1*, the kinase *CCaMK* or the transcription factor *CYCLOPS* and genes involved in the accommodation process itself, such as *VAPYRIN* and *SYP132a*, are all present in Bryophytes and Tracheophytes that are able to form AM symbiosis (*52*, *54*, *57*). Now, reverse genetics in a Bryophyte are needed to confirm their functional conservation. A suitable genetically

tractable model system first needs to be developed, as both the current model moss (*Physcomitrium patens*) and liverwort (*Marchantia polymorpha*) have lost AM symbiosis (*57*). As a close liverwort relative of *M. polymorpha* and with a sequenced genome, the AM fungal host *M. paleacea* is a promising candidate (**Table 1**).

As in all other species that have lost AM symbiosis - whether Angiosperms, Gymnosperms, ferns or Bryophytes - *M. polymorpha* has lost the signalling module, presumably via co-elimination (*57–59*). Co-elimination reflects the relaxed selection that any gene will experience when the only trait it has been selected for is lost in a given lineage (*60*). Symbiosis-associated genes that are retained in species lacking AM symbiosis likely fulfil additional, symbiosis-independent functions. For instance the transcriptional regulators DELLAs, which contribute to symbiotic signalling in angiosperms by physically bridging protein complexes, also participate in hormonal signalling regulating development (*61–63*). Symbiotic gene co-elimination differs between Bryophytes and Tracheophytes and may reflect the most pronounced differences between their life cycles: Bryophytes display an alternating phase lifecycle with a dominant gametophyte phase, while Tracheophytes have a dominant sporophyte generation (*64*). Thus, genes may have essential, non-symbiotic, roles in a free-living gametophyte but not in a reduced gametophyte such as those of most Tracheophytes. This is illustrated by *VAPYRIN* and *VPY-like*, two genes originating from an embryophyte-specific gene duplication. In angiosperm both genes are co-eliminated with the loss of AM symbiosis while they are, one or the other, maintained in Bryophytes irrespective of their symbiotic abilities (*57*, *65*). In the moss *P. patens*, inactivation of *VPY-like*, the paralog maintained in mosses, results in developmental defects in the gametophyte (*65*).

A clear case of gene retention is observed in the lineages that have lost AM symbiosis but have recruited alternative partners for their intracellular symbiosis (Fig. 3). These events

occurred in lupin, in the Ericaceae and the orchid (Orchidaceae, e.g. Serapias) families in angiosperms, in the liverworts of the Jungermanniales order, and in the moss *Takakia* (*57*). In all cases both the signalling and infection modules are maintained despite the loss of AM symbiosis. This indicates that intracellular symbiosis is a unique trait in embryophytes, defined by the plant-controlled accommodation of symbionts via the signalling and infection gene modules.

Convergence and lineage-specific innovations shape intracellular symbioses.

Lineage- and species-specific features contributed also to shaping intracellular symbiosis interactions. Parasponia or rice plants utilise single genes, PanLYK3 and OsCERK1 respectively, as receptors of immune-related and symbiosis-associated microbial signatures potentially representing an ancestral state (*66, 67*). By contrast, legume homologs of the CERK1 receptor acquired structural changes mainly in their extracellular LysM domain to bind highly modified Nod-factors, lipo-chitooligosaccharides involved in the perception of nitrogen-fixing rhizobia (*68*), which may have facilitated the switch from filamentous *Frankia* bacteria to rhizobial partners. Convergence also contributed to this transition, with the recruitment of different hemoglobin isoforms to protect rhizobia from oxygen which is required for efficient nitrogen-fixation to occur (*69*). Evidence for convergence has been also observed for the active transfer mechanisms that drive the nutrient exchange between microbial symbiont and their hosts. Some plant transporters required for the uptake of phosphate offered by AM fungi have been characterized. Genetics identified a single clade of transporters from the *PHT1* gene family, encompassing the Medicago *PT4* and rice *PT11* genes, as essential to fulfill this function (*70, 71*). In Solanaceae, transporters from non-*PT4/PT11* clades of the *PHT1* family are also transcriptionally up-regulated in arbusculated cells, indicative of potential convergent recruitment (*72*). In support, the liverwort *Lunularia*

cruciata upregulates *symPT*, yet another clade of *PHT1* transporter, upon AM fungal colonization (*52*).

Another case of clade-specific innovation is exemplified by the Myb transcription factor MYB1, which transcriptionally activates enzymes involved in the terminal stage of AM symbiosis in *M. truncatula*, when arbuscules degenerate (*73*). Although large-scale phylogenetic analyses have yet to be conducted, current evidence suggests that *MYB1* might be specific to a few angiosperm species (*73*), highlighting the need for further studies into the terminal stages of the interaction at a cellular level in diverse plant lineages. An extreme case of lineage-specific innovation has been reported in the Brassicaceae lineage that has lost the symbiosis signalling and infection modules. Instead, some plants of this clade associate with *Colletotrichum tofieldiae*, which is able to form limited intracellular structures and provides phosphate. These plants seem to limit the spread of the symbiont via indole glucosinolates, a class of defense compound specific to this clade (Fig. 3, *74*).

Symbiont shift, the convergent recruitment of PHT1 phosphate transporters and lineage-specific innovations can be seen as refinement of the ancestral, shared, state in embryophytes: intracellular symbiosis.

Independent but convergent evolution of intercellular symbioses.

Different degrees of adaptation are required for microbes colonising the intercellular apoplast versus those that require entry and establishment within plant cells. It is therefore anticipated that a greater number of less well adapted microbes is colonising the apoplast, resulting in interactions that are much more labile and variable between lineages. Supporting this hypothesis, community profiling of the endophytic compartment (obtained from washed plant tissues) and bulk soils revealed that the environment is the main driver of the diversity observed inside the plant (*75*). Consequently, specific intercellular symbionts that would be

conserved across land plants, if any, remain to be identified. Instead, the intercellular space, as a complex ecological niche, may favor the emergence of convergent and clade-specific interactions.

One such a case of convergence is the intercellular accommodation of nitrogen-fixing cyanobacteria. This association has been described in plant lineages as diverse as hornworts, the liverwort *Blasia pusilla*, the water fern *Azolla filiculoides* or cycads from the Gymnosperms (Fig. 2).

Although these plant species represent diverse lineages, the cyanobacteria are, in all cases, accommodated in cavities that are intercellular structures filled with a secondary metabolite and polysaccharide-rich mucilage (*76*). Once inside these cavities, chemical signals produced by the hosts promote the differentiation of the cyanobacteria into heterocysts to fix dinitrogen. Due to the lack of a model plant host, determining the molecular mechanisms behind these processes remains challenging. However, the genomes of three hornwort species (*Anthoceros agrestis*, *A. punctatus*,and *A. angutus*) and of the fern *A. filiculoides* were sequenced and assembled (*54*, *77*, *78*) and the authors investigated plant transcriptomic changes induced by the presence of the symbiotic cyanobacteria. In all three species, genes involved in the biosynthesis of flavonoids were found induced in presence of nitrogen-fixing cyanobacteria (*54*, *76*). Flavonoids are efficient inducers of a heterocyst-related gene in cyanobacteria (*79*). This suggests that flavonoids might have been recruited independently for symbiotic purposes in hornworts and ferns. Further testing the existence of molecular convergence in plant-cyanobacteria symbiosis now requires the development of genetically tractable models in hornworts and other host lineages.

Another case of convergence is Ectomycorrhizae, which collectively refers to fungal symbioses where the ascomycete of basidiomycete symbionts form a hyphal mantle on short

lateral roots and colonize exclusively the space in-between the epidermis and the first layers of the root cortex. Ectomycorrhizae evolved independently between multiple angiosperm and gymnosperm lineages, and more than seventy fungal lineages (*80*). Comparative genomics of the fungal partners identified convergent traits, such as the loss of cell-wall degrading enzymes (*81*). On the plant side, the sequencing of a diverse set of species including the Gymnosperms family Pinaceae and *Gnetum montanum* (*82*), and angiosperms such as poplar (*Populus trichocarpa*), *Casuarina glauca* or *Alnus glutinosa* opens the way for similar approaches to search for signs of molecular convergence (*53*, *83*). In parallel, a first set of studies on poplar started to reveal lineage-specific mechanisms involved in the ectomycorrhizal symbiosis formed with the ascomycete *Laccaria bicolor*. Silencing two genes of the intracellular symbiosis signalling module, *CCaMK* and *POLLUX*, lead to reduced level of interactions (*84*). Both genes are lost in the Pinaceae (*57*, *85*) which perfectly associate with ectomycorrhizal fungi, including *Laccaria bicolor*, hence this mechanism is likely poplar-specific. Using forward genetics in poplar, Labbé *et al*. identified a G-type lectin receptor-like kinase, *PtLecRLK1* that, when overexpressed in a non-host species *(Arabidopsis thaliana*), conferred some level of compatibility and the development of an extended fungal mantle (*86*). This gene likely originated from recent gene duplication and is missing from the closely related species *P. deltoides*. *PtLecRLK1* thus represents another event of lineage-specific innovation facilitating symbiosis.

From the bulk of commensals living as endophytes, convergent strategies are deployed by plants to tighten beneficial interactions with specific groups of microorganisms. Host lineage-specific innovations evolve from these first layers of interactions, leading to tightly controlled symbioses.

An evolutionary concept for plant microbe interactions

A diversity of plant-microbe interactions evolved within the green plant lineage through evolutionary processes at different time scales. Plant fitness relies on genes supporting general cell processes, defense against pathogens and support of symbiotic associations (Fig. 3).

Pathogens: an omnipresent threat to host fitness. Fossils of the streptophyte algae *Paleonitella*, an ancestor of extant Characeae, show infections by an oomycete-like filamentous microorganism (*87*) and major plant defense strategies predated the diversification of plants and are maintained in distant lineages (Fig. 1, *39*, *41*). A complex phenylpropanoid biosynthesis pathway, including homologs of enzymes involved in the production of defense compounds (*88*), was detected in the transcriptomes and genomes of multiple streptophyte algae, as were intracellular and extracellular immune receptors such as a diversified suit of LysM-RLK in the *Chara braunii* (Characeae) genome and proteins carrying NLR domains (*56*, *89*–*91*). Less clear is the role of some proteins supporting cellular processes in plant pathogen interactions. Recruitment of *M. polymorpha* SYP13B to intracellular pathogen structures (*92*) could either be the action of host-translocated microbial effector proteins, similar to the recruitment of autophagy processes in *N. benthamiana* (*93*), or of a stress management program by the plant to protect its cellular integrity. Functional studies in the absence of a microorganism offer the opportunity for testing the role of such proteins in plant integrity.

Intracellular symbioses have evolved by exploiting pre-existing core components.

It has been proposed that endosymbioses evolved by recruiting general cellular mechanisms (*94*). We extend this concept to propose that in a single evolutionary event during the emergence of embryophytes, genes from defense and general processes served as starting

points to evolve a set of genetic regulators (the symbiosis signalling and infection modules) that conferred the capability to benefit from and support symbionts within their cells (Fig. 3).

All plants from the most recent land plant ancestor to extant lineages have been equipped with both defense strategies, and general cell processes to support growth, development and interactions with the environment. The occurrence of most of the protection mechanisms in streptophyte green algae supports this view (Fig. 1, *4*). In addition, orthologs of support components were found in diverse streptophyte algae. This includes CCaMK and CYCLOPS, two central genes in symbiotic signalling that are biochemically compatible with a symbiotic function given that expression of either genes from Zygnematophyceae can complement symbiotic defects in the corresponding *M. truncatula* mutant (*52*). Because intracellular symbiosis has never been described in these algae, we propose that recruitment of these genes from an ancestral function occurred in embryophytes. This idea is illustrated by dn-OPDA and OPDA which both fulfil evolutionarily older, COI1-independent, roles in mediating thermotolerance in Bryophytes and the streptophyte alga *Klebsormidium nitens* prior to their recruitment into COI1-dependent signalling in embryophytes (*95*).

Symbiosis: a need, a partner and a home.

We propose that the exaptation - the recruitment - of the general cell process and defense gene ancestors into symbiotic processes must have been linked to favorable conditions.

During early land plant evolution, in a barren landscape, the need to access nutrients is considered a major driver of the emergence of symbiotic interactions (*3*). Recruitment of *C. tofieldiae* by Arabidopsis into a glucosinolate controlled interaction with phosphate transfer shows that nutrients may remain a major driver (*74*) including in plant lineages where alternative plant strategies substitute for the loss of symbiosis genes (Fig. 3).

To establish a plant fitness benefit, saprophytic or possibly weak pathogenic microbes became partially and then full plant-controlled partners held in check by defenses and balanced with support by symbiotic signalling and infection modules to allow their perception, entry, proliferation, establishment and control of their intracellular lifetime. A direct repression of defences by a master regulator of the phosphate stress response in *A. thaliana* is consistent with the ongoing plant priority for nutrition (*96*). In turn the microbial partners often commit to the relationship, illustrated by the loss of fatty-acid biosynthesis enzymes and the cell wall degrading machinery in AM fungi (*97, 98*), the later being also observed in more recently evolved fungal symbionts (*81*).

Complex tissues and organs allow plants to offer a perfect home in the form of dedicated, colonised structures and cells. They also give the plant control over access and direct carbohydrate provision such as in the cortex of angiosperm roots and the colonised storage cells of liverwort thalli, and facilitate the maintenance of mutualism (*99*). The complex multicellularity that evolved in embryophytes may have solved the conundrum represented by hosting an intracellular symbiont into a photosynthetic cell. From this multicellular body, cell differentiation into a symbiotic state was possible. We presume that this state followed intercellular or surface interactions that likely represent intermediate stages in the formation of intracellular symbiosis. Depending on the tissue where the intermediate intercellular symbiosis occurs, transition toward intracellular infection will utilize different cell types such as rhizoids or atrichoblasts, possibly in a generation-dependent manner (*100*).

Such transitions might be ongoing in the extant plant - cyanobacteria symbiosis. When plants host cyanobacteria, they keep them extracelluar. A notable exception is the

angiosperm genus *Gunnera* where nitrogen-fixing cyanobacteria are not only hosted in mucilage-producing glands in the stem but also intracellularly (*101*).

Conversely, pathogens take advantage of analogous plant architectures which evolved to facilitate gas exchange in photosynthetic tissues. Sponge parenchyma tissues of angiosperm leaves are frequently colonised by microbes with their stomata acting as entry or exit ports. Similarly, bacteria, fungi and oomycetes colonise the air chambers of the liverwort *M. polymorpha* using air pores for entry or exit (*37*). Coincidentally, the stomata of bryophytes only seem to serve roles in desiccation and are frequently lost (*102*).

Future perspectives

Although our concept for the evolution of plant - microbe interactions is supported by multiple pieces of evidence, a number of hypotheses need to be experimentally challenged. This will require the development of new genetic models covering the diversity of the Bryophytes and Tracheophytes, testing interspecies transfer of genes involved in support and protection mechanisms and exploring the diversity of plant - microbe interactions in terrestrial and aquatic ecosystems.

A greater diversity of genetically tractable plants.

Most of the current knowledge on the evolution of plant - microbe interactions results from genomic and genetic comparisons of a very limited number of Bryophytes to Angiosperms. To obtain a complete picture of the conserved and specific mechanisms underlying the interactions between plants and microorganisms more experimentally accessible plant - microbe systems in underrepresented clades are needed.

Model liverworts and hornworts will allow testing the universal function of the signalling and infection modules in intracellular symbiosis. Ferns that have free-living gametophytes and

sporophytes would be ideal to determine whether differences in the dominant phase may explain differential gene losses linked to the loss of symbiosis in Bryophytes versus Tracheophytes. Efficient gene transformation of Gymnosperms which only form ecto- but no endomycorrhizae or of the fern Azolla which associates with Cyanobacteria would aid studies into the convergent evolution of intercellular symbiosis mechanisms. Finally, as for many other questions linked to the origin of the first embryophytes, developing genetic models in streptophyte algae (in particular Characeae and the Zygnematophyceae) will pave the way to decipher the function of genes in lineages that did not - as far as we know - develop the traits their homologs are known for, such as the genes constituting the signalling module for intracellular symbiosis.

1001 plant - microbe interactions.

The diversity of described associations between plants and microbes, resulting in mutualism or parasitism, is astonishing and many more await discovery. High-throughput community profiling and metagenomics have told us that plant tissues host myriads of microorganisms. However, the level of interactions and the biology of these interactions cannot be defined without careful examination and approaches which maintain spatial resolution need to be included in such surveys (*103*). Interactions have mostly been described in crops and Angiosperms in general and, to some extent, in Bryophytes. By contrast, potential parasitic or symbiotic associations in streptophyte green algae, including Zygnematophyceae the closest algal relative to land plants, are scarce (*104*). Deciphering such associations in streptophyte algae would pave the way for understanding how much of the protection and support mechanisms found in extant embryophytes were already in place in their shared ancestor with extant green algae.

Conclusion

Decades of genetic and genomic dissection of the interactions between plants and microbes have unravelled mechanisms supporting or limiting them. Comparisons across non-vascular (Bryophytes) and vascular (Tracheophytes) species started to shed light onto ancient and conserved principles, convergent concepts as well as lineage-specific innovations underlying these supportive and protective mechanisms. Studies into molecular mechanisms of plant–microbe interactions are increasingly integrated into evolutionary studies enriching our understanding of plant-microbe evolution at maximum resolution (*105*).

The evolutionary trajectories of symbiotic and pathogenic interactions are constrained and interconnected. We hypothesise that all intracellular symbiotic associations rely on the same signalling and infection modules, and that new associations evolve from extant intercellular forms of symbiosis, providing microbial pathogens with more options for infection. The development of additional genetically tractable model systems in a range of plant lineages, including Zygnematophyceae, ferns, hornworts and symbiotic liverworts will enable testing these hypotheses. Finally, the engineering of improved symbiosis and protection mechanisms in crops using synthetic biology bears the potential to globally transform agriculture. The potential of crops to form mutualistic symbiosis could be broadened through recapitulating the diversity of support modules from other plants. This may improve their abilities to access nutrients in diverse environments. Transferring protection mechanisms from divergent plant lineages into crops to limit pathogen infection could help improving their disease resistance thereby decreasing the need for pesticides.

Prasino (*5*)

Chlamydomonas (*106*)

Mesostigma chlorokybus (*107*)

Klebso (*108*)

Chara (*56*)

Penium (*109*)

Spirogloea/meso (*55*)

Anthoceros (*54*, *78*)

Marchantia (*23*, *57*)

Physcomitrium (*110*)

Selaginella (*111*)

Azolla/salvinia (*77*)

Ceratopteris (*112*)

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Fig. 1. Gains of genes and processes for plant-microbe interactions. PNL, HNL, TNL, RNL, CNL, all Nucleotide binding site leucine rich repeat proteins with different N-terminal domains (protein kinase, alpha/beta-hydrolase, TIR-, RPW8-like, Coiled-coil). RLK, receptor-like kinase. LysM-RLK, Lysine Motif-RLK. MLD-RLK, Malectin-Like Domain RLK. CCaMK, Calcium and CalModulin dependent protein Kinase. DMI1, Doesn't Make Infection 1. PT, Phosphate Transporter. SymPT, Symbiotic PT. EFR, EF-Tu Receptor. FLS2 and 3, Flagellin Sensitive 2 and 3. OPR3, 12-Oxophytodienate Reductase 3. JAR1, JAsmonate Response locus 1. COI1, COronatine Insensitive 1. BAK1-like, pro-ortholog of BRASSINOSTEROID INSENSITIVE 1-Associated receptor Kinase 1. EIN2, EIN3, Ethylene Insensitive 2 and 3. ETR1, Ethylene Response 1.

Fig. 2. Examples of convergent plant traits relevant for microbes. Microbes are often accomodated in structures and cells such as stomata and air pores for pathogens, or auricle, cavities and zone for cyanobacteria. Metabolic defenses can be restricted to lineages-specific cell types evolved independently in some Tracheophytes (myrosin cells) and Bryophytes (oil bodies).

Fig. 3. Gains and losses during the evolution of symbioses. The diversity of extant symbioses, as illustrated by plant-fungal associations at the branch ends, was shaped by evolution. It started with a major gain of intracellular accommodation mechanisms including the recruitment of symbiosis genes from existing defense genes as well as general cell processes (I). Losses of several symbiosis genes occurred through co-elimination of intracellular symbiosis (II). Clade-specific alternative strategies for forming a symbiosis with microbes (III) arose in specific lineages. Pathogens suppress, recruit and hijack processes relevant for symbiotic associations and are thereby shaping their evolution.

Table 1.

Box 1 - Shared genes impacting on beneficial and detrimental interactions

Pathogenic and symbiotic filamentous microbes tap into general host cell and developmental processes during plant colonisation. Symbiosis-associated gene families arose early during land plant diversification and have been maintained in many lineages. Thus, it is likely that symbiosis-associated genes were vulnerable to pathogen manipulation and, in turn, were re-recruited into lineage-specific defense signalling.

Plant species with experimentally established symbiotic and pathogenic interactions allow for the identification of genes contributing to both processes in similar or opposite ways. To date, the *M. truncatula* genes *DMI1, LIN, LYK3, NFP, NSP1 and CERK1* have all been found to contribute positively to AM symbiosis, but negatively to filamentous fungal or oomycete pathogen infections. By contrast, mutations of *M. truncatula RAD1*, *RAM2* or RNA-interference of *ROP9* seem to have similar negative effects on both AM symbiosis and oomycete infections (*41*, *101*, *102*).

Another study suggests that the *Lotus japonicus* symbiosis signalling module genes *CCaMK* and *CYCLOPS* are implicated in supporting fungal biomass proliferation during early infection stages by a tomato endophytic *Fusarium solani* isolate K (*103*).

An *A. thaliana* ortholog of the symbiosis signalling module gene *POLLUX* as well as the SYMRK-homologous receptor kinases (ShRKs) have been implicated in limiting the reproductive success of the oomycete *Hyaloperonospora arabidopsidis*. Mutations in Arabidopsis *POLLUX* or *ShRK* genes did not elevate defense responses, but impacted on the timing and frequency of the expansion of intracellular haustoria, believed to be the

oomycete's structures for feeding and secretion of host transferred effector proteins (*104*).

Barley, wheat and *M. truncatula* genomes have maintained *MLO* genes of clade IV (inlcuding *HvMLO1*, *MtMLO8*) to support early AM fungal colonisation and endophytic interactions in roots, despite them being susceptibility genes for leaf infections by powdery mildews and oomycetes (*105–107*).

Such findings demonstrate that homologous or even orthologous members of protein families implicated in symbiosis can also fulfil roles associated with pathogen colonisation processes in specific plant lineages. Given that non-vascular plants such as the liverworts *Marchantia paleacea* and *Lunularia cruciata* are also colonised by AM fungi and filamentous pathogens it is possible to address the extent to which dual roles of genes in pathogenic and symbiotic interactions are conserved across these plant lineages.