Evolution of endophyte–plant symbioses

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All fungi invading plant foliage have an asymptomatic period in their life cycle that varies from an imperceptibly short period (e.g. pathogens) to a lifetime (e.g. *Neotyphodium* endophytes in grasses). Endophytic fungus–grass associations are generally treated separately from parasitic, pathogenic and saprophytic interactions and are viewed as mutualistic associations. However, endophyte–host interactions are based on mutual exploitation. Benefits to the partners are rarely symmetric and conflicting selection forces are likely to destabilize them. Unanswered questions are how (i) genetic diversity of the fungus and phenotypic plasticity in fungal life history traits, (ii) genetic combinations between the fungus and the host, and (iii) the fungus and host individually or in concert as a phenotypic unit, respond to changing selection pressures.

Although knowledge of the ecology, life history and phylogeny of endophytic fungi has accumulated rapidly during the past two decades, basic questions about the evolutionary origin, speciation and ecological role of endophytes remain largely unanswered [1]. Although the term 'endophyte' has been controversial since it appeared [2–4], it has become synonymous with mutualism (Box 1). However, recent studies show that the ecological role of even systemic grass endophytes can be complex and labile. Functionally, in terms of interactions with their host, different fungi are scattered throughout phylogenetic lineages [1,5,6]. Moreover, defense of the host plant via endophyte mycotoxins, the most often cited mechanism of mutualism, discovered in agronomic grasses, seems rare in most native grass– and tree–endophyte interactions [5–8]. Nonetheless, the majority of published studies are still based on the conventional wisdom that endophytic fungi are plant-defending mutualists, with fungus-produced, biologically active alkaloids as key to the evolution of the endophytic life-style of these fungi [1]. We propose that key elements for the evolution of the endophytic life-style of fungi are more complex, and involve multi-species interactions, multiple levels of causation and multidirectional flows of influence, and are influenced by stochastic events, such as abiotic and biotic environmental conditions, that drive the life histories of coevolving fungi and host plants.

Forces driving fungus–plant interactions

Like other host–parasite or host–predator, or host–mutualist interactions, endophyte–plant interactions project to the ecological surface of a dynamic fitness landscape with adaptive peaks and valleys occupied by the most and least fit fungus–plant genotype combinations within a population [9–11]. Highly integrated and specialized symbioses require well-matched architectural, morphological, physiological and life history traits of the fungus and of the host plant to evolve and persist [5,9]. In simplified agro-ecosystems, traits related to defensive plant mutualism can provide a selective advantage to the host plant, leading to highly integrated symbioses [12]. However, this human-driven selection, coupled with intensive and chronic grazing by livestock and consistently rich resource environments, usually does not occur in natural grasslands. In natural populations, selective forces are more variable and can operate simultaneously on several traits, or plasticity in traits, of the fungus, host or host–fungus unit [9–11,13,14]. Indeed, the paucity of the specialized, systemic endophyte interactions in grasses relative to the vast number of more generalized and opportunistic infections suggest this close matching is uncommon in nature. For example, whereas the focus on grass endophytes has been traditionally on specialized, systemic endophytes, these same grasses typically harbor hundreds of more generalized, HORIZONTALLY TRANSMITTED endophytic species (see Glossary) [15]. There are several reasons why matches between host and endophyte are uncommon. For example, conflicts between host and fungal reproduction, energetic costs of harboring the endophyte, suppression of immune systems of the host that allow the endophyte to subsist but that might increase the susceptibility to other fungal pathogens, and host control of fungal growth within the host [16], might

Glossary

Horizontal transmission: transmission of the fungus by sexual or asexual spores.
Pseudovivipary: production of offspring by apomictic or asexual propagules such as plantlets and bulbils.
Tillering: production of vegetative propagules, i.e. tillers, in grasses.
Vertical transmission: transmission of the systemic fungus from plant to offspring via host seeds.
Vivipary: the precocious and continuous growth of sexually produced offspring when still attached to the maternal plant.

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Box 1. 'Endophyte' has become synonymous with mutualism

Foliar tissues of all plant species examined to date harbor at least one, and more commonly, a wide diversity of fungi lurking within their plant tissues [3,40,41]. The majority of these endophytic fungi are localized infections by either latent pathogens or dormant saprophytes. A small fraction of endophytes found in some grasses are symbiotic fungi that form a lifelong, symptomless infection throughout the host plant [1,5]. Although the ecological role of these endophytic fungi vary from antagonistic to mutualistic [1,5–8], during the asymptomatic phase of the life cycle, all these fungi are commonly referred to indiscriminately as 'endophytes' [4]. The term became firmly established in ecological literature when livestock toxicoses in USA and New Zealand in the mid-20th century were demonstrated to be attributable to alkaloids produced by fungal endophytes belonging to the tribe Balansiae (Ascomycotina) in the 1970s [42].

Since then, asymptomatic infections of foliar fungi have attracted increasing attention among mycologists, ecologists and evolutionary biologists. Empirical evidence has demonstrated that the evolution of systemic endophytic fungal symbionts of some cool-season grasses of the subfamily Pooideae, in particular, the asexual genus *Neotyphodium* (formerly *Acremonium*) and their sexual antecedents in genus *Epichloë*, has involved many reciprocal benefits (Figure I) [1,5,28,42–44]. Systemic grass endophytes are now thought to affect virtually every type of plant–plant, plant–pathogen and plant–herbivore interactions in grassland communities [1]. However, most of the empirical evidence of strong plant–endophyte mutualism is based on studies of two economically important introduced grass species, tall fescue and perennial ryegrass, and their artificially selected cultivars [5,6,12].
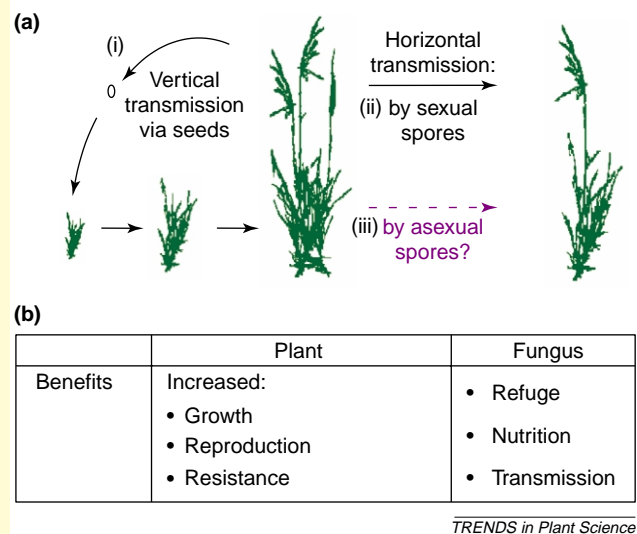
Recently, an increasing number of exceptions to the purported endophytic mutualism have been reported, particularly for woody plants and native grasses [5,6,8,19–21,45,46]. Non-systemic endophytes in woody plants are horizontally transmitted by spores from plant to plant and appear to rarely interact mutualistically with their hosts [5,7] (but also see Ref. [47]). Systemic and vertically transmitted *Epichloë* endophytes can shift from mutualistic to antagonistic symbioses when the fungus produces external stromata (providing both spermatia and female structures) surrounding some or all host inflorescences and aborting them [48], or by causing energetic costs to the host, particularly in resource-limited environments [5,6,8,19–21]. However, the term 'endophyte' has become synonymous with 'mutualism' in the literature.

Figure I. (a) Life cycles of systemic grass endophytes and (b) benefits to the partners. Hyphae grow internally and intercellularly throughout the above-ground tissues of the host plant and into the developing inflorescence and seeds and, thus, are transmitted vertically from maternal plant to offspring (i). *Neotyphodium* endophytes are assumed to be strictly vertically transmitted [1,28]. By contrast, *Epichloë* endophytes can also be transmitted sexually (spores) when the fungus forms external stromata with conidia around a developing inflorescence, causing abortion (ii). Contagious spread should not be ruled out even in *Neotyphodium* endophytes because they produce asexual conidia on growth media [50] and on living plants [51], and recent evidence indicates horizontal transmission in natural grass populations (iii). (b) Grass endophytes are generally considered to be mutualists because the fungus subsists entirely on the resources of the host. The fitness of an endophytic symbiont that has lost or limited opportunities for contagious spread by spores depends largely on the fitness of the host plant. The host receives benefits through increased resistance to herbivores, pathogens and drought and flooding stress, and enhanced competitive abilities [1].

destabilize the symbioses and lead to changes in reproduction allocation of the fungus and the host, and eventually to changes in reproduction strategies and speciation. This spectrum of endophytic interactions from relatively rare tightly integrated, VERTICALLY TRANSMITTED mutualist to more common opportunistic, horizontally transmitted antagonist nonetheless provides a fertile ground for ecologists and evolutionary biologists interested in levels of selection in coevolutionary processes, evolution of sex and species concepts.

Architecture and morphological adaptations, risk spreading and coevolution

The length of the endophytic phase, the ability to grow within plant meristems, reproduction and the transmission mode of foliar fungi are key traits that must match the morphological and life history traits of the host plants to persist in evolutionary time. The size and architecture, expected lifetime, and age of sexual maturity of the plant strongly constrain the fungal growth pattern within the host, the frequency of vertical and horizontal modes of transmission, and the length of the latency period of foliar fungi (Box 2).

Concordance of physical characters and life history traits of fungi and that of their host plants do not explain

why strictly maternally inherited fungi are only rarely found in annual grasses. The small size and relatively short life cycle of the annual grasses should provide more opportunities for systemic growth and subsequent vertical transmission to fungi than to perennial grasses. Thus, the lack of documented systemic, vertically transmitted endophytes in annual grasses is puzzling. Relatively few annual grasses have been surveyed for systemic endophytes, particularly those that are not important forage grasses. Apparently, well matched physical characters and life histories of the fungus and the host plant [1,9,17,18] fail to predict the fungal growth form, transmission and the length of latency periods. Instead, conflicting selective forces and genetic mismatches between host and endophyte might explain the disparity.

Conflicting forces in asexual grass endophyte symbiosis

Dependence of mutualism on environmental conditions, particularly resource availability in soils [8,19–21], suggests that mutualistic endophyte–plant symbiosis is based on mutual exploitation, like many species interactions, rather than reciprocal cooperation [9,22,23]. Thus, we propose that understanding the evolution of endophyte–plant symbioses requires broad perspectives

involving host–parasite interactions rather than specialized concepts developed for endophytes.

Our perspective that encompasses both host–parasite and mutualistic interactions suggests that benefits to the partners are only rarely symmetric, and that conflicting selective forces are likely to destabilize even highly specialized plant–fungus associations such as asexual, vertically transmitted *Neotyphodium* endophytes in grasses. These interactions are assumed to evolve toward mutualistic interactions primarily because the fitness of the fungus largely depends on the fitness of the host plant [1,5]. However, the converse does not necessarily hold, that is, the plant does not necessarily depend on the fungus, and in some environments, plant fitness increases when the fungus is absent [8,21]. This asymmetry in dependence can lead to host plant sanctions against less beneficial or mutualistic microbial strains. Plant sanctions (rewarding more beneficial and punishing less beneficial symbionts) have been demonstrated in plant–*Rhizobium* interactions [24,25]. Recent evidence [16] implies some control of host grasses over endophyte growth. *Epichloë* and *Neotyphodium* hyphae grow within developing leaves and cease apical extension and branching when leaf growth is completed. Apparently, growth of the endophyte is regulated by hormonal activity and production of chitinase by the host. We propose that seasonal changes in resource allocation among different plant meristems can also regulate the growth of the fungus [26,27]. The first foliage flush creates strong resource sinks until leaf growth is completed; fully grown leaves then become photosynthesizing sources. During reproduction, emerging culms are sinks and direct the resource flow into developing inflorescence and seeds, and the endophyte follows this sink into the developing flowers and seeds. The asymmetry in dependence has led to the general view that the host plant is largely in control of the interaction, particularly in the case of endophytes that have lost sexuality. These endophytes have been metaphorically perceived as 'trapped pathogens' that are 'slaves' to the host [1,28]. However, even asexual endophytes can be active participants and even exploiters in the interaction, and retain

control over host plant functions through several mechanisms.
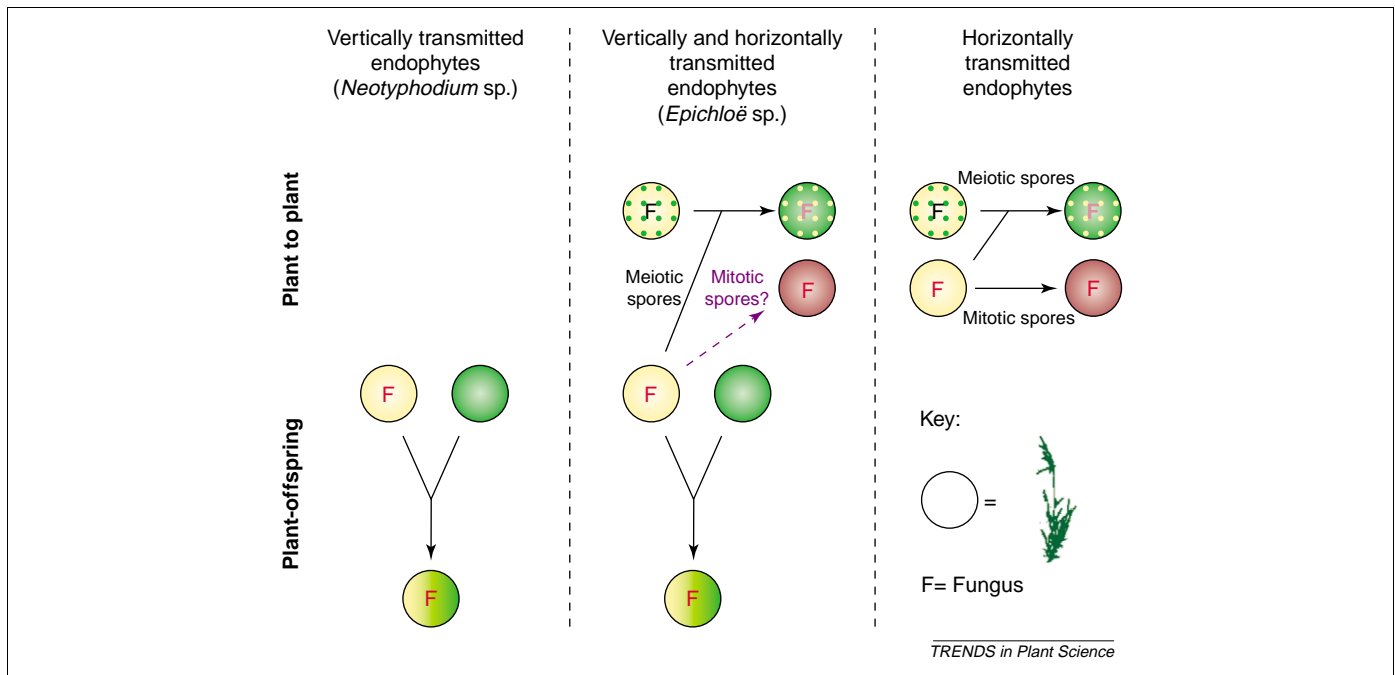
Transmission mode

Vertical transmission in systemic endophytes appears to reduce concomitantly the ability of the fungus to infect new uninfected hosts horizontally by either asexual or sexual spores [1]. In its extreme form, for example, *Neotyphodium* endophytes in grasses, the fungus has entirely lost its ability for contagious spread [1,28]. The fungus has presumably entirely lost the independent phase in its life cycle, and the symbiosis is essential for survival and reproduction of the fungus, but remains only conditional for the host. However, asexual endophytes can retain control over host plant reproduction by (i) increasing the proportional allocation to female functions, or (ii) increasing vegetative propagation by TILLERING, or (iii) inducing VIVIPARY and PSEUDOVIVIPARY of the host [8]. Although strong evidence for control of host functions by asexual endophytes is still unavailable, similar mechanisms of host manipulation are found in other vertically transmitted microbes, such as *Wolbachia* [29].

Sex

The loss of contagious spreading by spores is also noteworthy because of the associated loss of recombination potential through sexual reproduction (Figure 1). Indeed, in these associations, only one fungal genotype is transmitted to the seed progeny, which are usually produced by outcrossing in the host plant. Thus, at each plant reproductive event, the same fungal genotype is distributed in seeds that are genetically variable. In addition to accumulating deleterious mutations (Muller's rachet [30]), loss of fungal recombination can be disadvantageous because of restricted ability to respond to changing selection pressures. Thus, in theory, although loss of sexual reproduction might provide short-term benefits, it should decrease the chance of a highly specialized fungal lineage persisting. The fungus might be partly buffered from external environmental heterogeneity by residing within its host, although the host itself might change genetically. In addition, the fitness of fungus is intertwined with the fitness of novel genetic combinations of the outcrossing host plant that might evolve rapidly enough in the face of changing selection pressures. By contrast, recombination via fungal hybridization, presumably between *Neotyphodium* and sexual *Epichloë* that colonize the same host plant, might also occasionally provide inputs of genetic diversity [1].

Sex and genetic host specificity

Most systemic endophytes retain some form of sexuality, unlike the more extreme *Neotyphodium*, which has lost sexuality. *Epichloë*, for example, can be transmitted vertically (via hyphae growing into seeds) or sexually (via production of stromata that destroy host inflorescences), depending on endophyte species and environmental conditions [5]. Jean Pan and Keith Clay [31] showed that *Epichloë* infections can alter reproductive functions such as vegetative growth that promotes spread of the fungus. Here, the fungus might be in control of host

Figure 1. A comparison of the consequences of vertical versus horizontal transmission to the genetic interplay between the endophytic fungus and the host plant. The fill color patterns indicate genetic differences and inheritance in individuals. In vertically transmitted grass–endophyte associations, only one fungal genotype (red F) is transmitted vertically to genetically blended seed progeny (vertical shade from yellow to green) in an outcrossing maternal plant lineage. By contrast, horizontally transmitted endophytes typically infect genetically variable hosts (yellow with green dots, green with yellow dots and dark red), either by asexual mitotic spores (red F) or by sexual meiotic spores (pink F).

functions, and even asexual *Epichloë* in one plant might reduce outcrossing of the host to maintain favorable host plant genotypic combinations. It is likely that the host plant can also impose sanctions against *Epichloë* that would limit hyphal growth and reproduction.

The maintenance of sexual reproduction of the fungus should be favored because the fungus is continually confronted with new genetic combinations in the outcrossing host plant populations (Figure 1). This changing host genotype background could lead to an arms races between the fungus and the host – the Red Queen hypothesis, typically directed to host–parasite interactions, to explain the maintenance of sex in the host population as a response to parasites specializing on the most common host genotypes, reducing their fitness [9,32–34]. Contrary to past endophyte studies that mainly focused on what consequences the presence of the fungus had on the host plant, we emphasize the importance of genetic mismatch between the fungus and the host as a destabilizing force in endophyte–grass symbioses.

Comparisons of fungal and plant phylogenies suggest that systemic grass endophytes are host specific, leading to host-adapted fungal races that are compatible with only certain host genotypes. Genetic specificity is particularly high in strictly asexual and vertically transmitted fungi that inhabit grasses [1]. A high level of genetic specificity is probably tied to genetic incompatibility, constraining the diversity of successful genotype–genotype combinations of the systemic seed-borne endophytes and the host grasses. Sexual reproduction of the host plant should lead to a genetic mismatch with the asexual fungal partner.

If genetic mismatches occur commonly, then they might result in loss of infection, and might partially explain

differences in infection frequencies between (i) pioneering and established, older populations of perennial grasses, (ii) agronomic and natural grass populations, and (iii) perennial and annual grasses. For pioneer grass populations in a new habitat, seeds produced by outcrossing should have a high frequency of mismatches and incompatibilities between the host and fungus and hence lower infection frequencies than in established populations. In established populations, successful host–fungus combinations persist, reducing incompatible combinations. These matches are further propagated by clonal reproduction via host tillering in stable environments. Vegetative reproduction of host–fungus genotypic combinations could also suppress seedling establishment in dense, older grass populations, and thus maintain high infection frequencies of a few well-matched genotypic units. A similar situation can prevail in agronomic systems, where limited genetic diversity of a few well-matched cultivars with their endophytic partners and relative constant and enriched environments ensure continual persistence. This might explain why frequencies of asexual, seed-borne fungi are more variable in wild-grass populations than in agronomic ones [15,35]. Mismatching might also explain the puzzling lack of asexual, systemic infections in annual grasses relative to perennial ones. Annual grasses must reproduce by outcrossing at each generation. High recombination rates, with no vegetative reproduction or reduced inbreeding relative to perennial grasses, might prevent establishment of stable compatible genotype–genotype combinations between the fungus and annual hosts.

To keep pace in the arms race, at least in terms of compatibility, the fungus should have mechanisms to increase its genetic diversity, or alternatively to decrease

genetic diversity of the host, such as by increasing clonal propagation or self-pollination in its host. Systemic grass endophytes appear to be able to do both. Sexually reproducing strains of systemic endophytes (*Epichloë* spp.) cause destruction of host inflorescences through production of sexual structures (stromata). Some *Epichloë* can also increase vegetative reproduction by promoting tillering [31] or by increasing self pollination in the host [36]. In addition, molecular evidence suggests that genetic diversity of asexual, endophytic fungi can increase by parasexual hybridization [1].

Conclusions

Although endophytism can be mutually beneficial for the fungus and the host, specialized mutualism is likely to be an offshoot rather than an epitome of fungal interactions with the host plant. In spite of the possible short-term benefits to the fungus and the host in some cases, strictly asexual endophytic fungi that have lost contagious spreading can be evolutionary dead ends or intermediate steps [6] over the adaptive landscape. Because of the conflicting selection forces, life histories of foliar fungi are dynamic in evolutionary time (like the life histories of all organisms), and can occasionally lead to reproductive isolation and thus sympatric speciation of the fungus. Thus, we propose that instead of arguing whether endophyte−plant interactions are mutualistic or not, future studies should focus on how ecology and genetics interact to shift fungal life history traits between the extremes of sexuality and asexuality and pathogeneity and mutualism.

Ecological observations, coupled with phylogenetic and molecular analyses of foliar endophytic fungi, have provided glimpses of the degree of specialization among endophyte−plant interactions, and insights into their coevolution. Fungi have diverged into lineages that are sometimes considered as phylogenetic or taxonomic species [1,37] although the biological species concept might be difficult to apply to asexual fungi that inhabit multiple hosts [38]. Interestingly, comparisons across fungal taxa indicate that strictly vertically transmitted asexual groups forming endophytic infections have been repeatedly derived from sexual species in cool-season grasses, and that many of them are interspecific hybrids [1]. Even in the case of asexual *Neotyphodium* and its sexual form *Epichloë*, sexual reproduction (and consequently horizontal transmission) appears scattered across closely related phylogenetic fungal lineages, and asexual lineages show greater genetic host specificity [1]. Empirical evidence suggests that phenotypic changes from virulence to avirulence can be expeditious − even a mutation of a single locus can convert fungal phenotypes from pathogenic to that of a nonpathogenic endophytic symbiont [39]. Thus, these relatively small and rapid genetic changes can have great repercussions even in ecological time.

The central, and yet unanswered, questions are (i) how the processes of endophyte−plant interactions relate to their coevolutionary patterns and (ii) how the phenotypes of host−fungal units respond together or separately to changing environments. Because these questions require a more-comprehensive understanding of the genetic bases and phenotypic plasticity of traits of the fungus−plant unit, the use of controlled fungus−plant genotype combinations in different environments are essential in the future studies. Endophyte−plant interactions follow similar evolutionary and ecological processes as other host− mutualist, host−parasite or host−disease interactions, and therefore need not be treated differently.

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