

A Novel Framework for Decoding Fungal Endophyte Diversity

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CONTENTS

5.1	Introduction	65
5.2	A Dual-Axis Framework	66
5.2.1	Rationale	66
5.2.2	Mode of Transmission	66
5.2.3	Host Specificity	66
5.3	Applying the Framework	67
5.3.1	Community Members of the “Core” and “Rare” Endophytomes	68
5.3.2	Plant-Fungal Codiversification on Macroevolutionary Timescales	71
5.3.3	Functional Diversity—Plant-Infecting Clavicipitaceae as a Model System	72
5.4	Conclusions	74
	References	75

5.1 INTRODUCTION

Endophytic fungi are internal colonizers of aboveground tissues in all plant species studied to date (U’Ren et al. 2012). These cryptic organisms engage in a diverse set of symbioses and biological interactions with their plants hosts, from local infections of leaf tissues (Rodriguez et al. 2009) and bark (de Errasti, Carmarán, and Victoria Novas 2010) in herbaceous and woody plants to the systemic infections famously found in cool-season grasses (Clay and Schardl 2002). Moreover, endophytes range from mutualists to pathogens and saprobes, with great variation in the direction, the magnitude, and the frequency of fitness consequences for the host, as well as in their method of transmission from one host to the next through space and time (Clay and Schardl 2002; Porras-Alfaro and Bayman 2011).

AQ 1

This diversity of biological form and function has made it challenging to categorize these fungal symbionts in meaningful ways. As a result, previous classification systems of endophytes, while informative, are incomplete. Early efforts include categorization schemes focused solely on grass endophytes and their transmission mode (White 1988), ignoring herbaceous and woody

plant endophytes. Other schemes have attempted to classify a wider range of endophytes using taxonomic divisions, in particular by separating the clavicipitaceous endophytes of cool-season grasses from all other plant-endophyte associations (*sensu* “non-clavicipitaceous”) (Rodriguez et al. 2009). However, this system does not encompass the strikingly similar patterns of vertical transmission and high host specificity, recently identified in a number of alternative plant host systems, including fungal symbionts found in morning glories (*Periglandula*, Convolvulaceae; Steiner et al. 2011; Beaulieu et al. 2015) and locoweeds (*Undifilum*, Fabaceae; Baucom et al. 2012). Further, since these previous classification systems were proposed, there has been a proliferation of research on endophytes and plant-associated microbiome communities in general (Porras-Alfaro and Bayman 2011; Vandenkoornhuysen et al. 2015). New molecular technologies are revealing much greater fungal diversity than ever suspected (Zimmerman et al. 2014), and research has begun to examine how these symbiotic communities can be organized, particularly by employing theories for macro-organismal systems (Christian, Whitaker, and Clay 2015). Informed by these recent advances, we

suggest that a new framework is necessary for delineating the form and function of endophytic symbioses in nature.

In this contribution, we propose a novel framework for examining fungal endophyte biology. Notably, this framework does not rely on discrete categories, as in previous classification systems, but instead, it relies on two core axes of biological organization. With this framework, we may be better poised to explore key biological traits across the diversity of plant-fungal endophyte symbioses found in nature. We first describe our theoretical framework, outline the rationale, and then apply it to specific examples to better understand aboveground fungal endophyte diversity, spanning many host types and functional roles (e.g., pathogenic, saprotrophic, and mutualistic). We then overlay key examples from the literature onto this framework in order to generate hypotheses about the distribution of different biological characteristics of endophytes, such as population densities within hosts and the degree of mutualism seen in the interaction of species. We hope that this approach will serve to conceptually unify a wide range of endophyte symbioses and identify target areas for future research. Although we do not address belowground fungal symbionts, such as arbuscular mycorrhizal fungi and dark septate root endophytes, future work exploring belowground endophyte biology by using a similar framework may prove fruitful.

5.2 A DUAL-AXIS FRAMEWORK

5.2.1 Rationale

Our dual-axis framework is firmly rooted in endophyte biology and aims to explore the diversity of form and function in aboveground plant-fungal symbioses. The two axes that define this framework are (1) mode of host-to-host transmission and (2) degree of specificity to a particular host species or clade. We have limited our framework to these axes for two reasons. First, both axes represent a spectrum, offering more flexibility to describe and explore symbiotic interactions than what a binary framework could. Endophyte interactions may generally be categorized as falling within one or more of the four quadrants defined by the two perpendicular axes; however, the precise position within the quadrant can provide richer information about the symbiosis than a simple, categorical approach. Second, many of the traits commonly considered in plant-fungal symbioses, such as ecological role or trophic mode, are too context-specific and/or labile to form the basis of a robust, predictive framework (e.g., high lability in trophic mode; Delaye, García-Guzmán, and Heil 2013). Transmission mode and degree of host specificity, on the other hand, are traits that are relatively fixed in any given species interaction and can therefore be more reliably quantified.

5.2.2 Mode of Transmission

Host-to-host transmission mode has traditionally been described as a binary system: either strict vertical or strict

horizontal transmission. Vertical transmission is defined as direct passage of the symbiont from parent to offspring through the germ line, typically from the maternal plant to seedlings through seeds. Classic examples of vertical transmission in endophyte symbioses are many cool-season grasses (e.g., *Lolium arundinaceum*, tall fescue) infected by systemic clavicipitaceous endophytes (e.g., *Epichloë coenophiala*) that are transmitted to offspring through seeds (Clay and Schardl 2002). At the opposite end of the spectrum, plants can also acquire fungal endophytes horizontally from the surrounding environment. These fungi colonize host plants via spores or mycelia transmitted through diverse abiotic sources such as rain and wind or potentially via biological vectors such as insects, which can then germinate and penetrate into the leaf tissue. Horizontally transmitted fungal endophytes have been found to infect all plant species sampled to date (U'Ren et al. 2012). However, such a binary definition of host transmission is limited in that it does not account for transmission modes that bridge the gap between direct germ line transmission and environmentally acquired microbes. For instance, endophytes may be transferred indirectly from parent to offspring through host-associated leaf litter (Herre et al. 2007). When leaves senesce and fall to the ground, they often harbor living fungal endophytes and saprobes. These fungi can emerge, sporulate, and potentially recolonize the original host or host offspring through an “imperfect” vertical transmission process (Herre et al. 2007). Likewise, some species of *Epichloë* and related grass endophytes can exhibit both vertical transmission through seeds and horizontal transmission through spores, depending on the environmental conditions (i.e., “mixed” transmission) (White 1988; Clay and Kover 1996; Tintjer, Leuchtman, and Clay 2008). Further, some grass endophytes exhibit a form of “pseudo-vertical” transmission when they grow systemically into vegetative or clonal propagules (Clay 1986). Such gradations of transmission cannot be easily binned into one of the two binary categories, so our framework purposefully utilizes a spectrum between horizontal and vertical transmission poles.

5.2.3 Host Specificity

Fungal endophytes of plants exhibit a high level of variability in their host specificity. At one end of the spectrum, cosmopolitan and “weedy” fungi can colonize diverse host taxa and can be found across widespread geographic locales. These endophyte species do not exhibit strong evolutionary specificity to a particular host species or clade. For example, endophytes of tropical grasses on Barro Colorado Island, Panama, show no evidence of host or habitat specificity (Higgins et al. 2014). Similarly, some fungal pathogens (e.g., *Sclerotinia sclerotiorum*) can infect many dozens of plant families (Boland and Hall 1994). On the other hand, some endophytes are highly coevolved with a particular host species or genus. These tight interactions may thus influence host evolution and, potentially, speciation. Although best

known from the *Epichloë* symbioses in cool-season grasses, highly coevolved endophytic interactions are also found in other systems. For example, fungi in the genus *Periglandula* are symbiotic only with plants in four genera belonging to *Convolvulaceae*, the morning glory family. Falling in the middle of the spectrum would be fungal endophytes that are capable of colonizing phylogenetically disparate host species but are more specific to some host species over others. For example, numerous studies have found that *Colletotrichum tropicale* is consistently a dominant and functionally important endophyte in *Theobroma cacao*, the cacao tree (Mejía et al. 2008; Rojas et al. 2010). However, *C. tropicale* is also found to varying degrees in other tropical species, including other fruit trees (Lima et al. 2013; Álvarez et al. 2014), orchids (Tao et al. 2013), and grasses (Manamgoda et al. 2013).

5.3 APPLYING THE FRAMEWORK

In the following sections, we use this simple, dual-axis framework to explore the diversity of plant-endophyte symbioses. Specifically, we will examine how this framework predicts (1) the distribution of “core” and “rare” members within the fungal microbiome, (2) the evolutionary trajectories and codiversification of plant-endophyte interactions, and (3) the functional role of endophytes for their plant hosts. By visually superimposing key examples from the scientific literature onto our two axes (see Figures 5.1 through 5.3), we explore hypotheses about the role of transmission mode and host specificity in explaining ecological and evolutionary processes.

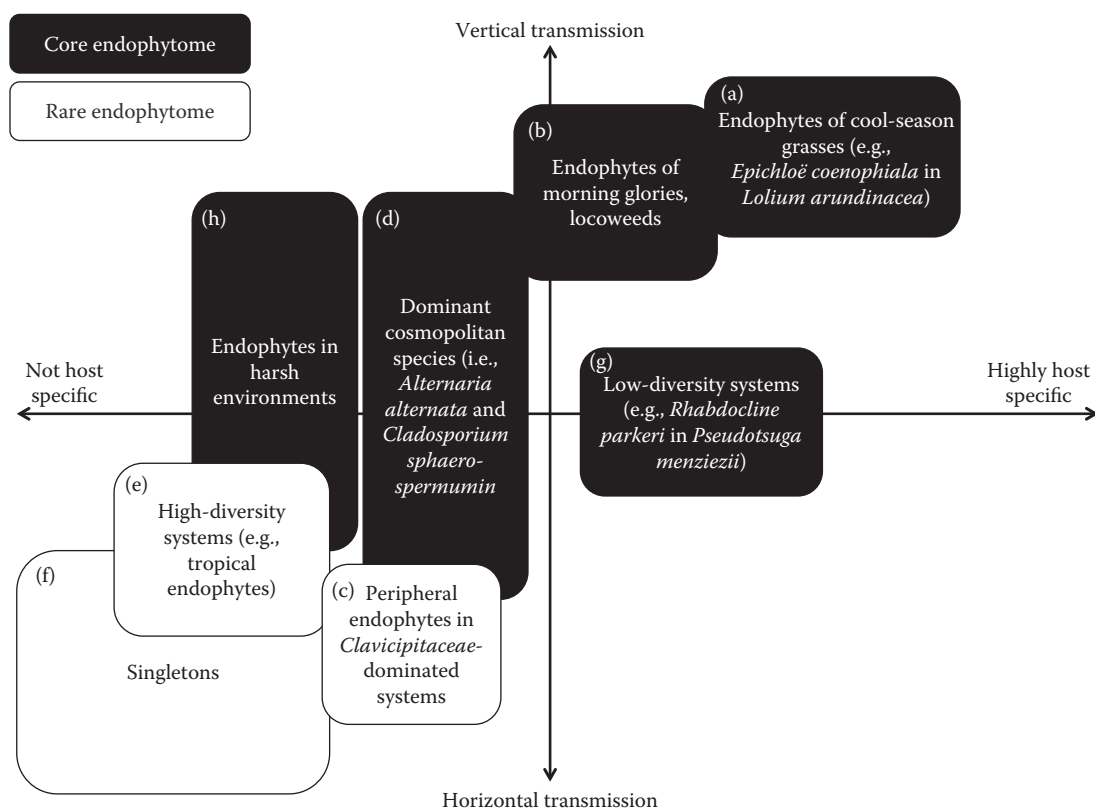


Figure 5.1 The dual-axis framework, overlaid with examples of core and rare endophytomes. The shape of each box indicates the breadth of each example with regard to transmission mode and host specificity. Black boxes represent core community members, and white boxes represent rare members. (a) Endophytes of cool-season grasses are host-specific, vertically transmitted, and found at high abundance within and across host populations, and they are functionally important. (b) Other host plants, such as morning glories and locoweeds, also have vertically transmitted core symbionts, but they have slightly broader host ranges. (c) Environmentally acquired, generalist endophytes may also colonize the tissues of plants dominated by vertically transmitted endophytes and constitute the rare, or peripheral, endophytome. (d) Horizontally transmitted, cosmopolitan endophytes may be core members of the microbiome and have multiple modes of transmission. (e) In high-diversity systems, such as the tropics, there are many rare taxa. For these horizontally transmitted endophytes, it may be a more beneficial strategy to be a rare colonizer of many host species. (f) Singleton endophytes, which are found only once in a system, are likely horizontally transmitted, non-host-specific, and may collectively dominate tissue, despite each individual species being very rare. (g) Low-diversity systems, such as monospecific stands of *Pseudotsuga menziesii*, are more likely to be dominated by one functionally important, core endophyte. (h) In harsh environments, the core endophytome may play an important role for stressed plants in both horizontally and vertically transmitted systems.

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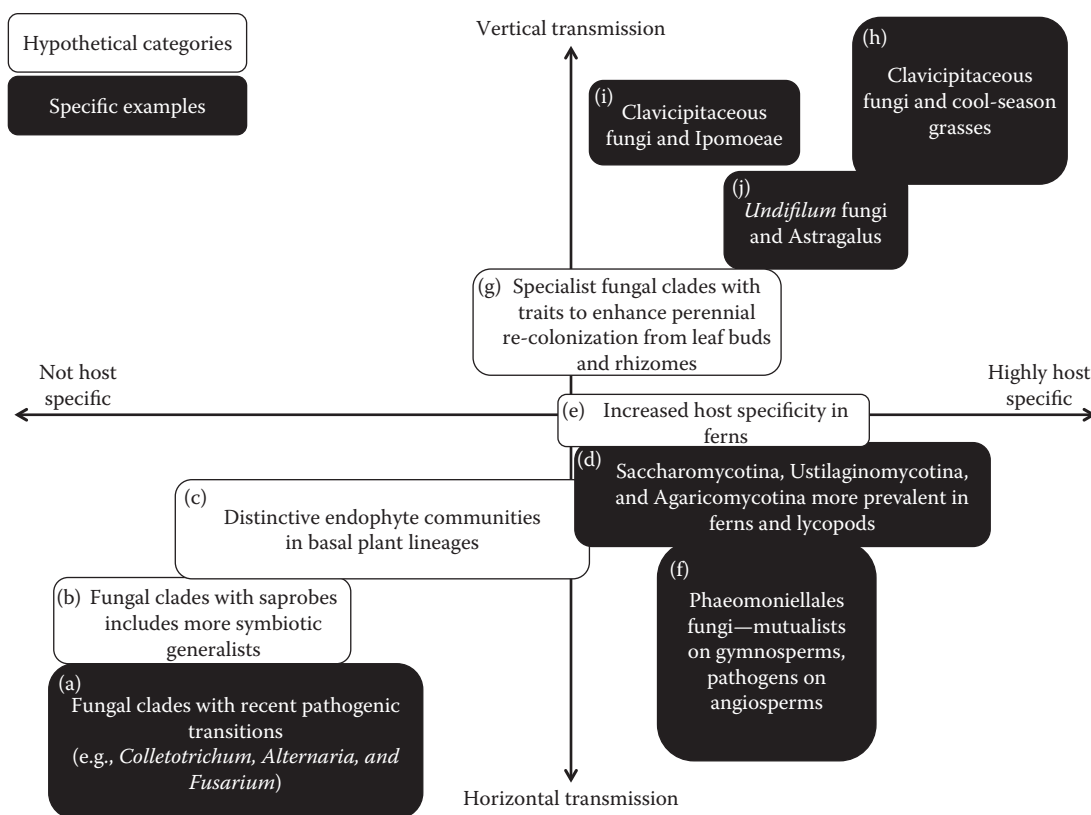


Figure 5.2 The dual-axis framework, overlaid with examples of how plant-fungal codiversification may have affected the evolution and ecology of specific plant and fungal clades. Black boxes represent specific examples of such codiversification, while white boxes represent hypothetical categories. (a) Endophyte genera that contain many pathogenic members often show very broad geographic distribution and low host specificity. (b) Similarly, fungal clades that contain many saprobic members may have evolved traits to be greater host generalists. (c) Basal plant lineages, such as the bryophytes, harbor distinctive endophyte communities. (d) Certain fungal lineages appear to be more abundant colonizers of ferns and lycopods. (e) Fern-endophyte communities are distinct from other vascular plants, possibly because of their unique leaf chemistries and structural morphologies. (f) The Phaeomoniellales fungi have host-specific functions; they typically act as mutualists on gymnosperms but as pathogens on angiosperms. (g) At a narrow taxonomic scale, certain fungal clades may be particularly adept at maintaining dormancy in perennial host organs and recolonizing during the growing season. (h) The Clavicipitaceous fungi and their cool-season grass hosts are highly coevolved compared with other fungal groups, which likely increased the rate of speciation for both groups. (i) Similarly, Clavicipitaceous fungi also interact with plants from the tribe Ipomoeae (Convolvulaceae), which is also an incredibly speciose group. (j) Species from the fungal genus *Undifilum* are vertically transmitted in *Astragalus* hosts, the largest genus of flowering plants in the world.

5.3.1 Community Members of the “Core” and “Rare” Endophytomes

An emerging concept in the field of microbiome biology is that of a “core” versus “rare” microbiome. Under this model, microbial community members can be designated as core, indicating either high abundance or consistent presence within the microbiome community, across space, time, or diverse host individuals and species (Shade and Handelsman 2012). Alternatively, community members can be rare, uncommon within the microbial community of a single host, or uncommon across hosts in space and time. However, while these definitions of “core” and “rare” microbiome members are widely used and discussed, they are not always consistent. For example, it is typically assumed

that the core microbiome plays an important functional role for the host; however, experimental tests of functional roles are less common than descriptive studies of community composition. Moreover, the rare microbiome may still have ecological importance, incongruent with its low abundance. Some endophytes could be rare at one time point but later increase in abundance and importance with changing environmental conditions (Shade et al. 2014). Although concepts of the core and rare microbiomes are typically applied to bacterial communities in hosts, the same principles may be applied to fungal endophytes within plants (i.e., the “endophytome”). We use our dual-axis framework to ask if transmission mode and host specificity of endophytic fungi predict the distribution of core and rare members in the plant-fungal microbiome.

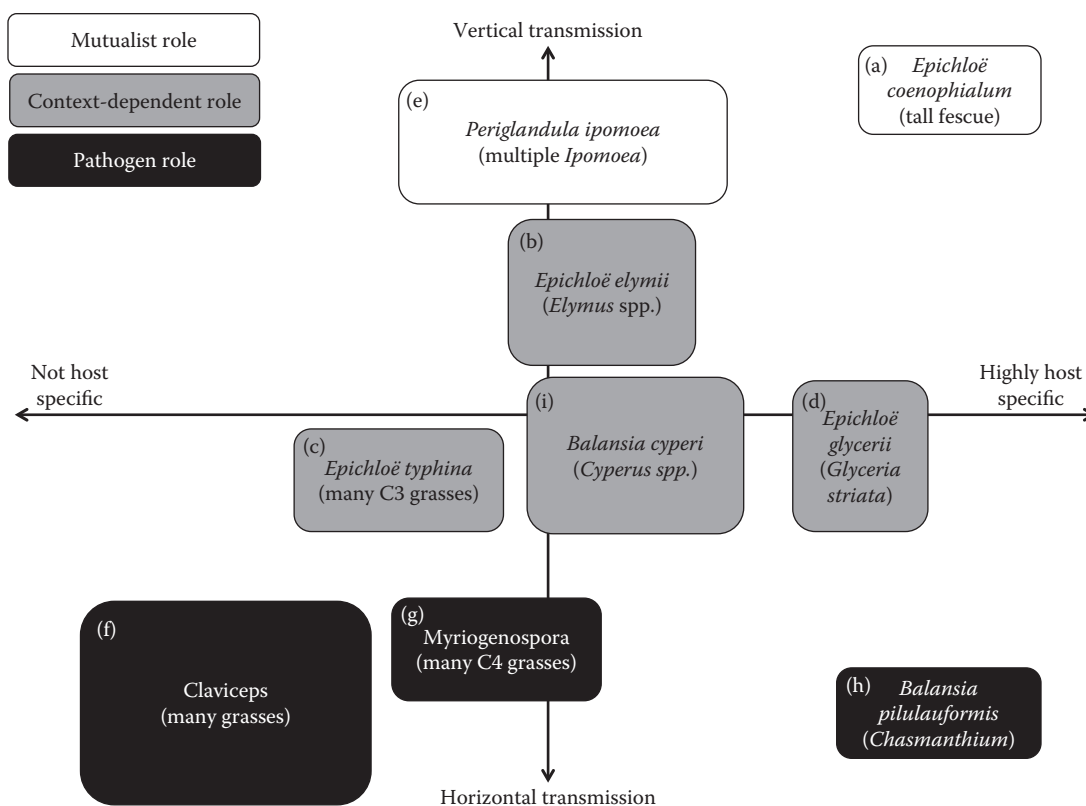


Figure 5.3 The dual-axis framework, overlaid with selected examples of plant-Clavicipitaceae fungal interactions. Black boxes represent more pathogenic interactions, white boxes represent more mutualistic interactions, and gray boxes represent more intermediate interactions. (a) The *Epichloë coenophiala*-*Lolium arundinaceum* (tall fescue grass) interaction is highly host-specific and is vertically transmitted via seed. Much literature indicates that it is highly mutualistic. (b) *Epichloë elymii* is vertically transmitted through seeds and horizontally transmitted by spores, entailing stroma production and castration of the reproductive tiller. It infects multiple species of *Elymus* grasses, and interactions are typically mutualistic (herbivore resistance and greater stress tolerance) but also reduce host fitness. (c) *Epichloë typhina* aborts host inflorescences when stromata and horizontally transmitted spores are formed and infects genera of cool-season grasses. (d) *Epichloë glycerii* infects the wetland grass, *Glyceria striata*, and is horizontally transmitted by spores. Host inflorescences are aborted, but host plants exhibit pseudo-vertical transmission by systemic spread of the fungus with increased clonal growth of the host. (e) *Periglandula*, a recently described genus, is vertically transmitted through seeds and forms symbioses with several genera in the dicotyledonous family Convolvulaceae. Reports of herbivore toxicity, caused by high levels of infection and activity of ergot alkaloids, suggest that is a mutualist. (f) Species of *Claviceps* (ergot) are horizontally transmitted ovarian parasites that infect a wide variety of grasses (multiple genera and subfamilies), as well as sedges and rushes. They reduce host fitness by replacing seeds with individual ergots. (g) *Myriogenospora* spp. infects multiple genera of warm-season grasses, aborts host inflorescences, and can spread clonally with host rhizomes, stolons, and so on, with no evidence of mutualistic effects on hosts. (h) *Balansia pilulaiformis* infects only two species of *Chasmanthium* in the southern United States. Like *Myriogenospora*, host inflorescences are aborted and the fungus is horizontally transmitted by spores. (i) *Balansia cyperi* infects multiple species of *Cyperus* (*Cyperaceae*) in the New World. Host inflorescences are aborted by fungal stromata, but some hosts exhibit pseudo-vertical transmission by clonal spread. Greater host growth and herbivore resistance have been reported, indicating some degree of mutualism.

The concept of a core endophytome is probably most exemplified by endophyte associations in cool-season grasses (Figure 5.1a). These systems, which are often characterized by strong vertical transmission and tight host specificity, are noted for consistently sharing a single dominant endophyte species across individuals within a single host population, among geographically disparate host populations, as well as between closely related host species. For instance, the highly host-specific, vertically transmitted endophyte *Epichloë coenophiala* is shared among approximately 98% of some

tall fescue (*Lolium arundinaceum*) populations (Saikkonen et al. 2000) and the majority of individuals within populations (Shelby and Dalrymple 1987). The high abundance of this endophyte across hosts, coupled with its well-documented functional importance in enhancing host survival, growth, and reproduction (Rudgers and Clay 2007), clearly qualifies it as a core endophyte within the tall fescue endophytome. Dominance by a core endophyte is also a common feature in non-grass systems characterized by vertical transmission, though with slightly broader host ranges (e.g., endophytes

of morning glories, locoweeds; Panaccione, Beaulieu, and Cook 2014; Figure 5.1b). Intriguingly, recent evidence shows that hosts dominated by vertically transmitted endophytes can also simultaneously host horizontally transmitted endophytes (Zabalgoeazcoa et al. 2013; Figure 5.1c). Although it is unclear what ecological role these less abundant, rare community members might play in the endophytome, analogous studies have been conducted in arthropod systems. In arthropods, the term primary symbiont (i.e., “core microbiome”) is typically used to refer to obligate, vertically transmitted bacterial symbionts that provide essential nutrients for the host. On the other hand, secondary symbionts (i.e., “rare microbiome”) are nonessential; they may, however, be common (Douglas 1998) and interact with primary symbionts. For instance, in the pea aphid *Acyrtosiphon pisum*, it has been shown that coinfection of hosts with the secondary symbiont *Rickettsia* significantly suppressed the population density of the primary obligate symbiont *Buchnera*, as well as host fitness (Sakurai et al. 2005). Future work should aim to characterize the rare fungal microbiome of plants and explore how vertically transmitted, systemic endophytes interact with environmentally acquired fungi within the host, as well as test the functional implications for plant hosts.

In herbaceous and woody plant systems, core microbiome members, as defined by their high abundance within host populations, may also be cosmopolitan and horizontally transmitted. For example, *Alternaria alternata* and *Cladosporium sphaerospermum* (Figure 5.1d) often dominate endophyte communities during seedling development, as well as at more mature growth stages. These cosmopolitan colonizers exhibit little host specificity and have been isolated as common endophytes of many plants. For example, *A. alternata* has been isolated from plants such as switchgrass (*Panicum virgatum*; Kleczewski et al. 2012), rice (*Oryza sativa*; O’Hanlon et al. 2012), and grape (*Vitis vinifera*; Musetti et al. 2006), while *C. sphaerospermum* has been identified in soybean (*Glycine max*; Hamayun et al. 2009) and pine (*Pinus spp.*; Chandra Paul and Yu 2008). There is also some evidence to suggest that *A. alternata* and *C. sphaerospermum* may occasionally be vertically transmitted (Figure 5.1d). For example, *A. alternata* was isolated from surface-sterilized seeds of six forb species (Hodgson et al. 2014), as well as the cotyledons and first true leaves of otherwise endophyte-free seedlings. Moreover, because *A. alternata* and *C. sphaerospermum* spores were found on and within pollen grains, the authors suggest that pollen was the source of transmission from parent to offspring. It remains to be seen whether this type of colonization by core, cosmopolitan endophytes during early seedling development creates any priority effects for later endophytome community assembly. *Alternaria alternata* has also been shown to be a dominant fungal symbiont of *Centaurea stoebe* when the plant is in its native range, but not in its invasive range, where no particular endophyte dominates

the community (Shipunov et al. 2008). This suggests that geographic location and the surrounding native plant community also influence the core constituents of the endophytome in a particular plant host.

Conversely, most herbaceous and woody plants have endophytomes that are highly skewed toward rare taxa, containing many endophytes that are both infrequently isolated within a single host and infrequently found across hosts in space and time. In tropical trees, for example, tens to hundreds of different fungal species may coexist within the foliage of a single host, where most of the endophyte taxa are rare (Gamboa, Laureano, and Bayman 2002). In these highly diverse plant communities, it may not be a beneficial strategy for endophytes to specialize on a particular host, given the difficulties in dispersing to a rare host, a phenomenon documented in fungal pathogens (Parker et al. 2015). Existing as a rare colonizer of a wide variety of plant hosts may be a more adaptive life style for the symbiont (Figure 5.1e). Thus, it is not unusual for over half of the horizontally transmitted fungal colonizers isolated from an individual host or host population to be singletons (i.e., only found once) and, likely, not host-specific (Arnold et al. 2000; Higgins et al. 2007; Davis and Shaw 2008; Sánchez Márquez, Bills, and Zabalgoeazcoa 2008). In some systems, the rare microbiome may even collectively dominate host tissues (Figure 5.1f). However, their functional importance for the host, their tendency to experience a rapid increase in abundance under certain environmental conditions, and their ability to drive changes in microbial community composition remain unclear. Studies that compare endophyte diversity of core and rare colonizers, as well as their intermediates, especially in the context of the surrounding plant community, would lend key insights into how biotic factors and species’ interactions structure endophytic communities. One hypothesis is that plants in high-diversity habitats, such as the tropics, are collectively dominated by rare microbiome members, while plants in low-diversity habitats are more likely dominated by a single endophyte species. For instance, in monospecific stands of *Pseudotsuga menziesii* (Douglas fir), the dominant endophyte *Rhodocline parkeri* has been isolated from virtually every individual tree (Carroll and Carroll 1978; Figure 5.1g). This core endophyte is also functionally important, promoting herbivore resistance in its host (Sherwood-Pike, Stone, and Carroll 1986; Carroll 1988).

A second hypothesis is that harsh environments represent important drivers for the distribution of core and rare members of the microbiome (Figure 5.1h). For instance, *Fusarium culmorum* confers tolerance to salt stress in *Leymus mollis* (dunegrass) in coastal habitats (Rodriguez et al. 2008), and *Curvularia protuberata* confers heat stress tolerance to *Dichanthelium lanuginosum* (panic grass) in geothermal habitats (Márquez et al. 2007). However, while these fungi are typically vertically transmitted in their host, the two species were able to promote similar functional responses when inoculated onto salt-stressed and heat-stressed tomato

plants (*Solanum lycopersicum*; Rodriguez et al. 2008). Thus, it appears that under harsh conditions, vertically or horizontally transmitted core members of the microbiome can serve important but non-host-specific functional roles. Deserts represent another type of stressful environment, where endophytes are rarely isolated in culture (2% of plant tissues) and are highly diverse (Massimo et al. 2015). In deserts, this rare microbiome could be favored as a bet-hedging strategy. However, another possibility is that stressed plants also harbor obligate, core symbionts that may be favored in the harsh desert environment. These core symbionts, like vertically transmitted endophytes, may be more difficult to culture, owing to their heavy reliance on plant chemistry and other aspects of a symbiotic life style, hence the low isolation frequency of endophytes in culture (Massimo et al. 2015).

Future studies should employ and directly compare both culture-based and culture-independent methods to assess the core and rare microbiomes through time, across plant community diversity gradients and across environmental stress gradients. Ultimately, assessing the relative functional importance of the core and rare microbiomes across space and time, especially with regard to a changing environment (e.g., developmental, seasonal, and pathogen-induced triggers) would help inform under what conditions certain endophytes are important for plant health and function.

5.3.2 Plant-Fungal Codiversification on Macroevolutionary Timescales

The plant and fungal kingdoms have been codiversifying since land colonization (Krings et al. 2007), leading to multiple, independent evolutionary origins of fungal endophytism. During this time, different plant species and clades interacted and evolved with their symbiotic partners. At these long timescales, we suggest that it is necessary to take a macroevolutionary perspective and examine how host specificity and mode of host-to-host transmission may have affected, or may have been affected by, the evolutionary trajectory of certain fungal lineages and their interactions within and among different plant clades.

Most endophytic fungi of plants occur within the Pezizomycotina sub-phylum of the Ascomycota (e.g., Sordariomycetes, Dothideomycetes, Pezizomycetes, Leticomycetes, and Eurotiomycetes), though some Saccharomycotina have also been documented as endophytes (Del Olmo-Ruiz and Arnold 2014). Fewer fungi within the Basidiomycota exhibit an endophytic habit; however, members of this phylum are consistently identified in endophyte surveys (Arnold et al. 2007; Pinruan et al. 2010; Martin et al. 2015). However, within all of these clades, multiple transitions to endophytism from non-endophyte ancestors are thought to have occurred. For example, research on the vertically transmitted clavicipitaceous fungi of cool-season grasses and morning glories suggests that these symbionts likely arose from insect pathogens to become plant parasites

and mutualists (Spatafora et al. 2007). The production of secondary compounds by these fungi, originally used to parasitize insects, was preadapted to defend plant hosts from insect attack. Host transitions from insects may also have been a source of the endophytic habit of horizontally transmitted fungi (Posada and Vega 2005; Ownley, Gwinn, and Vega 2010). In addition, many horizontally transmitted endophytic lineages have frequently reverted to necrotrophic lineages, and vice versa, as indicated by ancestral character state mapping (Delaye, García-Guzmán, and Heil 2013). Moreover, a transition from a previously endophytic fungal lineage to a biotrophic pathogen habit appears to be an evolutionarily stable strategy for environmentally acquired endophytes (Delaye, García-Guzmán, and Heil 2013). For example, many widespread plant pathogens are closely allied to commonly reported endophytes (e.g., *Alternaria*, *Colletotrichum*, and *Fusarium* species; Saikkonen et al. 1998; Figure 5.2a). Lastly, many other extant horizontally transmitted endophytes may have evolved from saprophytic (Carroll 1999; Schulz and Boyle 2005; Schoch et al. 2009; Figure 5.2b) or endolichenous (i.e., secondary symbionts, not primary mycobionts; Arnold et al. 2009) fungal ancestors, indicating high diversity within the evolutionary origins of plant endophytes. As our discovery of novel fungi continues to accelerate, expanding phylogenetic analyses may help provide insights into the evolutionary origins of endophytes and their functional roles.

Endophytic fungi, with their varied ancestral histories and functional roles, have different affinities for different plant clades. For example, endophyte communities colonizing bryophyta (mosses, hornworts, and liverworts), one of the most basal plant lineages found on the earth today, are consistently more diverse and encompass a larger number of distinct fungal taxa, when compared with more recently evolved plant taxa, such as gymnosperms and angiosperms (U'Ren et al. 2012). It is possible that over longer evolutionary timescales, more fungi have evolved the ability to colonize this basal plant group. Asymptomatic fungal communities living inside lichen thalli represent another example of ancient symbioses. Intriguingly, endolichenous and bryophyte endophyte communities are compositionally very similar to one another, suggesting that over time, very distinctive and specialized endophyte communities have evolved in these basal lineages (U'Ren et al. 2012; Figure 5.2c).

Most fungal endophyte studies focus on seed-bearing vascular plants (i.e., gymnosperms and angiosperms). By comparison, relatively few studies have characterized the endophyte communities in pteridophytes (ferns and fern allies), a nonseed-bearing vascular plant clade. Limited evidence suggests that ferns may host distinct fungal endophyte communities compared with other seed-bearing vascular plants at the same geographic location (U'Ren et al. 2012). For example, Saccharomycotina (Del Olmo-Ruiz and Arnold 2014) and the Basidiomycota lineages, Ustilaginomycotina

and Agaricomycotina (Chen et al. 2011; Del Olmo-Ruiz and Arnold 2014), are typically minor endophyte members of angiosperm hosts but may be more common in ferns and fern allies (Figure 5.2d). Pteridophytes evolved during the early Devonian period, before the evolution of seed-bearing plants (Kenrick and Crane 1997). Nevertheless, during the early period of lycophyte and fern radiation, alternative hosts such as bryophytes and early gymnosperms were present and similarly codiversifying with their potential fungal symbionts. It is possible that ferns host distinct endophyte communities owing to this early diversification or that specific fungal clades are more host-specific on ferns owing to their unique structural morphology and leaf chemistry relative to other plant groups (Markham, Chalk, and Stewart Jr. 2006; Del Olmo-Ruiz and Arnold 2014; Figure 5.2e). In vitro comparisons using leaf extracts from, or artificial inoculations onto, diverse plant host groups and fungal species may help illuminate what shapes these symbioses on ecological timescales.

Recent work also suggests that host-associated evolution within endophyte lineages is a result of the symbiotic interactions with distinct plant host groups. A recent multilocus phylogeny mapped endophytism within the Eurotiomycetes (Chen et al. 2015) and documented a previously unidentified fungal order, the Phaeomoniellales. Molecular clock calculations indicate that divergence and radiation for this order occurred around the time of gymnosperm diversification. Moreover, they demonstrated that while this fungal order generally occurs as an asymptomatic endophyte on gymnosperm hosts, it typically has a more pathogenic effect on angiosperm hosts (Figure 5.2f). Functional disparities between pathogenic and endophytic life styles, such as this one, may be the consequence of host transitions onto angiosperms from their initial adaptation and radiation on gymnosperms.

At a broad taxonomic scale, codiversification within certain host clades may define host-to-host transmission mode for fungal endophytes. To the best of our knowledge, there are no cases of true vertical transmission (i.e., transmission from the maternal plant to seeds) among the gymnosperms or analogous vertical transmission through spores in more ancient plant lineages such as ferns and bryophytes (though adaptation of specialist fungi for recolonization of perennial host organs is theoretically possible; see Figure 5.2g). Vertical transmission of fungal genera such as *Epichloë*, *Periglandula*, and *Undifilum* is unique to angiosperms and occurs in both monocotyledonous and dicotyledonous hosts (Figure 5.2h–j). Interestingly, the plant hosts also represent particularly speciose clades within the plant kingdom. For example, cool-season grasses, frequent hosts of vertically transmitted clavicipitaceous endophytes, are extremely diverse and globally distributed across numerous habitats (Soderstrom et al. 1987; Figure 5.2g). Similarly, approximately 900 species have been described from the tribe Ipomoeae, Convolvulaceae (known hosts of *Periglandula*

endophytes; Eserman et al. 2014; Figure 5.2h); *Astragalus* (i.e., the major genus of locoweeds) is the single largest genus of flowering plants on the earth today, consisting of upward of 2500 species (Sanderson and Wojciechowski 1996; Figure 5.2i). It is possible that the emergence of mutualistic, vertically transmitted symbionts increased the rate of speciation and adaptive radiation within these plant groups (Agrawal et al. 2009) and thus may function analogously to the evolution of novel traits.

In general, more research is needed to understand how plant-fungal codiversification at evolutionary timescales has shaped the host specificity and functional role of symbionts at ecological timescales. In particular, characterization of endophyte communities in non-seed and nonvascular plants needs to be expanded, particularly in habitats where all major plant clades are represented. Only then may large-scale studies incorporating systematics or meta-analyses uncover the macroevolutionary patterns and consequences of these symbioses at finer taxonomic resolutions within the plant and fungal kingdoms.

5.3.3 Functional Diversity—Plant-Infecting Clavicipitaceae as a Model System

Fungal endophytes exhibit a high diversity of functional roles in plant tissues and span the pathogen-mutualist spectrum. However, in general, their functional roles are often unclear, context-dependent, or lacking in-depth characterization. One exception is the Clavicipitaceae, a family of ascomycete fungi, whose functional roles have been the most intensively studied, given their ecological and economic importance. Clavicipitaceae consists largely of insect pathogens, where highly specialized endophyte lineages have radiated onto grasses, morning glories, and, to a lesser extent, other plant groups (Spatafora et al. 2007; Sung et al. 2007). Although associations between cool-season grass and endophyte have received the greatest attention, plant-infecting Clavicipitaceae represent a wide range of life cycles and host associations that goes well beyond the Class 1 categorization of Rodriguez et al. (2009) and also include endophytes of warm-season grasses, sedges (Cyperaceae; Diehl 1950; Plowman et al. 1990), morning glories (Convolvulaceae; Steiner et al. 2011; Beaulieu et al. 2015), Asteraceae (Bischoff and White 2003), Smilacaceae (Kobayasi 1981), and, possibly, other plant groups. In some cases, the endophytes are vertically transmitted through host seeds, while other taxa spread contagiously by spores. Likewise, some endophytes are highly host-specific, limited to one host species or genus, while others have much broader host ranges. While some taxa are clearly mutualists, enhancing fitness of their hosts (Rudgers and Clay 2007), others are more pathogenic and act as parasitic castrators, aborting host reproductive organs (Clay and Kover 1996), while yet others fall in between, with both pathogenic and mutualistic effects that can vary among closely related host

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species or environmental conditions (Schardl and Clay 1997; Tintjer, Leuchtman, and Clay 2008). Here, we use the Clavicipitaceae as a model system to look at the diversity of functional roles within this well-studied fungal family and specifically ask how transmission mode and host specificity predict function.

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The classic example of an endophyte infecting cool-season grasses (Class 1, *sensu* Rodriguez et al. 2009, or Type 3, *sensu* White 1988) is *Epichloë coenophiala* (Leuchtman et al. 2014), the endophyte of *Lolium arundinaceum* (tall fescue grass) (Figure 5.3a). This endophyte is highly host-specific and is completely vertically transmitted via seed (Rudgers and Clay 2007). In contrast, other species of *Epichloë* exhibit variable levels of host specificity and vertical transmission. For example, *E. elymii* can be vertically transmitted through host seeds and horizontally transmitted by spores (Type 2 of White 1988; Tintjer, Leuchtman, and Clay 2008) and infects multiple species of *Elymus* grasses (Schardl and Leuchtman 1999; Figure 5.3b). Other *Epichloë* species are only horizontally transmitted by spores and exhibit variable host ranges. For example, *E. typhina* infects multiple genera of cool-season grasses (Sampson 1933; Kohlmeyer and Kohlmeyer 1974; Figure 5.3c), while *E. glycerii* is reported to infect only *Glyceria striata* (Schardl and Leuchtman 1999). Interestingly, *E. glycerii* also exhibits a form of pseudo-vertical transmission via systemic spread into stolons, enhancing the vigorous clonal growth of its host (Pan and Clay 2003; Figure 5.3d). Thus, considering *Epichloë* endophytes of cool-season grasses, there does not appear to be a fixed relationship between host specificity and transmission mode.

Periglandula is a newly described genus of clavicipitaceous symbionts of morning glories (Convolvulaceae; Steiner et al. 2011) that appears to be transmitted only by vertical transmission through seeds (Beaulieu et al. 2015; Figure 5.3e). It represented the best documented example of Clavicipitaceae infecting dicotyledonous plants. Members of this genus may show broader host range than many *Epichloë* endophytes, interacting with several host species and genera within the monophyletic tribe Ipomoeae within the Convolvulaceae (Eserman et al. 2014). Current phylogenies suggest that this group is less diverse than the endophytes of cool-season grasses. However, relatively few taxa or plant-fungal associations have been critically examined to date, so our perspective on *Periglandula* diversity and host relations is likely to change with further study. These interactions are classified here as falling on the mutualistic end of the spectrum, primarily by extension from grasses, based on their high levels of bioactive alkaloid compounds.

At the more pathogenic end of the mutualism-parasitism spectrum are species from the genus *Claviceps* (ergot), which are horizontally transmitted by spores and infect a very wide variety of grass species (Brady 1962; Alderman, Halse, and White 2004; Figure 5.3f). Their wide host range may also reflect the fact that within one fungal species,

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multiple host-specific races may occur. Other unrelated endophytes may also represent host- or genotype-specific races specialized on particular host species in particular areas, as in the case of endophytes from New Guinea rainforest trees (Vincent et al. 2015). *Claviceps purpurea*, for example, exhibits host-specific races associated with particular grasses in different habitats (Fisher, DiTomaso, and Gordon 2005). Other *Claviceps* species also infect host species from the graminoid families, Cyperaceae and Juncaceae (Alderman et al. 2004). It could be argued that *Claviceps* species do not represent endophytes, given that they are localized ovarian parasites, differing from the systemic growth of most other plant-infecting Clavicipitaceae. However, many other endophytes of trees and herbaceous plants also form localized infections of particular host tissues. There is little evidence that *Claviceps* has any mutualistic benefits for host plants; however, Wäli et al. (2013) suggested that *Claviceps* infection might provide some protection to hosts from grazing mammals.

In addition, at the more pathogenic end of the spectrum are species of *Balansia* and *Myriogenospora* fungi in the genus *Myriogenospora* that infect multiple genera of warm-season grasses (Diehl 1950) and are not vertically transmitted through seeds but can spread clonally with host stolons (Figure 5.3g). Species of *Balansia* are parasites on a wide range of warm-season grasses and sedges and also are not known to be vertically transmitted through seeds. Nevertheless, many *Balansia* spp. exhibit high host specificity. For example, *B. obtecta* infects only grasses in the genus *Cenchrus* (Diehl 1950) and is transmitted via spores. Likewise, *B. pilulaeformis* infects only two species of *Chasmanthium* in the southern United States (Figure 5.3h), and *B. cyperi* infects a few species of *Cyperus* (Cyperaceae) in the New World (Figure 5.3i). In all of the aforementioned species, host plants are sterilized by infection and the fungus is horizontally transmitted by spores. Interestingly, in some *Cyperus* hosts, *B. cyperi* also exhibits pseudo-vertical transmission in that it can spread clonally via host plant bulbils and viviparous plantlets (Clay 1986; Stovall and Clay 1988). It is shown in Figure 5.3 as being less pathogenic than other *Balansia*, *Claviceps*, and *Myriogenospora* species, because empirical data suggest that host plants often grow faster and exhibit higher herbivore resistance than uninfected plants (Stovall and Clay 1988; Clay 1990). Thus, while many members of the Clavicipitaceae are horizontally transmitted by spores, there is no obvious relationship of transmission mode with host specificity in these other taxa as well. However, the degree of mutualism does appear to be more tightly correlated with host transmission mode where more mutualistic associations are characterized by vertical or pseudo-vertical transmission, which fits with theoretical predictions for the cost-benefit economy of symbioses (Ewald 1987; Lively et al. 2005).

The *Epichloë* endophytes of cool-season grasses and the *Periglandula* endophytes of morning glories may provide

insights into the long-term evolutionary trends in plant-Clavicipitaceae symbioses. A recent study reported finding a fossilized *Claviceps*-like fungus infecting a grass inflorescence preserved in amber, dating back to the early or mid Cretaceous period, 90–110 mya (Poinar Jr., Alderman, and Wunderlich 2015), and suggested that the interaction may have originated in the mid to late Jurassic. This date is consistent with the estimate of (Sung, Poinar Jr., and Spatafora 2008), based on molecular clock calculations, that grass-Clavicipitaceae interactions date back to at least 81 mya in the late Cretaceous. Thus, these plant-fungal symbioses are evolutionarily ancient and predate the evolution of leaf-cutter-ant-fungus symbioses, for example, by 30–50 million years (Schultz and Brady 2008). By contrast, Eserman et al. (2014) suggest that the tribe Ipomoeae (Convolvulaceae), which includes all of the known lineages containing symbiotic morning glories, dates to approximately 35 mya. They also concluded that having an association with *Periglandula* fungi is the ancestral condition in the Ipomoeae. This fossil and molecular evidence suggest that the grasses, and their interaction with clavicipitaceous fungi, potentially have had a longer period of evolutionary diversification of endophyte symbiosis, transmission mode, and degree of mutualism (Sung, Poinar Jr., and Spatafora 2008). However, modern grass genera such as *Festuca* and *Lolium*, which are highly endophyte infected, have more recent evolutionary origins (2–4 mya; Inda et al. 2014) than symbiotic morning glory lineages (~25 mya; Eserman et al. 2014), leading to the alternative hypothesis that more ancient endophyte symbioses are predominantly vertically transmitted.

Future research is required in several areas. While cool-season grasses and their *Epichloë* endophytes have been very well studied, much less data are available for *Periglandula*-morning glory interactions or for *Balansia* or *Claviceps*-host interactions, especially for wild, noneconomic plant species. Our understanding of general patterns is therefore likely to change as more information becomes available. Second, the function of these interactions for host plants requires additional investigation. For example, all of the plant-infecting Clavicipitaceae produce ergot alkaloids, but do the alkaloids play the same role in all endophyte interactions? Third, the endophytes of cool-season grasses exhibit considerable variation in host specificity, transmission mode, and degree of mutualism. They represent an ideal system to investigate the correlations and constraints among these three variables in a phylogenetically well-defined group of symbiotic interactions.

5.4 CONCLUSIONS

Previous classification systems have helped us understand general differences in types of plant-endophyte symbioses (see White 1988; Rodriguez et al. 2009), but they do not fully encompass the range of plant-fungal interactions

and their unique characteristics that we now know exist in nature. We hope that the novel framework described here will serve as a useful tool to generate hypotheses that explore and conceptually unify disparate components of plant-fungal symbioses. We see emergent patterns that suggest that transmission mode and host specificity explain ecological and evolutionary traits of endophytism. For example, the majority of the core and rare endophytome members fall along a diagonal axis (Figure 5.1), potentially representing a trade-off between transmission mode and host specificity that shapes the individual abundance and functional importance of endophytes within the endophytome. Specifically, the abundant and functionally important core members tend to be more vertically transmitted and host-specific, while rare members are more likely to be horizontally transmitted generalists. Similarly, plant-endophyte codiversification seems to be driven by an interaction between host specificity and host-to-host transmission mode, with most examples spanning the same diagonal axis (Figure 5.2). However, a handful of the examples that drive this pattern are hypothetical. We suggest that more in-depth characterization of plant-endophyte communities, especially in a phylogenetic context, will help add clarity and resolution to this emerging pattern.

By contrast, the functional role of endophytes may be driven primarily by transmission mode (Figure 5.3). In particular, vertically transmitted endophytes tend to be more mutualistic, with increasing pathogenicity seen with increasing horizontal transmission. The fact that both mutualists and pathogens may exist as either host specialists or generalists suggests that the host specificity axis does not shape the functional role of endophytic fungi, at least at the scale in question. Because we are examining functional role at a finer scale of taxonomic resolution, the transmission and host specificity axes may be more compressed for the Clavicipitaceae (Figure 5.3) than for more widely distributed endophyte associations. Patterns evident at larger taxonomic scales may be less evident at finer taxonomic scales, where other patterns may emerge more clearly. As an increasing number of studies continue to elucidate function of non-clavicipitaceous endophytes, it will be worthwhile to examine how transmission mode and host specificity shape functional role across the full range of fungal endophytes in nature and whether this change in taxonomic resolution affects the types of patterns that emerge.

Much endophyte research is at an early phase, describing which fungi are present in particular plant species and plant communities, yet there is a vast number of undescribed fungal symbioses in nature. In order to better understand the significance of plant-endophyte associations, we need to build upon this important foundational research and more fully explore how endophytes colonize their hosts; how specialized they are to particular plant tissues, species, and environments; and how they affect the fitness of host plants. A thorough grounding in theory, such as the framework that

we describe in this contribution, should allow researchers to use approaches such as meta-analytical techniques to synthesize and draw comparisons across diverse plant-endophyte interactions. Moreover, as we continue to add to the plant-endophyte literature, it will be important to purposefully choose focal fungal taxa for observational and manipulative experiments that span a wide range of the dual-axis space. By doing so, we will better understand the factors controlling the form and function of aboveground endophytic fungi in diverse host systems and environments. We believe that the framework described here serves as the appropriate conceptual foundation to both highlight gaps in our knowledge and guide future research.

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