

Evolutionary Origins and Ecological Consequences of Endophyte Symbiosis with Grasses

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ABSTRACT: Over the past 20 yr much has been learned about a unique symbiotic interaction between fungal endophytes and grasses. The fungi (Clavicipitaceae, Ascomycota) grow intercellularly and systemically in aboveground plant parts. Vertically transmitted asexual endophytes forming asymptomatic infections of cool-season grasses have been repeatedly derived from sexual species that abort host inflorescences. The phylogenetic distribution of seed-transmitted endophytes is strongly suggestive of cocladogenesis with their hosts. Molecular evidence indicates that many seed-transmitted endophytes are interspecific hybrids. Superinfection may result in hyphal fusion and parasexual recombination. Most endophytes produce one or more alkaloid classes that likely play some role in defending the host plant against pests. Hybridization may have led to the proliferation of alkaloid-production genes among asexual endophytes, favoring hybrids. The ergot alkaloid ergovaline, lolitrem, and lolines are produced by only a single sexual species, *Epichloë festucae*, but they are common in seed-transmitted endophytes, suggesting that *E. festucae* contributed genes for their synthesis. Asexual hybrids may also be favored by the counteracting of the accumulation of deleterious mutations (Muller's ratchet). Endophyte infection can provide other benefits, such as enhanced drought tolerance, photosynthetic rate, and growth. Estimates of infection frequency have revealed variable levels of infection with especially high prevalence in the subfamily Pooideae. Longitudinal studies suggest that the prevalence of seed-transmitted endophytes can increase rapidly over time. In field experiments, infected tall fescue suppressed other grasses and forbs relative to uninfected fescue and supported lower consumer populations. Unlike other widespread plant/microbial symbioses based on the acquisition of mineral resources, grass/endophyte associations are based primarily on protection of the host from biotic and abiotic stresses.

Keywords: endophyte, grasses, symbiosis, hybridization, alkaloids, herbivory.

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Symbiotic interactions of green plants with bacteria and fungi are widespread. Enhanced nutrient uptake, greater stress tolerance, and protection from pests are among many benefits obtained by infected hosts. Several questions occur in common across plant-microbial systems. What is the evolutionary origin of these interactions? What are the costs and benefits to each partner? How host specific is the interaction? How are the partners transmitted? Does the nature of the interaction vary among individuals, populations, or species?

Over the past 20 yr, research has brought to light a widespread interaction between grasses and systemic endophytes in the fungal family Clavicipitaceae. These interactions exhibit several unique features not found in other plant/microbial symbioses. Several important pasture and forage grasses are common endophyte hosts, and infection has major consequences for their biology, which has resulted in intense worldwide research efforts. Recent advances in endophyte systematics, genetics, chemistry, and ecology have provided a new perspective on grass/endophyte interactions. The purpose of this article is to examine the evolutionary origins of the symbiosis and its ecological consequences for both partners.

Studies of grass/endophyte interactions offer special insights into several general problems in the evolution of symbiotic and infectious relationships. The derived position of highly specialized mutualistic interactions within a larger clade of grass pathogens indicates that mutualists have evolved from pathogens and suggests mechanisms by which this occurred. Studies have revealed unexpectedly high levels of hybridization among endophytes, suggesting that vertically transmitted endophytes frequently arise from this genetic recombination and possess novel ecological traits. A key feature of grass/endophyte interactions is the endophyte's capability for vertical or horizontal transmission and the correlation of transmission mode with host fitness. Endophyte production of alkaloid toxins provides a clear mechanism for altered host physiology and ecology. Controlled and natural experiments suggest that endophyte symbiosis can have large effects on plant communities and their associated consumers.

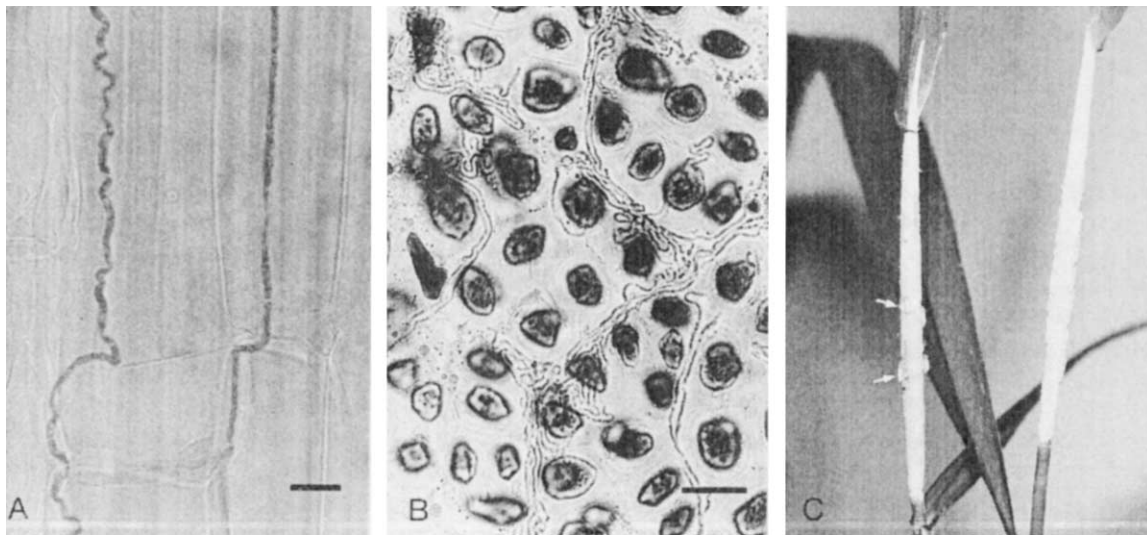


Figure 1: Fungal endophytes of grasses. *A*, Endophytic hyphae of *Epichloë clarkii* in intercellular spaces within leaf sheath of *Holcus lanatus* (bar = 10 μm). *B*, Mycelium of *Neotyphodium lolii* in seed of perennial ryegrass *Lolium perenne* (bar = 40 μm). *C*, Stromata of *Epichloë typhinae* on aborted flowering culms of *Elymus virginicus*. Arrows point to larvae of fly that vectors conidia and effects fungal fertilization. From Clay and Leuchtman (1997).

Endophyte Symbiosis

“Endophyte” is a generic term for any organism that lives inside of a plant, analogous to an epiphyte living on the plant surface. There has been semantic disagreement over usage of the term endophyte, with the suggestion that the word implies a mutualistic relationship that may not exist and that other words may be better (Wennstrom 1994). For example, some plant pathogens like smuts may exist internally and asymptotically within host plants for many years before they finally become evident. Nevertheless, a large variety of heterotrophic organisms exist internally within plants, at least during part of their life cycle, without producing any disease symptoms. Most attention has been focused on fungal endophytes that exist in leaves, stems, and reproductive organs of host plants. The straightforward technique of surface sterilizing a leaf or leaf segment and plating it out on nutrient agar will generally result in the outgrowth of one or more fungi even if the leaf was asymptomatic. Asymptomatic, endophytic fungi may be ubiquitous in the plant kingdom, rivaling insects in their species diversity (Carroll 1988; Arnold et al. 2000). But we know relatively little at present about the distribution and diversity of endophytes in different plant groups and plant communities, outside of a few well-studied examples.

Grass Endophytes

Grass endophytes may constitute a monophyletic clade with the fungal family Clavicipitaceae (Ascomycota; Kul-

dau et al. 1997), but even with recent data it remains unclear if this is a monophyletic clade. Clavicipitaceous fungi include parasites of the grass family (and occasionally sedges) that can form pathogenic or mutualistic relationships with their hosts. Three of the four tribes infect only grasses or sedges, while the fourth tribe, Cordycipieae (genus *Cordyceps*), is pathogenic on insects or other fungi (Kuldau et al. 1997). The tribe Clavicipieae (i.e., *Claviceps*) parasitizes a wide range of grasses where it forms infections of single grass florets and replaces the seed with individual sclerotia. These are the well-known ergot pathogens that produce toxic ergot alkaloids (Groger 1972). The most diverse tribe is the Balansieae, consisting of several genera forming systemic infections of host grasses that also produce alkaloids (Bush et al. 1997). One genus (*Epichloë*) has spawned a diversity of asexual forms (*Neotyphodium* species) that have radiated in association with cool-season grasses in the subfamily Pooideae (Schardl 1996). Our primary focus is on these associations, reflecting the substantial research efforts devoted to this group.

Growth in the Host

Growth of clavicipitaceous endophytes in grasses exhibits several distinctive features. Growth is systemic throughout the aboveground tissues of their hosts (fig. 1A). Sparsely branched hyphae grow parallel to the long axis of plant cells in intercellular spaces where they likely subsist on sugars and amino acids released into the apoplast. Infec-

tions are perennial such that plants will remain infected throughout their life span, although sectoring and loss of infection in particular segments of host plants can be occasionally observed. During host flowering, the fungus grows into ovules and seeds (fig. 1B) or it proliferates to form a fruiting body (fig. 1C, and see "Life History and Reproduction"). Molecular evidence suggests that most endophyte hosts are infected by only a single fungal genotype (Kover et al. 1997; Meijer and Leuchtman 1999). Multiple infections can occur occasionally and may be highly significant because they afford the opportunity for fungal hybridization (Scharndl et al. 1994; Tsai et al. 1994). Experimental inoculations with multiple strains show that all but one strain is eventually excluded at either the whole plant or tiller level (Wille et al. 1999; Christensen et al. 2000). In other endophytic associations with nongrass hosts, infections are typically highly localized and may consist of only a few epidermal cells. One leaf or one plant may be infected by dozens or hundreds of distinct fungal species (An et al. 1992; Saikkonen et al. 1998; Arnold et al. 2000).

Host grasses infected by fungal endophytes do not typically exhibit any type of defense response despite the extensive ramification of hyphae throughout the plant body. Unlike many pathogens, endophytes do not produce haustoria and so do not directly cause cell damage. However, inoculations of seedlings with endophytes isolated from distinct host species can induce localized cell death more typical of plant-pathogen interactions (Koga et al. 1993). A range of inoculation studies (Leuchtman and Clay 1993; Chung et al. 1997) also point to a high level of host specificity under genetic control of the fungus. Host tissue deformations are known to occur in conjunction with fruiting body production, resulting in increased rates of nutrient uptake by the fungus (White et al. 1997).

An interesting variant of the typical endophyte growth form is epiphytic growth where fungal mycelium is concentrated on the surfaces of young leaves, buds, and meristematic regions and on reproductive structures (Leuchtman and Clay 1988; White and Glenn 1994). Meristematic regions may provide high concentrations of resources before the leaf cuticle becomes well developed. New plant growth becomes infected at a very early developmental stage, allowing the fungus to maintain its systemic infection. The close phylogenetic placement of epiphytic and endophytic species within the Clavicipitaceae (e.g., congeneric species of *Balansia*; Clay and Frenz 1993) indicates that the evolutionary transition between superficial (epiphytic) growth of a parasite on its host and intercellular, systemic growth within the host is not difficult. In fact, there is no clear-cut evolutionary trend toward either endophytic or epiphytic growth, suggesting that these growth habits may switch over evolutionary time

(Kuldau et al. 1997). Bertoni et al. (1997) recently described a fungal parasite of the shrub *Baccharis coridifolia* (Asteraceae) that grows in a very similar manner. Interestingly, the host plants are reported to be toxic.

Life History and Reproduction

The generalized endophyte life cycle involves perennial and systemic growth within the host plant for most of the year. At the time of flowering, the fungi typically produce fruiting bodies and spores and inhibit or abort the host's reproductive organs ("choke disease"; White et al. 1991a). However, in many cool-season grasses, the fungus does not produce any external structures and is transmitted vertically through the seeds of infected plants (Sampson 1933; fig. 2).

There is a dichotomy between endophyte life histories for a series of correlated traits (table 1). The symptomatic life cycle entails contagious spread of the fungus by meiotic ascospores and the reproductive sterilization (castration) of the host. The fungi are heterothallic and require transfer of conidia (spermatia) between mating types for successful reproduction (Bultman and White 1988). This strategy is referred to as Type I by White (1988) and is not fundamentally different from other plant pathogens. At the other end of the spectrum is the asymptomatic life cycle (Type III) where the fungus remains internal throughout the season, including during flowering. No sexual spores (ascospores) are produced, so there is no regular mechanism of genetic recombination for the fungus. Lineages of single fungal genotypes are transmitted vertically through seeds by hyphal growth into developing ovules (Freeman 1904), providing greater opportunities for coevolutionary interactions. The rate of vertical transmission can be extremely efficient, approaching 100%.

Reproduction of endophytes infecting cool-season grasses can be extremely plastic, with individual plants producing both fungal fruiting bodies and inflorescences simultaneously. White (1988) refers to this mixed mechanism as Type II. For example, in red fescue (*Festuca rubra*) both endophyte-infected seeds and aborted inflorescences with fungal fruiting bodies can be produced simultaneously on the same plant (Scharndl 2001). Scharndl et al. (1997) called fungi producing this mixed state "pleiotropic symbionts" because they are transmitted both vertically through seeds and horizontally by spores. Such a situation would appear to maximize potential of the symbionts to exert mutualistic effects on their hosts, while still permitting the symbiont to exhibit the sexual state associated with an antagonistic characteristic, namely, choke disease. These pleiotropic associations are of particular interest because they possibly reflect a mixed evolutionary strategy and a pathway by which contagiously transmitted path-

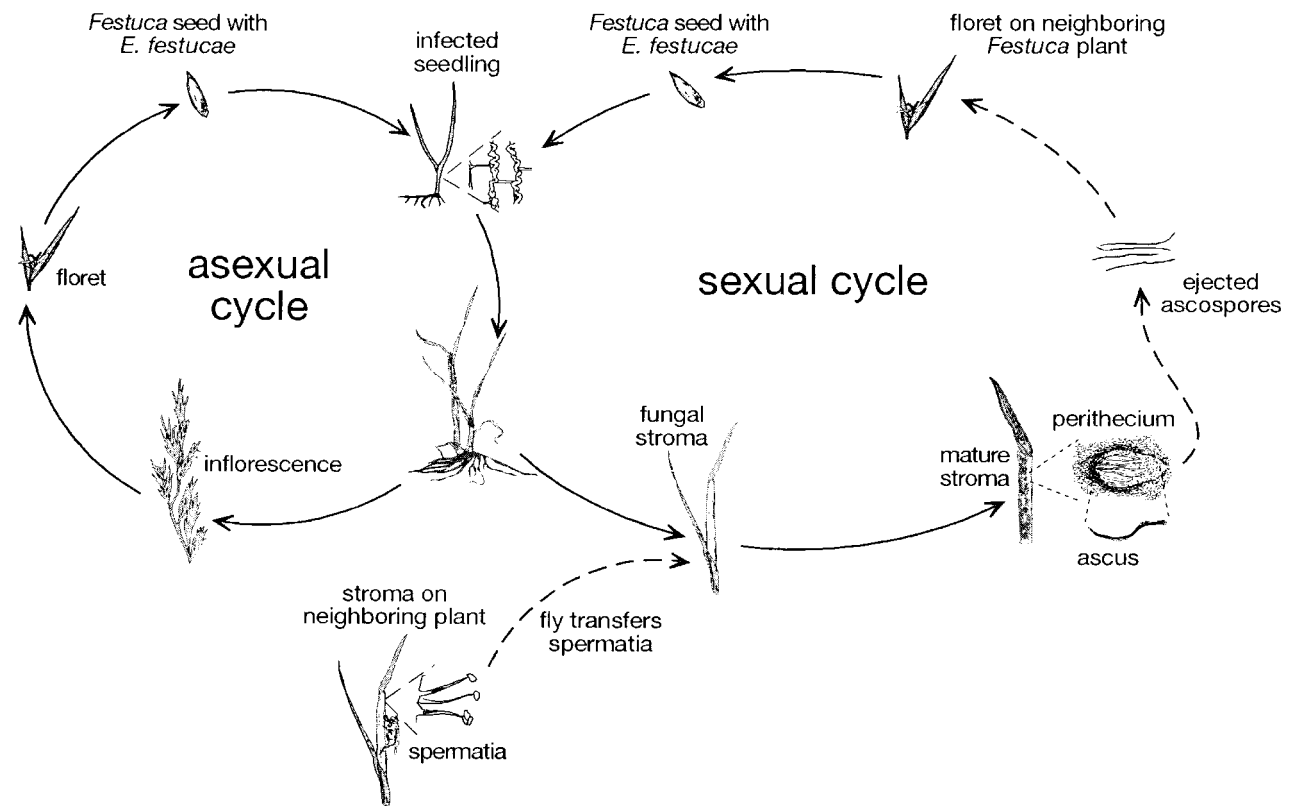


Figure 2: Type II life cycle of *Epichloë festucae* with alternative sexual and asexual life cycles corresponding to horizontal and vertical reproduction, respectively.

ogens become vertically transmitted symbionts. This evolutionary transition may be quite plastic because even castrating endophytes may provide certain host benefits like increased growth (Clay 1986; Groppe et al. 1999) and enhanced resistance to herbivores (Cheplick and Clay 1988).

Bucheli and Leuchtman (1996) found evidence of genetic differentiation between horizontally transmitted (symptom-causing) endophytes and vertically transmitted endophytes coexisting within single host species or populations. However, a single Type II fungal genotype can adopt both reproductive strategies simultaneously with each tiller, producing either fungal stroma or endophyte-infected seeds (Kover and Clay 1998; Schardl 2001). Inoculation studies suggest that sporulation is under genetic control of the endophyte (Leuchtman and Clay 1993). The relative proportion of symptomatic and asymptomatic inflorescences can change from season to season in response to plant size and/or environmental conditions (Sun et al. 1990). True Type II endophytes may be hard to distinguish from Type I endophytes that only partially infect a single plant, leading to chimeric individuals with both aborted and healthy inflorescences (Groppe et al.

2001). In Type I endophytes, seeds are fungus-free and give rise to uninfected plants.

It is only within the clade of *Epichloë* species and derived *Neotyphodium* species that the total range of reproductive variation and regular vertical transmission occurs. Most other endophyte genera form only Type I, symptomatic associations. The species *Atkinsonella hypoxylon* is somewhat anomalous, given that it is always symptomatic with fruiting body production and also vertically transmitted through dimorphic cleistogamous seeds (Clay 1994). Kover et al. (1997) found that most fungal genotypes in *Atkinsonella* populations arise by contagious spread of ascospores.

For clonal plant species, systemic fungal growth represents a special form of vertical transmission where the same endophyte genotype is transmitted by vegetative propagation of new plant parts (Clay 1986; Clay and Kover 1996). Unlike vertical transmission through seeds, it is not limited to cool-season grasses. There may be less selective pressure for vertical transmission through seeds where extensive clonal growth is possible through stolons, rhizomes, bulbils, and so forth. Endophyte infection can di-

Table 1: Contrasting characteristics of Types I, II, and III grass/endophyte associations

	Symptomatic (Type I)	Mixed (Type II)	Asymptomatic (Type III)
Fungus:			
Reproduction	Sexual	Both	Clonal
Transmission	Horizontal	Both	Vertical
Propagule	Ascospores	Both	Seeds
Host:			
Reproduction	Sterile/clonal	Partial sterility	Sexual
Interaction	Pathogenic	Intermediate	Mutualistic
Infection frequency	Low-moderate	Intermediate	High
Taxonomy	Entire grass family	C3 pooid grasses	C3 pooid grasses

rectly alter the host plant's propensity for clonal reproduction (Stovall and Clay 1988; Pan and Clay 2002).

The expected population genetic consequences of sexual or asexual reproduction are supported by empirical studies. Leuchtmann and Clay (1997) found that the ratio of distinct genotypes to isolates sampled, a measure of the clonal structure of fungal populations, was highest in species exhibiting symptomatic Type I associations and lowest in asymptomatic Type III associations. Vertically transmitted, asexual endophytes exhibit less genetic variation than sexually recombining endophytes. Host populations may be infected by only a single endophyte genotype, which remains constant over repeated host generations. The accumulation of deleterious mutations over time (Muller's ratchet) may represent a barrier for their long-term persistence.

The frequency of infection within host populations appears to parallel reproductive strategy with Type I infections occurring at the lowest frequency and Type III infections occurring at up to 100% frequency (Clay and Leuchtmann 1989; Bier 1995; Clay 1997).

Transmission Mode and Virulence

Endophyte symbioses offer insights into the relationship between parasite transmission mode and virulence. Theories of parasite virulence assume that there is a positive correlation between transmission rate and virulence, the negative effects of parasite infection on host survival or reproduction (Bull 1994). Greater transmission by the parasite may require increased exploitation of the host, reducing host survival or growth and, therefore, resources available to the parasite (Mackinnon and Read 1999). Thus, there may be a trade-off between parasite transmission and host survival, which could lead to intermediate levels of virulence (Lipsitch et al. 1995). More complex models incorporating superinfection, where an infected host becomes additionally infected by another parasite strain, suggest that selection can favor increased

levels of virulence relative to single parasite infections (Lenski and May 1994; Nowak and May 1994).

Predicted levels of virulence are less clear when a parasite is capable of both vertical and horizontal (contagious) transmission, as in the case of Type II endophytes. Selection may favor greater virulence and contagious transmission if there is a mechanistic link between virulence and vertical transmission levels. In one grass-endophyte system, vertical transmission requires a minimum level of virulence (Kover and Clay 1998). Effective colonization of seeds may require a minimum fungal biomass, or titer, within the host plant, as has been found for some plant viruses. Lipsitch et al. (1996) pointed out that when high rates of horizontal transmission lead to high infection rates within the population, vertical transmission is then favored, since there are few uninfected individuals capable of becoming infected.

Purely maternally transmitted endosymbionts cannot persist in host populations if they reduce host fitness relative to uninfected individuals (Fine 1975; Lipsitch et al. 1995). Because the endosymbiont reproduces only through eggs or seeds, hosts infected by symbionts that reduce seed production will be replaced over time by uninfected hosts with higher rates of seed production. Therefore, maternally transmitted symbionts are indirect evidence that infected hosts have higher fitness than uninfected hosts. Type III, seed-transmitted endophytes should therefore be mutualistic. Empirical evidence from nonendophyte systems supports the predicted correlation between parasite transmission mode and host fitness (Bull et al. 1991; Herre 1993). Vertically transmitted endophytes may possess unobserved mechanisms for occasional contagious spread (Moy et al. 2000), which could obscure the predicted association between transmission mode and parasite virulence. In addition, clavicipitaceous endophytes may interact with other types of endophytes infecting grasses, along with herbivores and plant pathogens (An et al. 1992; Schulthess and Faeth 1998).

Other types of endophytic fungi infecting plants appear

to be horizontally transmitted but are nonpathogenic (Carroll 1988; Arnold et al. 2000), reflecting complexity in the nature of transmission/virulence correlation. How horizontally acquired mutualists originate and are maintained represents a general problem in ecology (Wilkinson and Sherratt 2001; J. Bever, unpublished manuscript).

Grass/Endophyte Cophylogeny

Endophyte groups and reproductive strategies map at various levels onto the phylogeny of grasses. Type II and Type III seed-transmitted endophytes occur only in grasses with the C3 photosynthetic mechanism in the subfamily Pooideae (Schardl et al. 1997). Other endophyte genera exhibit only Type I contagious spread and are generally found in the warm season C4 grasses. Greater levels of specialization also exist. For example, *Atkinsonella hypoxylon* infects only eastern North American species of *Danthonia* (Leuchtman and Clay 1996) and *Balansia cyperi* infects only New World *Cyperus* (Plowman et al. 1990).

The phylogenetic distribution of seed-transmitted Type II endophytes is strongly suggestive of co cladogenesis with their plant hosts. Outgroup rooting splits the genus *Epichloë* into two major clades (Craven et al. 2001b; fig. 3). One of these clades, designated the *Epichloë typhina* complex, is diverse, interreticulated, and contains three described species, *E. typhina*, *Epichloë clarkii*, and *Epichloë sylvatica*. Because of the very broad host range of *E. typhina*, and interreticulation with *E. clarkii* and *E. sylvatica*, there is no indication of co cladogenesis between the *E. typhina* complex and hosts. The other major clade, designated the main clade, presents a strong contrast with at least seven phylogenetic species each associated with a single host tribe. Interestingly, the sister relationship of grass tribes Aveneae and Poeae (Soreng and Davis 1998) reflects the sister relationships of the associated *Epichloë* species *Epichloë baconii*, *Epichloë amarillans* (both on Aveneae), and *Epichloë festucae* (on Poeae). The same is true of sister species *Epichloë elymi* and *Epichloë bromicola* on the sister tribes Triticeae and Bromeae, respectively. The most deeply rooted *Epichloë* species is *Epichloë brachyelytri*, known only from the host tribe, Brachyelytreae, which is itself the most deeply rooted in the Pooideae (Catalán et al. 1997).

In a phylogeny inferred from introns of the beta-tubulin gene, *tub2*, the relationships among the main clade *Epichloë* species matched those of the corresponding host tribes, with the sole exception of *Epichloë glyceriae* (Schardl et al. 1997), which is consistent with the idea that *E. glyceriae* arose in a jump to a new host taxon. However, combining the *tub2* phylogeny with that of another housekeeping gene, *tefl*, encoding translation elongation factor 1-alpha, and again using the variable intron sequences, leads to a phylogeny in which there is no obvious host

jump in the main clade (fig. 3). Rather, around the time or shortly after emergence of the *E. brachyelytri* lineage there appears to have been a "species duplication," giving rise to two lineages that then may have independently coevolved with the grasses. One of these lineages gave rise to *E. festucae*, *E. amarillans*, and *E. baconii*, whereas the other gave rise to *E. glyceriae*, *E. elymi*, and *E. bromicola*. Which of these scenarios is more likely, and, indeed, whether co cladogenesis adequately explains relationships among *Epichloë* species, require more detailed analysis aimed at discerning the relative timing of host and symbiont cladogenesis events.

Co cladogenesis may be indicative of coevolution in the sense of Janzen (1980), whereby evolution of the symbiont would respond to evolution of the host, and evolution of the host to evolution of the symbiont. It is likely that endophytes provide critical components of diversity and adaptability among the Pooideae, thus influencing host evolution and perhaps even speciation. However, co cladogenesis alone need not imply coevolution, and endophytes might track host evolution simply by selection to maintain compatibility. Indeed, when these endophytes are moved even among closely related hosts they can elicit varying degrees of incompatibility (Koga et al. 1993; Christensen 1995). Genetic evidence also suggests that the *Neotyphodium* and *Epichloë* endophytes must closely coadapt to their host species. Progeny of *Epichloë* matings between strains adapted to different host species can exhibit reduced compatibility to both hosts (Chung et al. 1997). The resulting selection against mating between host-associated populations should promote *Epichloë* speciation (Thompson 1987).

The fact that the best indications of co cladogenesis involve Type II associations suggests that closer physiological adaptation of the symbiont to the host is required for the pleiotropic symbiosis than for either Type I or Type III symbiosis. It is especially interesting that the strictly vertically transmitted endophytes show no indications of co cladogenesis because in documented examples of co cladogenesis in other systems the symbiont (whether mutualistic or parasitic) is exclusively or almost exclusively transmitted vertically (Hafner and Nadler 1988; Munson et al. 1991; Chapela et al. 1994; Distel et al. 1994; Hinkle et al. 1994). Phylogenetic studies of several systems have demonstrated that highly evolved mutualisms do not necessarily involve co cladogenesis (Chapela et al. 1994; Gast and Caron 1996; Wilkinson et al. 1996; Janz and Nylin 1998; Van Hoek et al. 2000). Furthermore, as discussed below, endophytes that are only vertically transmitted (Type III) also fail to exhibit co cladogenesis with their hosts. Nevertheless, vertical transmission may play a partial role in co cladogenesis of Type II endophytes. For example, a recent study suggests some phylogenetic tracking

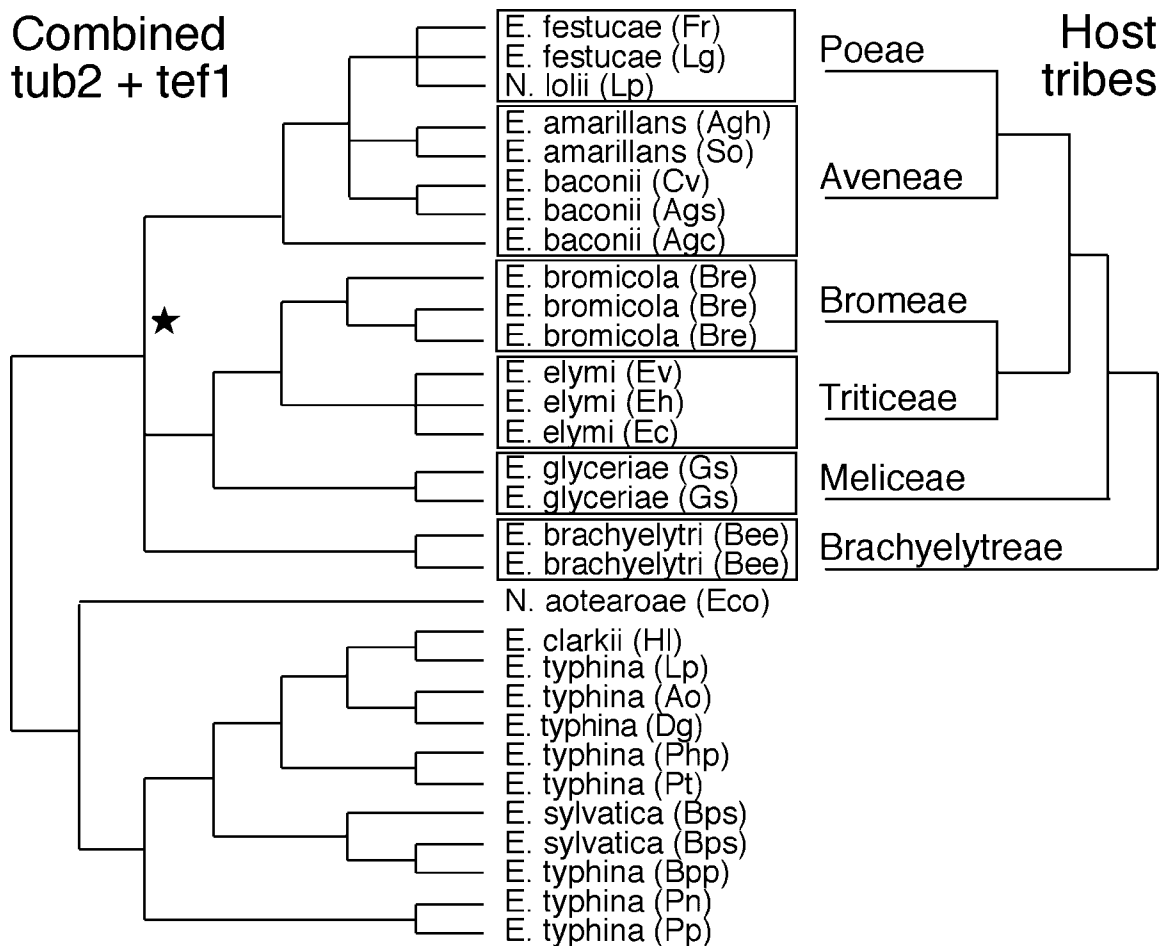


Figure 3: Phylogenetic relationships of *Epichloë* and nonhybrid *Neotyphodium* species juxtaposed against the phylogeny of their host tribes based on results of Catalán et al. (1997) and Soreng and Davis (1998). The endophyte phylogeny is the consensus of the four most parsimonious trees identified by branch-and-bound search (for exact solutions) on a combined dataset consisting of aligned intron sequences from the translation elongation factor 1-alpha (*tef1*) and beta-tubulin (*tub2*) genes (Craven et al. 2001b; Moon et al. 2002). Host tribe relationships are indicated for the *Epichloë* main clade but not for the *Epichloë typhina* complex (*Epichloë clarkii*, *Epichloë sylvatica*, and *E. typhina*) because *E. typhina* infects multiple host tribes. The star represents a possible cladogenesis of endophytes without corresponding cladogenesis of the hosts, as discussed in the text. Host species are abbreviated in parentheses following each species name as follows: Fr (*Festuca rubra*); Lg (*Lolium giganteum*); Lp (*Lolium perenne*); Agh (*Agrostis hiemalis*); So (*Sphenopholis obtusata*); Cv (*Calamagrostis villosa*); Ags (*Agrostis stolonifera*); Agc (*Agrostis capillaris*); Bre (*Bromus erectus*); Ev (*Elymus virginicus*); Eh (*Elymus hystrix*); Ec (*Elymus canadensis*); Gs (*Glyceria striata*); Bee (*Brachyelytrum erectum*); Eco (*Echinopogon ovatus*); Hl (*Holcus lanatus*); Ao (*Anthoxanthum odoratum*); Dg (*Dactylis glomerata*); Php (*Phleum pratense*); Pt (*Poa trivialis*); Bps (*Brachypodium sylvaticum*); Bpp (*Brachypodium pinnatum*); Pn (*Poa nemoralis*); Pp (*Poa pratensis*).

of *E. festucae* with species of the grass genus *Festuca* (Tredway et al. 1999), and a survey of natural populations of *E. festucae* indicates the importance of clonal propagation (which is coupled with vertical transmission) in *E. festucae* population structure (Arroyo García et al. 2002).

Speciation of Sexual Endophytes

The tendency of *Epichloë* species to be associated with individual host tribes or even individual genera, regardless

of whether they have tracked host phylogeny, suggests that host specialization is an important factor in their speciation. Recent host-driven speciation was evident in the main clade when it was observed that an *Epichloë* isolate from *Koeleria* sp. was apparently unable to mate with *Epichloë festucae* on *Festuca* species despite the fact that these endophytes have nearly identical gene sequences and, therefore, are very close relatives (Craven et al. 2001b). There is also support for several host-specialized populations in the *Epichloë typhina* complex that may be newly

emerged or emerging species. For example, *Epichloë sylvatica* does not appear to be a distinct phylogenetic species because it does not form a monophyletic clade separate from *E. typhina*, yet there is very little interfertility between *E. sylvatica* and *E. typhina*. Although such fertility barriers do not exist or are less extreme between other host-associated populations, phylogenetic evidence suggests a high degree of genetic distinction between *E. typhina* associated with certain hosts such as *Poa nemoralis*, *Poa pratensis*, *Brachypodium pinnatum*, and *Holcus lanatus* (the latter having been described as *E. clarkii*; Craven et al. 2001b). Although these results suggest that there may be additional species within the *E. typhina* complex, neither the application of a biological species concept (Mayr 1999) nor a popular phylogenetic species concept (Avice and Wollenberg 1997) clearly identifies them. Population genetic analysis appears to be the most powerful means to identify these otherwise cryptic species (Leuchtmann and Schardl 1998).

Even with these host-associated cryptic or incipient species, much of the *E. typhina* complex appears to be a single, broad host range interbreeding population. A large clade in the complex includes pathogens of *H. lanatus*, *Lolium perenne*, *Anthoxanthum odoratum*, *Dactylis glomerata*, *Phleum pratense*, *Poa trivialis*, and probably others (fig. 3). All are Type I endophytes that cause complete abortion of all host inflorescences and lack the capability for seed transmission. Craven et al. (2001b) speculated that the frequent requirement of these Type I endophytes to colonize new host plants selects both for their high level of virulence and broad host range. Further, since frequent recombination of *E. typhina* genotypes might aid in colonizing multiple hosts, as suggested by Chung et al. (1997), selection should favor continued fertility among strains in different host species. Broad host range should slow or stop evolution of barriers to fertility between diverse genotypes of the species. Thus, in contrast to most *Epichloë* main clade species, *E. typhina* (with *E. clarkii*) appears to have a very long evolutionary history with almost no emergence of intersterile populations.

Geographical isolation is also likely to promote speciation among the sexual endophytes. For example, there is intersterility between the closely related species, *Epichloë baconii* and *Epichloë amarillans*, even though both are associated with grasses in genus *Agrostis* and closely related genera (Leuchtmann and Schardl 1998) but *E. baconii* has only been found in Europe and *E. amarillans* only in North America.

Hybridization and Speciation of Asexual Endophytes

Since a biological species concept cannot practically apply to asexual organisms (Mayr 1999), it is preferable to em-

ploy some form of phylogenetic species concept for the Type III endophytes and, on that basis, elucidate the speciation process. Several recent studies take this approach (Schardl et al. 1994; Tsai et al. 1994; Moon et al. 2000, 2002; Craven et al. 2001a), with some surprising results. Given the tendency of Type II endophyte relationships to reflect host relationships, it is tempting to suggest that cocladogenesis might result from vertical transmission. Phylogenies of the asexual endophytes demonstrate that this is an oversimplification because these endophytes, which are strictly vertically transmitted (Type III), fail to show any evidence of host cocladogenesis. Type III endophytes have arisen repeatedly and independently from sexual ancestors, and often via interspecific hybridization.

The endophyte that has sparked the greatest research interest is *Neotyphodium coenophialum* (formerly *Acremonium coenophialum*), which is a ubiquitous symbiont of tall fescue (*Festuca arundinacea* = *Lolium arundinaceum*), contributes a wide range of host benefits, and produces three of the four known classes of endophyte-associated antiherbivore alkaloids (table 2). Phylogenetic evidence suggests that this endophyte is an interspecific hybrid with three *Epichloë* ancestors, providing a basis its multifarious capabilities (Tsai et al. 1994). The reasons for inferring a hybrid origin of *N. coenophialum* are its tendency to possess multiple loci for isozymes that are single copy in *Epichloë* species (Leuchtmann and Clay 1990), phylogenetic evidence that each of its three *tub2* genes is derived from a different *Epichloë* species (Tsai et al. 1994; fig. 4), similar implications for its two *tefl* genes (K. D. Craven and C. L. Schardl, unpublished data; fig. 4), and a genome size nearly twice that of related *Epichloë* species (Kuldau et al. 1999). Two additional unnamed endophyte species (designated FaTG-2 and FaTG-3) have been identified in Mediterranean tall fescues (Christensen et al. 1993) and have also been characterized as interspecific hybrids (Tsai et al. 1994).

Detailed phylogenetic analyses have also been conducted on *Neotyphodium lolii* from perennial ryegrass; *Lolium perenne* (Schardl et al. 1994), another perennial ryegrass endophyte designated LpTG-2 (Christensen et al. 1993; Schardl et al. 1994; Collett et al. 1995); *Neotyphodium uncinatum* from meadow fescue; *Lolium pratensis* (Gams et al. 1990; Craven et al. 2001a) and *Neotyphodium tembladerae* from *Poa huecu* (Cabral et al. 1999; Moon et al. 2002). Of these, all but *N. lolii* are apparent interspecific hybrids. Furthermore, descriptions of several new endophyte species have been based, in part, on their hybrid origins and relationships to *Epichloë* species: *N. occultans* from annual ryegrasses (Moon et al. 2000), *Neotyphodium siegelii* from meadow fescue (Craven et al. 2001a), *Neotyphodium australiense* from Australian *Echinopogon ovatus* and *Neotyphodium melicicola* from South African *Melica*

decumbens (Moon et al. 2002). The relationships of *tub2* and *tefl* genes of these endophytes and their nonhybrid (mainly sexual) relatives are shown in figure 4. In these studies an apparent nonhybrid endophyte, *Neotyphodium aotearoae*, was also identified from New Zealand and Australian *E. ovatus* populations. Interestingly, one of the ancestors of the hybrid *N. mellicicola* is closely related to *N. aotearoae* (fig. 4), suggesting the existence of an unsampled or recently extinct *Epichloë* species that contributed to the evolution of these two Southern Hemisphere endophytes. Phylogenetic analysis is a convenient tool for investigating evolutionary origins of the hybrid endophytes, but allozyme and microsatellite analyses provide a more facile survey of many more loci. Indeed, all allozyme (Leuchtman and Clay 1990; Schardl et al. 1994; Craven et al. 2001a) and microsatellite (Moon et al. 1999, 2000) studies have indicated the tendency for asexual endophytes to have multiple loci for genes where their sexual counterparts have only single loci. These studies have confirmed the particular hybrid origins inferred by phylogenetics. Furthermore, the prediction that hybrids should have larger genomes than their nonhybrid counterparts was confirmed by Kuldau et al. (1999). Nuclear genome sizes of nonhybrids *Epichloë festucae*, *Epichloë typhina* and *N. lolii* were estimated at 29 Mb, but the genome size for LpTG-2, an inferred *N. lolii* × *E. festucae* hybrid, was approximately 55 Mb. Likewise, the *N. coenophialum* genome was estimated at 57 Mb. Interestingly, the mitotic spores (conidia) of hybrid endophytes tend to be larger and sometimes distinctly different in shape from those of nonhybrids (Kuldau et al. 1999; Craven et al. 2001a).

The asexual endophytes tend to be slower growing in pure culture than the sexual endophytes (White et al. 1991b). Interestingly, most of the slowest-growing *Neotyphodium* species are apparent nonhybrids: *N. aotearoae* (Moon et al. 2002), *N. lolii* (Latch et al. 1984), *N. typhinum* var. *canariense* (Moon et al. 2000), and the endophyte of *Achnatherum inebrians* (Miles et al. 1998; C. D. Moon and C. L. Schardl, unpublished data). A notable exception is the hybrid *N. occultans*, which cannot be maintained in culture (Moon et al. 2000) and, thus, represents the extreme among the hybrids. It is noteworthy that most aneuploid and polyploid derivatives of *Emericella nidulans* (*Aspergillus nidulans*) grow slowly relative to the haploid, which is the usual ploidy status for most filamentous ascomycetes (Käfer and Upshall 1973; Upshall 1981). Although the mechanism for this is unknown, a similar phenomenon may underlie the slower growth of the heteroploid hybrid endophytes relative to the haploid *Epichloë* species. In some circumstances, slow growth may put endophytes at a disadvantage. However, growth in culture might not predict growth *in symbio* and survival of the endophyte depends on the fitness of its host plant.

Enhanced host fitness may dictate the high frequency with which heteroploid hybrid endophytes are identified, relative to other filamentous ascomycetes.

Endophyte Alkaloids

Most seed-transmitted endophytes produce alkaloids that likely play some role in benefiting the host plant against pests (table 2). Several distinct classes of alkaloids are known from clavicipitaceous endophytes including ergot alkaloids similar to those from *Claviceps* (Groger 1972). Other classes include saturated aminopyrrolizidine (loline) alkaloids, indole diterpenoid (lolitrem) alkaloids and pyrrolopyrazine (peramine) alkaloids. Examples of chemical structures are shown in figure 5. Siegel et al. (1990) found that peramine was present in the majority of endophyte-infected host grasses followed by ergot alkaloids (50%), loline alkaloids (35%), and lolitrem (10%). Some of these alkaloids are known only from *Epichloë* and *Neotyphodium* species but ergot alkaloids are widespread in the plant parasitic Clavicipitaceae. A number of detailed reviews of endophyte alkaloids have been published (Porter 1994; Bush et al. 1997; Siegel and Bush 1997).

Ergot alkaloid toxicity (ergotism) has been known for many centuries (Groger 1972) and several ergot alkaloids have pharmaceutical applications. Many ergot alkaloids are derived from variations on the basic ergoline ring structure (see Siegel and Bush 1997; fig. 5). Ergot alkaloids have been detected in a wide variety of endophyte-infected grass species, but specific chemical composition and concentrations vary with the symbiotic combination. For example, in tall fescue infected with *Neotyphodium coenophialum* the predominant ergot alkaloid is ergovaline (Arechavaleta et al. 1992), whereas in sleepygrass (*Achnatherum robustum* = *Stipa robusta*) and *Achnatherum inebrians* infected by other *Neotyphodium* species, the predominant ergot alkaloids are lysergic acid amide (Petroski et al. 1992) and ergonovine (Miles et al. 1996), respectively. Ergobalansine is the major alkaloid component produced by *Balansia cyperi*, which infects a variety of tropical and subtropical sedges, and *Balansia obtecta*, which infects sandbur grass, *Cenchrus echinatus* (Powell et al. 1990).

Plant genotype, tissue type, season, and other abiotic environmental conditions can all influence alkaloid concentration *in planta*. Lyons et al. (1986) found that ergot alkaloid concentration in greenhouse-grown tall fescue was significantly higher with nitrogen fertilizer applications and was higher in leaf sheaths compared to leaf blades. Belesky et al. (1988) also found that ergot alkaloid concentrations in the field were highest in spring and autumn with a substantial decrease in concentration in midsummer. Soil phosphorus levels and drought stress also may influence ergot alkaloid concentration in the host (Are-

Table 2: Characteristics of selected *Epichloë* and *Neotyphodium* species

Endophyte spp.	Host grass	Host tribe	Geographic origin ^a	Closest nonhybrid relatives ^b	Alkaloids ^c					Antivertebrate activity	Anti-insect activity	Sources
					EV	EA	LA	ID	PM			
<i>Epichloë amarillans</i>	<i>Agrostis</i> , <i>Sphenopholis</i> , <i>Calamagrostis</i> spp.	Aveneae	NA	Eam	+	nt [§]	–	–	+	nt	+	Siegel et al. 1990; White 1994
<i>Epichloë baconii</i>	<i>Agrostis</i> , <i>Calamagrostis</i> spp.	Aveneae	NA	Eba	–	nt	–	nt	–	nt	nt	White 1993; Leuchtman et al. 2000
<i>Epichloë brachyelytri</i>	<i>Brachyelytrum erectum</i>	Brachyelytreae	NA	Ebe	nt	nt	nt	nt	nt	nt	nt	Schardl and Leuchtman 1999
<i>Epichloë bromicola</i>	<i>Bromus</i> spp.	Bromeae	EU	Ebr	–	nt	–	nt	++	nt	nt	Leuchtman and Schardl 1998; Leuchtman et al. 2000
<i>Epichloë clarkii</i>	<i>Holcus lanatus</i>	Poeae	EU	ETC	–	nt	–	nt	–	nt	nt	White 1993; Leuchtman et al. 2000
<i>Epichloë elymi</i>	<i>Elymus</i> spp.	Triticeae	NA	Eel	–	nt	–	–	+++	nt	+	Siegel et al. 1990; Schardl and Leuchtman 1999
<i>Epichloë festucae</i>	<i>Festuca</i> , <i>Lolium</i> spp.	Poeae	EU	Efe	+	+	+	+	+	+	+	Siegel et al. 1990; Leuchtman et al. 2000; Wilkinson et al. 2000
<i>Epichloë glyceriae</i>	<i>Glyceria striata</i>	Meliceae	NA	Egl	nt	nt	nt	nt	nt	nt	+	Cheplick and Clay 1988; Schardl and Leuchtman 1999
<i>Epichloë sylvatica</i>	<i>Brachypodium sylvaticum</i>	Brachypodieae	EU	ETC	–	nt	–	nt	–	nt	+	Leuchtman and Schardl 1998; Leuchtman et al. 2000; Brem and Leuchtman 2001
<i>Epichloë typhina</i>	Many	Aveneae, Brachypodieae, Poeae	EU	ETC	–	nt	–	–	+	nt	nt	Siegel et al. 1990; Leuchtman and Schardl 1998; Leuchtman et al. 2000
<i>Neotyphodium aotearoae</i>	<i>Echinopogon ovatus</i>	Aveneae	AU	Nao	–	–	+++	–	nt	nt	nt	Miles et al. 1998; Panaccione et al. 2001; Spiering et al. 2002
<i>Neotyphodium australiense</i>	<i>E. ovatus</i>	Aveneae	AU	Efe, ETC	nt	nt	nt	nt	nt	nt	nt	Moon et al. 2002
<i>Neotyphodium coenophialum</i>	<i>Lolium arundinaceum</i> (= <i>Festuca arundinacea</i>)	Poeae	EU, NAF	Eba, Efe, ETC	++	++	+++	–	+++	+	+	Cheplick and Clay 1988; Clay 1990b; Siegel et al. 1990; Christensen et al. 1993; Craven et al. 2001a

<i>Neotyphodium inebrians</i> ^d	<i>Achnatherum inebrians</i>	Stipeae	EU	Nin	–	+++	nt	–	nt	+	nt	Miles et al. 1996
<i>Neotyphodium lolii</i>	<i>Lolium perenne</i>	Poeae	EU	Efe	++	++	–	++	+++	+	+	Siegel et al. 1990; Christensen et al. 1993; Schardl et al. 1994
<i>Neotyphodium</i> sp. LpTG-2	<i>L. perenne</i>	Poeae	EU	Efe, ETC	+++	+++	–	–	+++	+	+	Christensen et al. 1993; Schardl et al. 1994
<i>Neotyphodium</i> sp. FaTG-2	<i>L. arundinaceum</i>	Poeae	EU, NAF	Eba, Efe	+++	nt	–	+	–	nt	–	Christensen et al. 1993; Schardl et al. 1994
<i>Neotyphodium</i> sp. FaTG-3	<i>L. arundinaceum</i>	Poeae	EU, NAF	Efe, ETC	–	nt	+++	–	+++	nt	+	Christensen et al. 1993; Schardl et al. 1994
<i>Neotyphodium melicicola</i>	<i>Melica decumbens</i>	Meliceae	SAF	Efe, Nao	nt	nt	nt	–	nt	+	nt	Moon et al. 2002
<i>Neotyphodium occultans</i>	Annual <i>Lolium</i> spp.	Poeae	EU	Eba, Ebr	–	–	+++	nt	nt	nt	nt	TePaske et al. 1993; Moon et al. 2000
<i>Neotyphodium siegelii</i>	<i>Lolium pratense</i> (= <i>Festuca pratensis</i>)	Poeae	EU	Ebr, Efe	–	–	+++	–	–	nt	nt	Craven et al. 2001a; K. Hignight (Advanta Seeds Pacific), personal communication
<i>Neotyphodium</i> sp. <i>Neotyphodium tembladerae</i>	<i>Achnatherum robustum</i>	Stipeae	NA	nt	–	+	+	nt	nt	+	nt	Petroski et al. 1992
	<i>Poa huecu</i>	Poeae	SA	Efe, ETC	nt	nt	nt	nt	+++	+	nt	Cabral et al. 1999; Moon et al. 2002
<i>Neotyphodium uncinatum</i>	<i>L. pratense</i>	Poeae	EU	Ebr, ETC	–	–	+++	–	–	+	–	Leuchtmann et al. 2000; Craven et al. 2001a

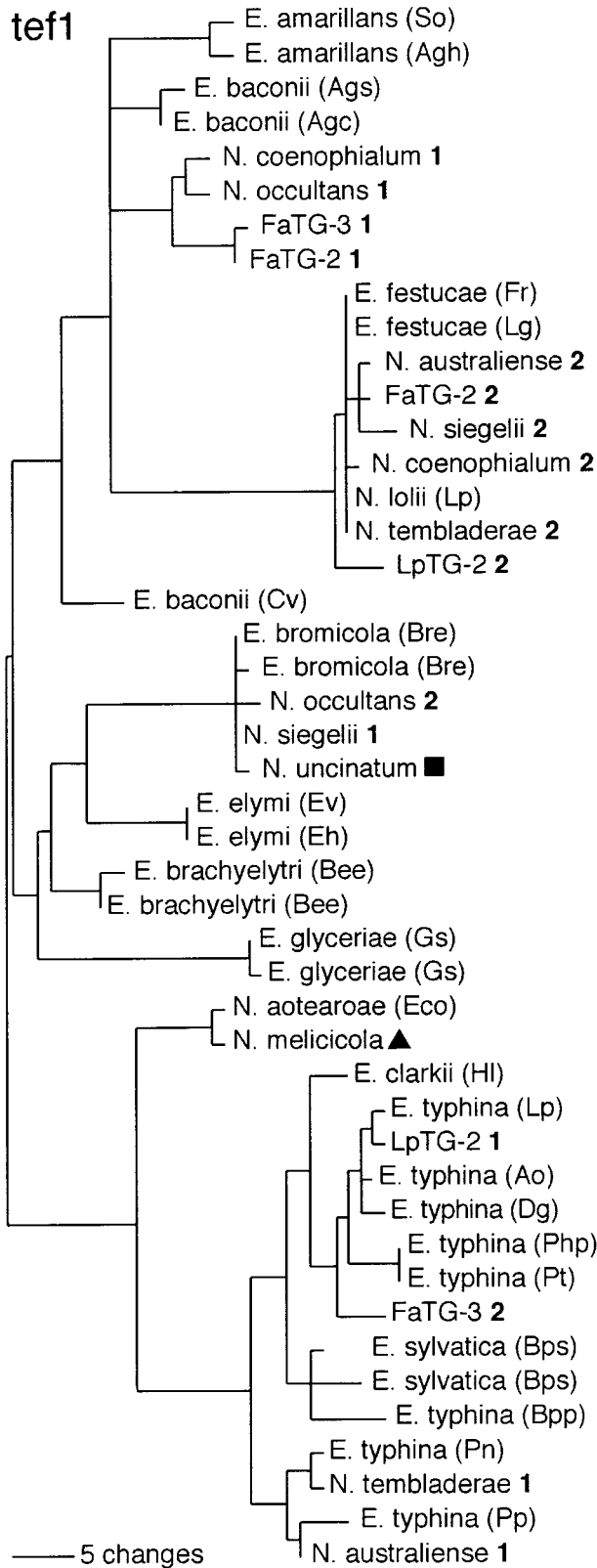
^a AU = Australasia, EU = Eurasia, NA = North America, NAF = North Africa, SA = South America, SAF = South Africa.

^b Eam = *Epichloë amarillans*, Eba = *E. baconii*, Ebe = *E. brachyelytri*, Ebr = *E. bromicola*, Eel = *E. elymi*, Efe = *E. festucae*, Egl = *E. glyceriae*, ETC = *E. typhina* complex, Nao = *Neotyphodium aotearoae*, Nin = *N. inebrians*.

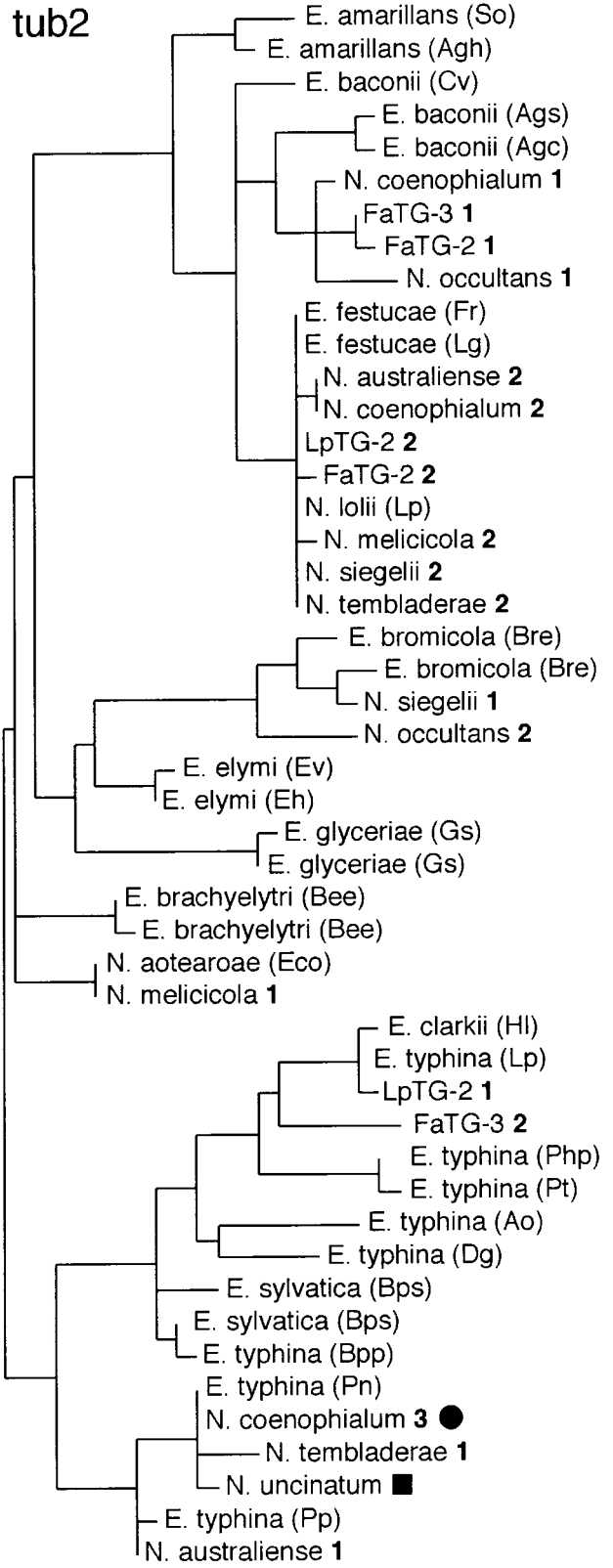
^c EV = ergovaline, EA = other (generally simpler) ergot alkaloids, LA = loline alkaloids, ID = indole-diterpenoids (including lolitrems), PM = peramine. – = undetected from any isolates tested, + = detected from some isolates, ++ = detected from most isolates, +++ = detected from all tested isolates, nt = not tested.

^d Tentative name; to be proposed elsewhere.

tef1



tub2



chavaleta et al. 1992; Malinowski et al. 1998). In a series of reciprocal inoculations where the same endophyte genotypes were introduced into different tall fescue genotypes (Hill et al. 1991), plant genotype had a major effect on alkaloid concentration in the plant. Reciprocal crosses between tall fescue genotypes associated with high and low alkaloid levels resulted in F1 progeny with intermediate levels (Agee and Hill 1994). Genetic modification of an endophyte to knock out the gene for ergopeptide lactam synthesis resulted normal host colonization but ergovaline-free infected plants (Panaccione et al. 2001).

Loline alkaloids are less widely distributed among endophyte-infected grasses than ergot alkaloids but are generally found in higher concentrations than other alkaloids (exceeding 1% of plant dry weight biomass; Siegel and Bush 1997). As with the ergot alkaloids, loline alkaloids vary with plant tissue, age, season, and water stress (Hardy et al. 1986; Bush et al. 1993; Siegel and Bush 1997). Lolitrems are tremorgenic neurotoxins primarily known from endophyte-infected perennial ryegrass where they cause the ryegrass staggers syndrome of sheep. Considerable research has been conducted in New Zealand where ryegrass staggers is a major economic problem. Lolitrems concentrations vary with tissue type and season, and inoculation studies indicate that endophyte genotype is the major determinant of alkaloid production, with a smaller effect of host plant genotype (Siegel and Bush 1997). Peramine, the only pyrrolopyrazine alkaloid found in endophyte-infected grasses, is more widely distributed in *Epichloë* and *Neotyphodium* host grasses than other alkaloids. Peramine has a relatively even distribution in the plant and over the growing season, and there is evidence of host genotype control of peramine concentrations (Siegel and Bush 1997).

All four classes of alkaloids have been produced by pure fungal cultures (Porter 1994; Blankenship et al. 2001) and are absent in uninfected grasses, clearly demonstrating their fungal origin. However, as discussed above, there is considerable evidence that the host plant can modify al-

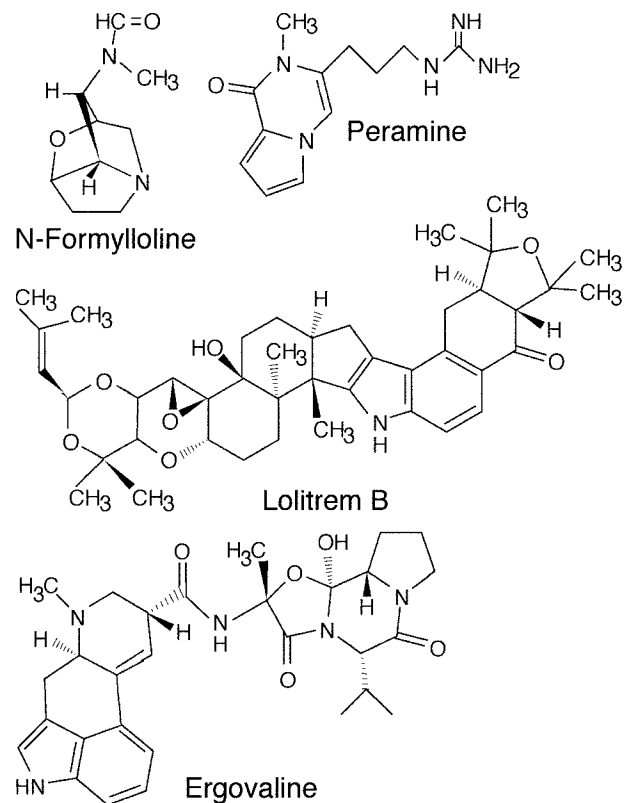


Figure 5: Examples of the four major classes of endophyte alkaloids. Lolitrems, peramine, N-acetyl loline, and ergotamine represent indole-terpenoid, pyrrolopyrazine, aminopyrrolizidine and ergot alkaloids, respectively. Individual infected grasses may have various mixtures of alkaloids.

kaloid expression. Many host species infected by single endophyte genotypes produce multiple classes of alkaloids. A study of tall fescue-*N. coenophialum* symbiotes indicated that alkaloids of different classes (peramine and ergovaline) can be independently regulated (Roylance et al. 1994), but in a study of perennial ryegrass-*N. lolii*, the

Figure 4: Gene trees generated by maximum parsimony search on aligned intron sequences of the *tef1* and *tub2* genes from *Epichloë* and *Neotyphodium* species. Only a single *tef1* and a single *tub2* gene sequence was identified in each *Epichloë* (sexual) isolate. Where multiple genes were identified in *Neotyphodium* (asexual) species, the different genes are represented by bold numerals after the endophyte species name. Squares represent single *tef1* and *tub2* genes in *Neotyphodium uncinatum* that show different relationships to different *Epichloë* species. This situation suggested that *N. uncinatum* was a hybrid, as confirmed by identification and phylogenetic analysis of its two actin genes (Craven et al. 2001a). The triangle indicates the single *tef1* gene in an endophyte that possesses two *tub2* genes, and the circle represents a third *tub2* gene in *Neotyphodium coenophialum* for which there is no corresponding third *tef1* gene. Host species for *Epichloë* isolates are abbreviated in parentheses as in figure 3. Host species for *Neotyphodium* species are indicated in the text. Three endophytes that have not yet been formally described are designated FaTG-2, FaTG-3, and LpTG-2, according to Christensen et al. (1993). Trees were drawn with the inferred midpoint root at the left edge. The *tef1* tree shown is one of 16 identified by heuristic search with branch-swapping by tree-bisection-reconnection; tree length = 210; consistency index (CI) = 0.8619; retention index (RI) = 0.9656; rescaled consistency index (RC) = 0.8323. The *tub2* tree shown is one of 16 identified by heuristic search with branch-swapping by tree-bisection-reconnection; tree length = 157; CI = 0.7898; RI = 0.9305, RC = 0.7349. These and all other most parsimonious *tef1* and *tub2* trees were concordant with the consensus tree shown in figure 3.

levels of peramine and lolitrem B correlated with each other and with density of endophyte hyphae (Ball et al. 1995). In a survey of 48 grass samples infected by a variety of *Epichloë* and *Neotyphodium* species, 43 (90%) produced at least one type of alkaloid (Siegel and Bush 1997). It should be noted that analytical techniques may not detect certain alkaloids and that other undiscovered groups of endophyte alkaloids may exist (Jones et al. 2000; Brem and Leuchtman 2001). No plant-endophyte combination producing all four classes of alkaloids has been found. However, five grass-endophyte symbiota had three alkaloid classes, 19 contained two, and 19 contained only one type. Thus, alkaloid toxins are widely distributed in endophyte-infected host plants but with considerable variation in profiles and concentrations, and they are under the influence of plant genotype, tissue, age, and growing conditions.

Alkaloids and Herbivores

Much attention has been focused on endophyte alkaloids because they represent a potential mechanism of ecological benefit to their hosts and an important economic problem in agricultural systems. The intense research focus on endophyte-plant interactions over the past 25 yr was largely stimulated by the discovery that a common toxic syndrome of cattle grazing tall fescue grass and of sheep grazing perennial ryegrass was strongly correlated with endophyte infection (Bacon et al. 1977; Latch 1993). However, additional examples of other endophyte-infected grasses causing livestock toxicity continue to be reported (Miles et al. 1996, 1998; Cabral et al. 1999). For example, the Chinese grass *Achnatherum inebrians* (drunken horse grass), infected by a *Neotyphodium* endophyte, is rapidly increasing its geographic range and frequency of afflicted livestock (Miles et al. 1996). Indigenous livestock rarely eat the grass because they have learned to avoid it, but naive animals are frequent victims of intoxication or death. Endophyte-infected *Echinopogon* species native to Australia are reported to cause stock poisonings very like those seen with perennial ryegrass (Miles et al. 1998).

Endophyte infection can be an impediment to livestock production but chemical effects are not limited to domestic animals. Wild herbivores such as voles and rabbits are negatively affected by endophyte-infected tall fescue (Giuliano et al. 1994; Fortier et al. 2000). Conover and Messmer (1996) found that Canada geese grazing infected tall fescue plots lost body mass relative to control birds grazing uninfected tall fescue and exhibited a preference for uninfected plots after experience grazing infected plots. Conover (1998) also found a similar situation with meadow voles where initially they did not discriminate but after a period of feeding they preferred uninfected plants. In a study with five passerine birds, four species significantly preferred

endophyte-free tall fescue seeds in choice tests (the fifth species also preferred uninfected seeds in a 2 : 1 ratio), and average weight loss was strongly correlated with the amount of infected seed in their diet (Madej and Clay 1991). Several rodent species also suffer various ill effects from consuming infected versus uninfected tall fescue seed in laboratory settings (Clay 1991). Detrimental effects of endophyte-infected grasses on wild vertebrates are harder to observe because animals are apt to move and select alternative food plants, unlike captive livestock.

Many insect herbivores are also sensitive to endophyte alkaloids in grasses as well (Clay et al. 1985; Rowan et al. 1986; Latch 1993; Brem and Leuchtman 2001). Heterogeneity in responses to infected grasses exists, often in relation to the particular alkaloid constitution of the infected grasses. For example, Siegel et al. (1990) and Wilkinson et al. (2000) found that two aphid species differed in their response to particular grass-endophyte combinations differing in alkaloid profile. Several insects were unaffected by the endophyte of Arizona fescue, but strongly affected by the endophyte of tall fescue (Knoch et al. 1993; Lopez et al. 1995). Siegel and Bush (1997) did not report any alkaloids from *Festuca arizonica* whereas infected tall fescue (*Festuca arundinacea*) produced three of the four known classes of endophyte alkaloids.

Different alkaloid classes may have different effects depending on the herbivore. Ergot alkaloids are primarily active against vertebrate herbivores, although some activity against insects has been documented (Bush et al. 1997). Symptoms of livestock toxicity are similar to the known pharmacological activity of these alkaloids (e.g., vasoconstriction, reproductive difficulties; Plowman et al. 1990). Reproductive inhibition of prairie voles has also been demonstrated (Fortier et al. 2000). There is no evidence of any inherent resistance by vertebrate herbivores to ergot alkaloids, suggesting that consumption of any infected grass with significant alkaloid concentration will result in toxicity and/or avoidance. Lolitrems are also primarily associated with mammalian toxicity, particularly of herbivores grazing endophyte-infected perennial ryegrass. In contrast, the primary effect of peramine is as an insect feeding deterrent; for example, the Argentine stem weevil, a major pasture pest in New Zealand, is extremely sensitive to the presence of peramine in host grasses (Rowan et al. 1986). Loline alkaloids may have some activity against vertebrates but are most active as toxins and feeding deterrents for a range of insect herbivores (Bush et al. 1997).

It has been suggested that strong antiherbivore effects of endophyte infection are limited to a small number of agronomic grasses subject to strong artificial selection (Saikkonen et al. 1998). Most research on grass/endophyte interactions has been conducted with tall fescue or perennial ryegrass, reflecting both their economic importance

and extensive range. But published reports on antiherbivore effects of endophyte infection in a wide range of grasses, both cultivated and wild, suggest that, instead, the alkaloid chemistry of host grasses and not their history of domestication is the primary determinant of antiherbivore effects. In the absence of bioassays, alkaloid identity and concentration in host tissues may provide a useful surrogate for toxic effects on herbivores.

Alkaloids and Hybrids

Many asexual, seed-transmitted endophytes are of hybrid origin: 19 of 29 species examined thus far have clear evidence of hybridization (table 2; C. Schardl, unpublished data). Further, most examples of highly toxic grasses are infected by hybrid endophytes. Surveys suggest that asexual, seed-transmitted endophytes produce more types of alkaloids at higher concentrations than sexual endophytes (Siegel et al. 1990; Siegel and Bush 1997; Leuchtman et al. 2000; Craven et al. 2001b). Does hybridization lead to the proliferation of alkaloid-production genes among asexual endophytes?

No ergot alkaloids are produced by *Cordyceps* pathogens of insects and fungi, but the grass-infecting species of the family Clavicipitaceae are broadly capable of ergot alkaloid production, including clades associated with C3 grasses (*Claviceps*, *Epichloë*, *Neotyphodium*) and with C4 grasses (*Balansia* and *Myriogenospora*; Glenn and Bacon 1997). The majority of hosts in the *Epichloë/Neotyphodium* clade contained ergovaline or other ergot alkaloids (Siegel et al. 1990; Siegel and Bush 1997). Peramine was also widely distributed but lolitrem B was primarily associated with endophyte-infected *Lolium* species (Siegel et al. 1990; Siegel and Bush 1997). None of these alkaloids appear to be associated with hybridization.

In contrast, loline alkaloids suggest an important role for hybridization in the evolution of bioprotective endophytes. Lolines are found almost exclusively with grasses infected by *Neotyphodium* endophytes, and they exhibit broad-spectrum insecticidal activity (Bush et al. 1997; Siegel and Bush 1997; Wilkinson et al. 2000). While mammalian sensitivity to lolines has been noted, concentrations of loline alkaloids found *in planta* are generally not toxic to mammalian herbivores.

Siegel et al. (1990) found that some endophytes produced high concentrations of lolines (>1,000 ppm). We now know that most of these endophytes are hybrids. In a survey of European grasses, Leuchtman et al. (2000) found that lolines were produced only in symbioses with *Epichloë festucae* or *Neotyphodium* endophytes. Recently, Craven et al. (2001b) found that endophytes associated with meadow fescue, *Lolium* (= *Festuca*) *pratense*, were

primarily hybrids producing high levels of loline alkaloids (up to 20,000 ppm).

As the number of known endophyte species has grown over the past decade, further surveys of alkaloid production (TePaske et al. 1993; Leuchtman et al. 2000; and see reviews by Bush et al. 1997; Lane et al. 2000) have revealed an interesting pattern (table 2). Peramine is produced by many of the sexual (*Epichloë*) and asexual (*Neotyphodium*) species. In contrast, the ergopeptine alkaloid, ergovaline is produced by only a single sexual species, *E. festucae*. Likewise, *E. festucae* is the only sexual species known to produce lolitrem B or lolines. No single isolate of *E. festucae* produces all four alkaloids, and this genetic variation in alkaloid profiles has made it an ideal genetic system to demonstrate that loline alkaloid expression is controlled by a single genetic locus in *E. festucae* crosses (Wilkinson et al. 2000). The locus contains genes that are unique to loline alkaloid-producing endophytes (Spiering et al. 2002).

Given these observations, the widespread occurrence of all four alkaloid classes among asexual endophyte species, and the hybrid origins of many such endophytes, it is reasonable to speculate that *E. festucae* may have been the ancestor that contributed genes for synthesis of ergovaline, lolitrem B, and lolines. This expectation seems valid for producers of ergovaline or lolitrem B, but not for loline alkaloid producers. For example, *N. occultans*, *N. uncinatum* and *N. aotearoae* all produce lolines but in none of these species are housekeeping genes, *tub2*, *tef1* and *act1*, derived from *E. festucae* (see fig. 4). It is possible that hybridization with *E. festucae* occurred in their ancestry, but other *E. festucae* genes were lost while loline-production genes were retained. Alternatively, an *E. festucae* lineage may have acquired loline genes by hybridization with another species, but subsequent loss of most redundant chromosomes may then have so thoroughly reconstituted the *E. festucae* genome as to permit its sexual expression. Thus, *E. festucae* and/or other endophytes may have acquired alkaloid production genes by horizontal transfer (Prade et al. 1997; Walton 2000).

Selection for Hybrids and Hybridization

Interspecific hybridization appears rare in fungi outside of *Neotyphodium* endophytes where evidence for hybridization is frequent and dramatic. Are asexual grass endophytes particularly prone to hybridization, or does their particular ecological niche favor hybrids once they occur?

A significant barrier to interspecific hybridization among fungi is vegetative incompatibility. In most fungal species, allelic identity at several loci, designated *vic* or *het* loci, is a prerequisite for hyphal fusion and generation of heterokaryotes (Glass and Kuldau 1992; Leslie 1993). *Magnaporthe grisea* is an exception in that it is reported to lack

such a vegetative incompatibility system (Crawford et al. 1986). To test for vegetative incompatibility in sexual *Epichloë* species, Chung and Schardl (1997b) paired nitrate-utilization mutants on culture plates with nitrate as the sole nitrogen source. When the mutations affected different loci, they complemented each other even when the mutants were of different species. No vegetative incompatibility was evident and fusion of hyphae and formation of heterokaryons is readily accomplished by *Epichloë* species. However, hybrid *Neotyphodium* species are not only heterokaryotic but also have hybrid nuclei (Schardl et al. 1994; Tsai et al. 1994; Kulda et al. 1999; Craven et al. 2001b), and no such hybrids were generated in these complementation tests. Furthermore, in coinfections the endophytes tend to segregate from one another on a tiller-by-tiller basis (Meijer and Leuchtman 1999; Wille et al. 1999). Nevertheless, Christensen et al. (2000) found coinfecting tillers several months after establishing superinfection, but no nuclear hybrids were obtained (W. Hollin and C. L. Schardl, unpublished data). Nuclear hybridization is clearly not an inevitable consequence of two endophytes coinfecting the same plant.

The large number of hybrid endophytes identified with limited surveys suggests that hybrids are positively selected, at least in some circumstances. Two likely bases for selection include the pyramiding of favorable genes and the counteracting of Muller's ratchet. By acquiring several favorable characteristics from multiple endophyte ancestors, a hybrid endophyte has higher fitness than its nonhybrid ancestors. As described, asexual endophytes tend to have greater variation in alkaloid profiles than *Epichloë* species, and expression of two or three different alkaloid classes is much more frequent. An interesting counterexample is *N. lolii*, a nonhybrid asexual species that produces three of the four alkaloid classes. However, this endophyte is derived from the only sexual species (*Epichloë festucae*) known to produce all of these alkaloid types. Indeed, *E. festucae* contributes to seven of 10 interspecific hybrids characterized to date (fig. 4). The three hybrids that lack an *E. festucae* contribution all produce insecticidal loline alkaloids. These observations constitute circumstantial evidence that asexual endophytes with strong antiherbivore activities are favored by selection. Adaptive alkaloid-production genes may proliferate among asexual endophytes similar to antibiotic-resistance genes among bacteria. Further, given the gene duplication inherent in the hybridization process, asexual endophytes may accumulate, or pyramid, alkaloid-production genes, allowing higher alkaloid expression in host plants.

Another potential factor selecting for interspecific hybrids, not mutually exclusive with the first, is Muller's ratchet, the accumulation of marginally deleterious mutations in clonal lineages (Muller 1964; Lynch et al. 1993;

Moran 1996; Bidochka 2001). Such mutations can be eliminated more effectively if genomes recombine, at least occasionally (Felsenstein 1974). The best known, and most organized, means of genome recombination in eukaryotes is by meiosis in a sexual life cycle. However, parasexual processes are well known in some fungi (Caten 1981). Current evidence suggests that endophyte hybridization is a parasexual process (Tsai et al. 1994). It is also noteworthy that the only demonstrated means of contagious spread hosts in the *Epichloë* clade is via sexually derived spores (Chung and Schardl 1997a; Brem and Leuchtman 1999). Thus, an asexual endophyte that accumulates deleterious mutations may gain a full complement of functional house-keeping genes by hybridization with a sexual *Epichloë* species.

Muller's ratchet may be difficult to demonstrate in endophytes. In the apparently asexual fungal symbionts in arbuscular mycorrhizae, tests of the ratios of synonymous and nonsynonymous mutations support the action of Muller's ratchet (Kuhn et al. 2001). But *Neotyphodium* species are too closely related to *Epichloë* species to make this type of analysis practical. Further surveys of endophytes could provide information as to the relative ages of genomes in hybrid versus nonhybrid asexuals. Based on Muller's ratchet, nonhybrid, asexual endophytes are predicted to be phylogenetically closer to sexual species than hybrids. Consistent with this is the relationship of the tall fescue and annual ryegrass endophytes, all of which have a set of genes (designated copy 1 in fig. 4) that are not closely related to those of any *Epichloë* species. These may represent ancient asexual lineages that have survived this long because of their ability to hybridize with a variety of *Epichloë* species: *E. festucae* in the cases of *N. coenophialum* and FaTG-2, *E. typhina* in the cases of *N. coenophialum* and FaTG-3, and *E. bromicola* in the case of *N. occultans*. Also suggestive of Muller's ratchet is the observation that most hybrid endophytes (excepting *N. occultans*) grow faster in culture than nonhybrid asexual species.

Other Effects of Endophytes on Host Plants

Our focus has been on the production of alkaloids and their role as defensive compounds as a mechanism for mutualism in the grass/endophyte symbiosis. But endophyte infection can provide other benefits to their hosts besides protection from herbivory, whether they produce alkaloids or not.

Protection against plant pathogens is another possible benefit of endophyte infection. For example, infected tall fescue is more resistant to seedling blight caused by *Rhizoctonia* (Gwinn and Gavin 1992) and crown rust caused by *Puccinia coronata* relative to uninfected plants (Ford and Kirkpatrick 1989). Mahmood et al. (1993) found that the

incidence of barley yellow dwarf virus was twice as frequent in uninfected versus infected tall fescue, reflecting deterrence of aphid vectors of the virus. The wetland grass *Panicum agrostoides* had lower levels of infection by *Alternaria triticina* when infected by *Balansia henningsiana* (Clay et al. 1989), while *Epichloë*-infected timothy (*Phleum pratense*) plants were resistant to *Cladosporium phlei* (Shimanuki 1987). Inhibition of plant pathogens in pure cultures by endophyte isolates has been documented (Siegel and Latch 1991; Stovall and Clay 1991), as has enhanced resistance to nematodes (West et al. 1988; Kimmons et al. 1990). Endophyte infection has no effect on resistance to some pathogens (Welty et al. 1991; Burpee and Bouton 1993) but, as with herbivory, blanket resistance to all pathogens is not required to provide some benefit to host plants.

Enhanced drought tolerance is a well-known benefit of endophyte infection in tall fescue and perennial ryegrass (Arechavaleta et al. 1989; Bouton et al. 1993; Lewis et al. 1997; Malinowski et al. 1997a; but see Cheplick et al. 2000). For example, Lewis et al. (1997) examined 523 perennial ryegrass populations from 15 European countries and found that infection frequency was significantly related to water-supply deficit, an indicator of drought stress. The most highly infected populations were located in Mediterranean regions subject to summer drought stress. West et al. (1993) found that the growth advantage of infected tall fescue over uninfected fescue in experimental plots was significantly higher in water-stressed versus well-watered plots. Greater drought tolerance may result from altered stomatal behavior and osmotic adjustment, leading to better turgor maintenance (West 1994). Siegel and Bush (1997) suggested that the accumulation of loline alkaloids could affect osmotic potential and, therefore, drought stress. Increased tolerance to other environmental stresses like heat (Marks and Clay 1996), low light (Bier 1995), and low soil fertility (Malinowski and Belesky 2000) have also been reported.

Enhanced growth of infected plants, independent of apparent biotic or abiotic stresses, has been found in many grass/endophyte systems (Clay et al. 1989; Rice et al. 1990; Malinowski et al. 1997b). For example, 15 of 17 studies with tall fescue found increased productivity of infected plants relative to uninfected plants (Clay 1997). However, infected plants may grow more poorly under some nutrient-poor conditions (Cheplick et al. 1989) and in some Type I associations, there is no effect or a negative effect on host growth (Clay 1990a, 1990b; Pan and Clay 2002). However, most studies, albeit from a few widespread species, point to an invigorating effect of endophyte infection on plant growth.

Endophyte infection causes morphological changes in many host species that result in increased clonal growth. These changes may be adaptive when seed production is

limited or where seedling establishment is rare, as in heavily grazed systems. Diehl (1950) and Bradshaw (1959) noted the tendency toward greater lateral spread by infected species of *Cenchrus* and *Agrostis*, respectively. The sedge *Cyperus rotundus* produced significantly more subterranean bulbils when infected by *Balansia cyperi* (Stovall and Clay 1988) and *C. virens* exhibited proliferation of viviparous plantlets when infected by the same fungus (Clay 1986). Recently, Pan and Clay (2002) demonstrated that clones of the wetland grass *Glyceria striata* were significantly more stoloniferous when infected by *E. glyceriae*, and that this effect disappeared when the fungus was eliminated by fungicide applications (J. Pan and K. Clay, unpublished data). In all of these cases, infection causes inflorescence abortion (Type I), so increased clonal growth could reflect resource reallocation away from flowering. However, De Battista et al. (1990b) also found increases in rhizome production in tall fescue where the endophyte is completely seed transmitted.

The underlying mechanisms for these changes may include biochemical and hormonal alterations to host plants by endophyte infection. In tall fescue, enhanced photosynthetic rates with endophyte infection are known (Richardson et al. 1993; Marks and Clay 1996). Photosynthetic rate could reflect differences in water relations and stomatal behavior, or changes in photorespiration (Marks and Clay 1996). The endophytes of tall fescue and other grasses produce auxin (Porter et al. 1985; De Battista et al. 1990a), which can influence a variety of morphological and physiological responses. Additionally, fungal endophytes are sinks for sucrose and convert it rapidly into sugar alcohols (Thrower and Lewis 1973; Smith et al. 1985), possibly reducing feedback inhibition of photosynthesis, thereby allowing greater growth than uninfected plants.

Endophyte infection entails physiological costs that need to be outweighed by benefits in a mutualistic relationship. Endophytes must obtain all of their nutrients from host plants, including precursors in the synthesis of secondary metabolites. Reduced growth of infected tall fescue under low-nutrient conditions (Cheplick et al. 1989) and the association of infected *Danthonia spicata* with higher soil ammonia patches (McCormick et al. 2001) may reflect the greater resource demand of infected plants.

Population Dynamics of Endophyte Infection

Population dynamics of infection within populations provide insights into the selective pressures acting on endophyte symbiosis. In Type III interactions with complete seed transmission, changes in infection frequency within populations directly reflect the relative fitness of infected versus uninfected plants. Seed-transmitted pathogens should decrease in frequency in the host population while

mutualistic endophytes should increase. Host populations with 100% infection frequency are common (Clay 1997). Completely uninfected host populations should remain so, barring long-distance seed dispersal from another population. In between are mixed populations where, besides infection frequency, we wish to know whether this value is changing and in what direction. Population dynamics of infection in Type I and Type II associations with contagious spread are not so straightforward.

Three types of data are available. Static measures of infection frequency in host populations provide a slice-in-time view without information on trajectory. Surveys of herbarium specimens for endophyte infection have proven useful for identifying host species and prevalence within a species (White 1987; Clay and Leuchtman 1989). Comparative data on infection frequency in host populations of differing age assume that on average this is the only factor that differs. Longitudinal data on infection dynamics are obtained from following populations over time.

One-time estimates of infection frequency have revealed variable levels of infection within and among species. In a study of native and naturalized fescues, Spyreas et al. (2001b) found that in southern Illinois two native species (*Festuca paradoxa* and *Festuca obtusa*) were nearly 100% infected, while the introduced *Festuca rubra* was infection-free. Populations of tall fescue in Illinois and England exhibited variable infection rates. Many were at or near 100% infection while a smaller number were endophyte-free. Saikonen et al. (2000) found that populations of tall fescue in Finland were all highly infected (96%–100%). Over 95% of collections from northern Africa and Sardinia were infected with a mean infection rate per collection of 77% (Clement et al. 2001). Vinton et al. (2001) recently reported that virtually all populations of *Elymus canadensis*, a native prairie grass, were 100% infected. In Europe 62% of 523 perennial ryegrass populations contained some infected plants with more populations between 1% and 50% infection than between 50% and 100% infection (Lewis et al. 1997). Several other studies report infection frequencies in endophyte host populations (Clay and Leuchtman 1989; Bazely et al. 1997; Miles et al. 1998; Schulthess and Faeth 1998; Zabalogeazcoa et al. 1999).

A compilation of published studies from many host species (Clay 1997) revealed that in many species and/or populations, infection frequency was 100%, suggesting a selective advantage for infection; in most other cases, infection frequencies were high (50%–99%); and infection frequencies were generally higher in Type III, seed-transmitted endophytes than in sexual, contagiously spread endophytes. The symbiosis is worldwide in distribution and new host species are continually being reported. For example, infection by *Neotyphodium* endophytes was recently reported in two wild wheat (*Triticum*) species from

Turkey (Marshall et al. 1999). Our knowledge of the distribution of endophytes in grasses is incomplete. Whereas Aldous et al. (1999) reported that endophytes in the genus *Neotyphodium* are not found in Australian native grasses, Miles et al. (1998) and Moon et al. (2002) reported two *Neotyphodium* species from Australian grasses. We know that many species do not serve as hosts and uninfected species may be underreported. Most grass species have probably never been examined. We cannot say at present what proportion of grasses serve as endophyte hosts except that it is substantial.

There is also considerable variation in infection frequency within host species, raising the question of whether intermediate infection frequencies are transient or stable. Relatively few data are available on changes over time in endophyte infection frequency and most comes from tall fescue and perennial ryegrass. Comparative studies of perennial ryegrass pastures in Tasmania and the United Kingdom suggest that infection frequencies rise over time. Both Lewis and Clements (1986) and Cunningham et al. (1993) found strong trends for older pastures to have higher infection rates than younger pastures. Tasmanian pastures planted with 80% infected seeds increased to 100% in 4 yr (Cunningham et al. 1993). In New Zealand, Francis and Baird (1989) documented rapid increases in infection frequency in recently sown pastures. In one variety of ryegrass, infection went from 3% to 67% in 3 yr while another variety went from 3% to 83% in the same period. Infection frequencies in several Type I associations also tend to increase with host population age (Diehl 1950; Bradshaw 1959). For example, increases in infection by *Epichloë typhina*, which aborts host inflorescences, have been reported in seed fields of *Dactylis glomerata* (Large 1952; Pfender and Alderman 1999).

Several studies with tall fescue suggest gradual increases in frequency over time (Thompson et al. 1989; Read and Walker 1990; Shelby and Dalrymple 1993). In Alabama, Shelby and Dalrymple (1993) followed infection frequency changes over 4 yr in experimental plots sown with known frequencies of infected seed. Plots sown with endophyte-free seed remained uninfected as expected, whereas infection increased in all other plots, with the greatest rate of increase in plots with the lowest initial infection level. More rapid changes in infection frequency occurred in southern Georgia when uninfected fescue suffered complete mortality over one summer during a severe drought while infected fescue persisted well (Bouton et al. 1993).

In a recent study, 60 experimental plots (5 × 5 m) were each sown with a 50 : 50 mix of infected and uninfected tall fescue seed. Different treatments were applied to examine the role of herbivory and infection dynamics (K. Clay, unpublished data). Some variation in the initial infection frequency of established seedlings occurred due to

random chance and/or sampling error. Tiller samples were then taken from each plot in the spring and fall ($N = 50\text{--}100$) for 54 mo and scored for infection. The results are illustrated in figure 6. All experimental treatments are combined. If there were no changes in infection frequency, all plots would fall along the diagonal line. A few plots decreased in estimated infection frequency, but the large majority (51 of 60) increased in infection frequency (fig. 6). Overall, there was a highly significant increase in infection frequency of nearly 20% ($P < .01$). These data are consistent with previous studies, showing that infection rates increase over time, as expected with a beneficial, seed-transmitted symbiont. It is not known whether this dynamic would eventually lead to 100% infection or whether the rate of increase would slow as infection frequencies became higher. We are not aware of any studies documenting consistent decreases in Type III infection frequency over time. The population dynamics of Type I or Type II infections will reflect their ability for both vertical and horizontal spread, and they may exhibit density dependence, unlike Type III associations.

Populations of seed-transmitted endophytes with intermediate infection frequencies may reflect transient dynamics toward complete infection or loss of infection, or, alternatively, evidence of selection for or against infection under some environmental conditions. Endophyte infection might be most highly selected during periods of intense stress, such as during an insect outbreak or extreme drought stress, leading to rapid fixation (see Francis and Baird 1989; Bouton et al. 1993). If vertical transmission is relatively high and infection provides some minimal benefit, or minimal cost, during nonstress periods, infection may be maintained until it becomes highly selected once again. Intermediate infection frequencies could also represent imperfect vertical transmission to seeds (Ravel et al. 1997). However, pure infected seed stocks are widely available (K. Clay, personal observation). Long-term seed dormancy could result in reduced infection rates since endophyte viability in dormant seeds decreases more rapidly than seed viability itself (Latch 1983; Rolston et al. 1986), but seedlings rarely become established in dense swards. One further possibility, hinted at by the results of Shelby and Dalrymple (1993), is frequency-dependent selection where the advantage of infection decreases with an increasing infection rate. This would support a biotic stress mechanism of mutualism rather than an abiotic stress mechanism. In a stand where 90% of the grasses are endophyte-infected, herbivores might sample a few and then decide to forage elsewhere, leading to associational resistance of uninfected plants to herbivory without cost. It is not obvious how a similar benefit to uninfected plants would arise from greater drought tolerance of infected plants, for example.

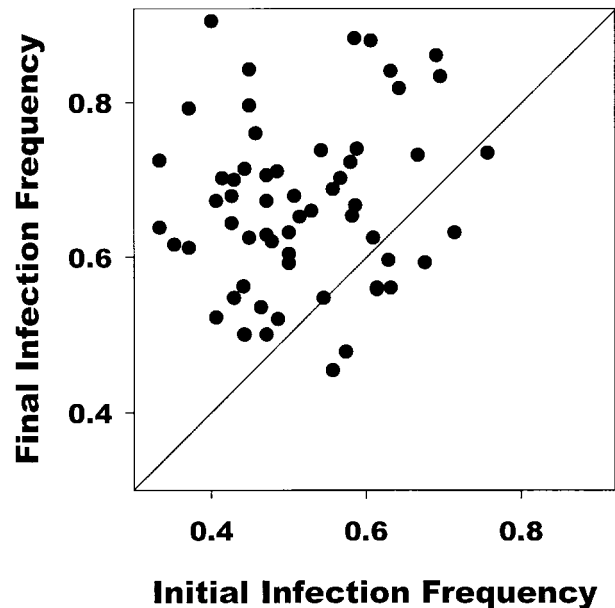


Figure 6: Change in infection frequency in tall fescue over a 4-yr period. Sixty plots were repeatedly sampled. Each point represents starting and final values for one plot. Diagonal line represents no change in infection frequency (K. Clay, unpublished data).

Community and Ecosystem Consequences of Endophyte Infection

Endophyte infection affects the biochemistry and physiology of host plants and these changes may have cascading effects on co-occurring plant species and their consumers. Grasses and grasslands are dominant features of our landscape and some endophyte symbioses potentially have large-scale consequences. For example, there are 18,000,000 ha of tall fescue grass-dominated grasslands in the eastern United States (Ball et al. 1993).

Greenhouse experiments suggest that endophyte infection can alter competitive interactions with other plants (Cheplick et al. 1989; Marks et al. 1991; Malinowski et al. 1997b). Altered interactions with co-occurring plant species could influence the structure and dynamics of vegetation. Funk et al. (1983) found that infected ryegrass maintained greater cover in turf plots and was less heavily invaded by weeds compared to uninfected ryegrass. Similarly, white clover (*Trifolium repens*) was competitively suppressed by perennial ryegrass in infected pastures but not in uninfected pastures, making endophyte-infected grass/clover mixtures difficult to sustain (Sutherland and Hoglund 1990). Other studies have found no effect of endophyte symbiosis on the ryegrass/clover interaction (Lewis and Clements 1990). In surveys of perennial ryegrass in Europe, infection frequency was significantly cor-

related with the abundance of ryegrass in the stand (Lewis et al. 1997), suggesting that more highly infected ryegrass populations better exclude competitors.

In large field plots sown with either infected or uninfected tall fescue seed, infected fescue was more dominant and suppressed other grasses and dicots relative to uninfected fescue, which was largely replaced by other grass species over a 4-yr period (Clay and Holah 1999). Species richness was significantly lower in infected versus uninfected plots (fig. 7). Especially noteworthy was the near-complete exclusion of forbs from infected plots and the more rapid rate of invasion by woody plants into uninfected plots. However, there was no difference in overall productivity between plot types (Clay and Holah 1999). After 7 yr, total basal area of woody plants was over 10 times higher on average in uninfected plots (K. Clay, unpublished data), suggesting that uninfected plots were undergoing secondary succession more rapidly. In another recent study, Spyreas et al. (2001a) examined patterns of diversity in tall fescue grasslands subject to mowing and fertilizer treatments. There was no overall relationship between species richness and endophyte-infection frequency but species richness decreased with infection frequency in unmowed plots and increased with infection frequency in mowed plots. These results indicate that the relationship between endophyte infection and diversity may be influenced by environmental factors like mowing. Grazing is likely to be more selective than mowing.

Changes in vegetation may reflect interacting effects of competition and herbivory. For example, Watson et al. (1993) found that white clover yields were higher in uninfected ryegrass pastures due to greater herbivore damage to the grass. In infected pastures there was less damage to ryegrass and less clover. In greenhouse studies, the outcome of competitive interactions among several grass species was influenced by herbivory and endophyte infection (Clay et al. 1993). For example, infected perennial ryegrass was also a superior competitor with Kentucky bluegrass (*Poa pratense*) compared to uninfected ryegrass, but only with insect herbivory.

Endophyte infection could affect the total numbers or biomass of consumers, the number of species, and/or the herbivore assemblage by making plant tissues less attractive or less nutritious. Potential consumers also include plant pathogens. The magnitude of these effects will vary with the relative abundance of infected plants in the community, the degree of host specificity exhibited by herbivores, and the alkaloid chemistry of the endophyte. In agricultural systems, endophyte-infected tall fescue and perennial ryegrass support less consumer biomass in the form of dairy and meat production than uninfected grasslands (Hoveland 1993). A similar relationship may hold in unmanaged situations. Regional declines in the abun-

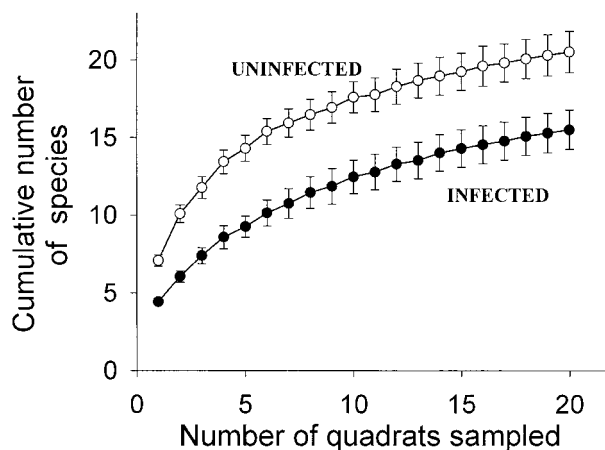


Figure 7: Species-area curve for infected versus uninfected tall fescue plots in year 4 (1998) of field experiment (see Clay and Holah 1999). Mean cumulative species number per plot (\pm SE among plots) is shown. A random subsampling procedure was repeated 500 times for each plot. One quadrat per plot was selected at random, and number of species was determined. Then a second quadrat was selected at random, and the cumulative number of species in both quadrats was determined, and so forth, through 20 quadrats.

dance of rabbits has been attributed to increasing density of endophyte-infected tall fescue in grassland habitats (Giuliano et al. 1994), and fewer small mammal species may be found in areas of highly infected versus uninfected tall fescue (Coley et al. 1995). Bazely et al. (1997) suggested that periodic population crashes of feral sheep in the St. Kilda Islands of Scotland might result from high levels of endophyte infection in red fescue *Festuca rubra*. In an experimental study, sex ratio and the reproductive status of female prairie voles was significantly affected by endophyte infection in field plots of tall fescue (Fortier et al. 2000). Laboratory studies with several passerine birds found strong avoidance of endophyte-infected tall fescue seeds in choice tests, and reduced survival and weight gain in nonchoice tests (Madej and Clay 1991). All of these cases are consistent with local or regional declines in the numbers of vertebrate herbivores.

Insect numbers and/or species richness may be influenced by endophyte infection of grass species as well. Loline alkaloids have broad-based anti-insect effects while peramine is also an antifeedant to some species (Siegel et al. 1990; Siegel and Bush 1997). Laboratory feeding and choice studies have indicated strong effects of endophyte infection in some systems (Clay et al. 1985; Latch 1993; Brem and Leuchtman 2001). However, no apparent effects of infection have been found with other grass and/or insect species (Lewis and Clements 1986; Lopez et al. 1995). In field studies, Funk et al. (1983) found signifi-

cantly fewer turfgrass insect pests in plots with infected perennial ryegrass compared to uninfected ryegrass. Similarly, in New Zealand pastures dominated by endophyte-infected ryegrass there were significantly fewer Argentine stem weevils compared to uninfected pastures (Latch 1993). Omacini et al. (2001) found that field plots of infected Italian ryegrass (*Lolium multiflorum*) supported fewer *Rhopalosiphum padi* aphids than uninfected plots but there were no differences in numbers of *Metopolophium festucae* aphids. No data on alkaloid profiles were reported but the results were similar to those of Siegel et al. (1990) where differential response of two aphid species reflected their sensitivities to loline and peramine alkaloids. In 20 fields of tall fescue in Missouri with different infection frequencies, three leafhopper and one beetle species had reduced abundances with increasing endophyte infection while one leafhopper and one isid species were positively correlated with endophyte-infection level (Kirfman et al. 1986). Experimental tests or field samples of the herbivore fauna on endophyte-infected versus uninfected conspecifics are needed for a wider range of species.

Changes in the abundance or richness of primary consumers could affect the structure of the secondary consumer (predator or parasitoid), but few studies have addressed this issue. Bultman et al. (1997) fed fall armyworm larvae (*Spodoptera frugiperda*) foliage from infected or uninfected tall fescue, and then allowed two parasitoid species to parasitize the larvae. Endophyte infection resulted in reduced pupal mass of the parasitoids, but had no effect on developmental time or survival. Pure loline alkaloids added to artificial armyworm diet also reduced parasitoid survival. In another study with insect herbivore/parasitoid food chains, Omacini et al. (2001) found that endophyte infection of the food plant (*L. multiflorum*) resulted in a proportional decrease in the rate of aphid parasitism and a reduction in the rate of secondary parasitism. There were more aphids on endophyte-free grasses, they had a higher rate of parasitism and supported a more diverse assemblage of parasitoids, including a generalist hyperparasitoid not found with infected grasses.

Clearly, endophyte infection has the potential of affecting multiple trophic levels. Given the large land area covered by endophyte-infected grasses, especially tall fescue and perennial ryegrass, this is not only an academic issue but also one that bears on agricultural productivity and conservation of biodiversity.

Conclusions

Endophyte symbiosis is widespread in the grass family, especially in the cool-season grasses of the subfamily Pooideae. The close relationships of mutualistic endophytes to plant pathogens, their propensity for hybridization and

vertical transmission through seeds, and the chemical protection of host plants against herbivores are features that distinguish this from other plant/microbial interactions. In the most widespread relationship, endophyte symbiosis with tall fescue can have dramatic effects on the structure of vegetation and the animal communities dependent on that vegetation. Unlike other plant/microbial symbioses, grass/endophyte associations are not directly involved with the acquisition of mineral resources but instead are based primarily on protection of the host.

Endophyte symbiosis may have evolutionary consequences for grasses and for fungi. Hybridization is rampant in the grass family, with up to 70% of all species being polyploid. Endophyte symbiosis could provide adaptive benefit to hybrids, allowing their persistence and increase. Endophytes also provide a mechanism of surrogate horizontal gene transfer and the movement of traits like alkaloid synthesis genes among discrete groups of grasses by shared or related endophytes. Certain pooid clades are very speciose (e.g., *Agrostis*, *Festuca*, *Poa*) and have high levels of seed-transmitted endophyte infection. Could endophyte symbiosis facilitate speciation in grasses? Compared to other large plant families like the legumes or composites, grasses more frequently lack secondary chemistry, perhaps because that niche is already occupied by alkaloid-producing endophytes. Given their role in grass metabolism and physiology, endophyte symbiosis may enhance the host's ability to exploit novel or extreme habitats such as the hot, dry regions of northern Africa or the southeastern United States that are typically inhospitable to perennial C3 grasses.

Endophytes have diversified across the grass family in parallel with their hosts. In contrast to other interactions where symbiosis is obligate, grasses can survive without endophytes, at least under benign conditions. The completely asymptomatic and seed-transmitted life history of Type III endophytes is a unique niche for parasitic fungi that may shed light on the origin of intracellular symbiosis and organelles. The ability to produce biologically active alkaloid compounds appears to have been key for the evolution and diversification of this group of fungi. The switch between vertical and horizontal transmission is relatively easy, as are correlated changes in virulence and host benefits.

Recent discoveries of asymptomatic infections of other plant groups by distinct groups of endophytic fungi that produce physiologically active mycotoxins suggest that grass/endophyte associations may be the best known of a more widespread type of interaction. Additional research on the evolution and ecology of plant/microbial interactions promises to be fruitful.

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