Abstract

_**Relationships of Epichloë typhina** isolates from different host grasses_

_C.L. SCHARDL, A. LEUCHTMANN, & B.A. MCDONALD_

1University of Kentucky, Department of Plant Pathology, 201 PSB, 1405 Veterans Dr., Lexington, KY 40546-0312, U.S.A.
2ETH Zurich, Institute of Integrative Biology, Plant Ecological Genetics, CHN G29 Universitaetstrasse 16, CH-8092 Zurich, Switzerland
3ETH Zurich, Institute of Integrative Biology, Plant Pathology, LFW B16, Universitaetstrasse 2, CH-8092 Zurich, Switzerland

Keywords: _Brachypodium_ species, _Clavicipitaceae_, _Dactylis glomerata_, _Epichloë_, _epichloë endophytes_, _grass_, _host specificity_, _molecular phylogenetics_, _Poaceae_, _Poa_ species, _population genetics_

Introduction

The _Epichloë_ and _Neotyphodium_ species are, respectively, sexual and asexual fungi that maintain constitutive, systemic symbioses with many grasses. These endophytes range from the more virulent and contagious sexual species, to mutualistic but asexual species that are only transmitted vertically. These fungal species and their host grasses have been an interesting model for the evolution of symbiotic systems. Results of such studies indicate a strong relationship between host specificity and phylogenetic species. Phylogenetic inference of _Epichloë_ species relationships has mainly used sequences of intron-rich segments of genes encoding beta-tubulin (tubB), translation elongation factor 1-alpha (tEF1A), and actin (ACTG) (Craven et al. 2001). Distinct phylogenetic species of _Epichloë_ are associated with each of the host genera _Brachyelytrum_ (Epichloë brachyelytii), _Glyceria_ (Epichloë glyceriae), _Elymus_ (Epichloë elymi), and _Bromus_ (Epichloë bromicola). The phylogenetic species _Epichloë amarillans_ is associated with closely related host genera _Agrostis_ and _Sphenopholis_, and possibly also _Calamagrostis_ and _Cinna_ species, all within the tribe _Aveneae_. Whereas _E. amarillans_ has been found only in North America, _Epichloë baconii_ has been identified in _Agrostis_ and _Calamagrostis_ species in Europe. However, the sequenced isolate from _Calamagrostis villosa_ groups separately from a clade of isolates from _Agrostis_ species. Despite this apparent contradiction with the phylogenetic species concept, _E. baconii_ has been circumscribed to include isolates from both genera because they are interfertile. In contrast, the phylogenetic species, _Epichloë festucae_, includes isolates from _Festuca_ and _Lotium_ that are interfertile, as well as a stroma-forming isolate from _Koeleria_ sp. that was not interfertile with the others. Yet, all _E. festucae_ isolates to date have been highly similar in sequences of _actG_, _tEF1A_, and _tubB_ introns, and the rDNA-ITS regions. Interestingly, _E. typhina sensu stricto_, which has been defined mainly on interfertility relationships, is associated with several genera in three different grass tribes: _Poeae_, _Aveneae_, and _Brachypodioideae_ (Leuchtmann & Schardl 1998). This is a genetically diverse species, which is parapatric to _Epichloë sylvatica_ and _Epichloë clarkii_. Although _E. clarkii_ is interfertile with _E. typhina_, a morphological difference in the ascospores and its restriction to the host species _Holcus lanatus_ has justified its specific binomial (White 1993). Likewise _E. sylvaticum_ is restricted to a single host species, _Brachypodium sylvaticum_ (Leuchtmann & Schardl 1998). Interestingly, _E. typhina_ isolates from the congener, _Brachypodium pinnatum_ are closely related to _E. sylvatica_. Surprisingly, whereas _E. sylvatica_ isolates appear not to be interfertile with those from _B. pinnatum_, the latter are interfertile with more distantly related _E. typhina_ isolates associated with _Dactylis glomerata_ (Craven et al. 2001; Leuchtmann & Schardl 1998). Isolates of _Epichloë_ spp. from different host genera almost never have identical _tubB_, _tEF1A_, and _actG_ sequences sequences. To date, the only exceptions have been _E. festucae_ isolates. The possibility that _E. typhina_ is a single, broad host range species might need reconsideration if this pattern holds in a more extensive sampling of _E. typhina_ isolates from several common hosts. Therefore, we conducted surveys of isolates from four hosts: _Brachypodium pinnatum_, _Dactylis glomerata_, _Poa nemoralis_, and _Poa trivialis_, in a transect of Switzerland, to determine the extent of gene flow between populations defined on a host basis.

Methods

Plants of _B. pinnatum_, _D. glomerata_, _P. nemoralis_ and _P. trivialis_ exhibiting choke disease were identified in several locations in France and Switzerland, on a transect from North 46°20.6’ East 6°04.9’ (near Versany, France) to North 47°45.7’ East 8°36.9’ (near Merishausen, Switzerland (Table 1). Stromata were selected from plants at least 1 m apart. Stromata were lyophilised. 

<table>
<thead>
<tr>
<th>Host</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. pinnatum</em></td>
<td></td>
</tr>
<tr>
<td><em>D. glomerata</em></td>
<td></td>
</tr>
<tr>
<td><em>P. nemoralis</em></td>
<td></td>
</tr>
<tr>
<td><em>P. trivialis</em></td>
<td></td>
</tr>
</tbody>
</table>

*Locations are Versany in France, and Cantons Vaud (VD), Zürich (ZH) and Schaffhausen (SH) in Switzerland.*
Figure 1  Phylogenetic relationships of *Epichloë typhina* isolates from populations of *B. pinnatum*, *D. glomerata*, *P. nemoralis* and *P. trivialis* based on comparisons of *tubB* intron sequences. Also included is the sequence from *Epichloë clarkii*, a species that is associated with *Holcus lanatus* and is infertile with *E. typhina*.
Figure 2  Phylogenetic relationships of tubB introns sequences from Epichloë species and selected Neotyphodium species. Each sequence is shown once, except those from E. festucae isolates from different host species. The LAE clade represents one of the genomes from four interspecific hybrids, for which the other alleles are not shown. Isolates in the E. typhina complex include those of E. clarkii and E. sylvatica, where indicated, or E. typhina as currently described.
for storage and later DNA isolation. Stems from the stroma-bearing tillers were surface disinfested, then placed on nutrient agar medium to grow out endophyte cultures as previously described (Leuchtmann 1994; Leuchtmann & Clay 1988). DNA was isolated from lyophilised stromata or fungal mycelium with the DNAeasy plant minikit or DNAeasy 96 plant kit from Qiagen (Valencia, California), per manufacturer’s instructions. A segment of E. typhina tubB (previously tub2), including the first three introns, was amplified and sequenced from each sample as previously described (Craven et al. 2001) Sequences were aligned with Pileup (GGC 1998), with gap creation penalty = 0 and gap extension penalty = 1. Alignments were adjusted by eye. Phylogenetic trees were inferred with PAUP® software (Swoford 1998), by parsimony with unweighted and unordered DNA character states, and indels treated as missing information.

Results
Each E. typhina isolate gave a single tubB haplotype, in keeping with the haplode nature of this species (Fig. 1). In total, 4 tubB haplotypes were identified in the 38 isolates from B. pinnatum, 3 haplotypes in 26 isolates from P. nemoralis, and 2 haplotypes in 79 isolates from P. trivialis. The most genetic diverse group was that associated with D. glomerata, with a total of 24 haplotypes identified in 117 isolates.

Haplotypes in isolates from P. nemoralis and P. trivialis formed distinct clades, but despite the generic relationship of the hosts, these clades were not closely related. Surprisingly, populations associated with D. glomerata and B. pinnatum did not form monophyletic clades (Fig.2). Instead, those from B. pinnatum were paraphyletic to the rest of the E. typhina complex, and those from D. glomerata were paraphyletic to the P. trivialis-associated clade, as well as to sequences in isolates from the hosts Lolium perenne, Anthoxanthum odoratum, and Holcus lanatus (the latter are classified as E. clarkii). Some isolates from B. pinnatum had haplotypes that were very similar (each with a single nucleotide substitution difference) to haplotypes of E. sylvatica.

Discussion
The lack of any shared haplotypes in our study strongly indicates that E. typhina populations associated with different host species rarely or never mate productively. Mating of the Epichloë species are mediated by species of Botanophila, anthymiid flies that vector spermatia between stromata, and which depend on the resulting ascocmal development for larval nutrition (Bultman et al. 1995). However, analysis of conidia from frass indicates that the flies are not entirely specific for stromata on individual host species (Leuchtmann & Bultman 2001). Therefore, another genetic isolation mechanism may be at work. A possibility is that hybrids from matings of E. typhina strains on different hosts are rarely capable of survival in one or the other parental host. This hypothesis was largely supported by a study of host compatibility of progeny obtained when E. typhina from D. glomerata was mated with E. typhina from L. perenne (Chung et al. 1997). However, host compatibility appeared to be a complex, quantitative trait. Furthermore, such matings gave rise to some recombinant ascospores that infected L. perenne (Chung & Sardall 1997), and even a hybrid that was capable of producing stromata on D. glomerata (Chung et al. 1997). Thus, past studies suggest that host species barriers are not absolute, although such barriers are apparently sufficient to prevent substantial genetic exchange between host-specialised populations. The relative diversity and paraphyletic relationships of populations on B. pinnatum and D. glomerata suggest that E. typhina parasitism of those hosts may be relatively ancient, and that populations on other hosts arose by jumps or shifts. Some of these jumps were between hosts in different tribes, such as may have occurred between Brachypodium spp. (Brachypodiceae) and Poa spp. (Poaceae). In other cases it is unclear if host jumps occurred or, instead, if E. typhina populations diverged along with the speciation of the hosts. So, for example, it is possible that the closely related isolates of E. sylvatica on B. sylvaticum and E. typhina on B. pinnatum may represent cocladogenesis driven by host specialisation. The lack of detectable genetic exchange between populations on different hosts suggests cryptic speciation driven by host specialisation. However, the instances of paraphyly pose a problem for phylogenetically based recognition of such species (Taylor et al. 2000). Such paraphyletic relationships may be expected if new niches (in this case, new hosts) are colonized rarely, and by few individuals from larger ancestral populations (founder effect), and if hybrids between individuals specialised on different hosts are strongly disfavoured. The large number of host species with epichloë endophytes suggests that colonisation of new hosts is common on evolutionary time scales. However, the majority of such endophytes are strictly asexual, and are maintained by vertical transmission in host seeds. Perhaps the majority of host jumps result in asymptomatic symbioses, and relatively few give symbioses in which the endophyte can express the sexual state (choke). Indeed, many asexual endophytes appear to be derived either from E. typhina or from diploid or polyploid hybrids possessing an E. typhina-derived genome (Gentile et al. 2005; Moon et al. 2004). Ironically, most of the hosts that support stroma formation by E. typhina do not support vertical transmission. In such cases, stromata are necessary to provide the inoculum (ascospores) to spread the fungus in the host population. The only known exception to date is P. nemoralis, on which E. typhina is capable of both vertical and horizontal transmission (Sardall & Leuchtmann 2005). Not surprisingly, E. typhina genotypes on P. nemoralis are closely related to non-hybrid asexual endophytes in several hosts (Gentile et al. 2005; Moon et al. 2004). In contrast, jumps to new hosts of other E. typhina genotypes will probably be dead ends unless the sexual state is expressed on the new hosts (or the endophyte hybrids with a resident, vertically transmissible endophyte). Thus, we speculate that a major selection for host specialisation is the requirement for stromal expression to maintain a viable, contagious population.

ACKNOWLEDGEMENTS
This study was supported by a Visiting Professorship stipend provided to CLS by the ETH Zürich, Switzerland.

REFERENCES


