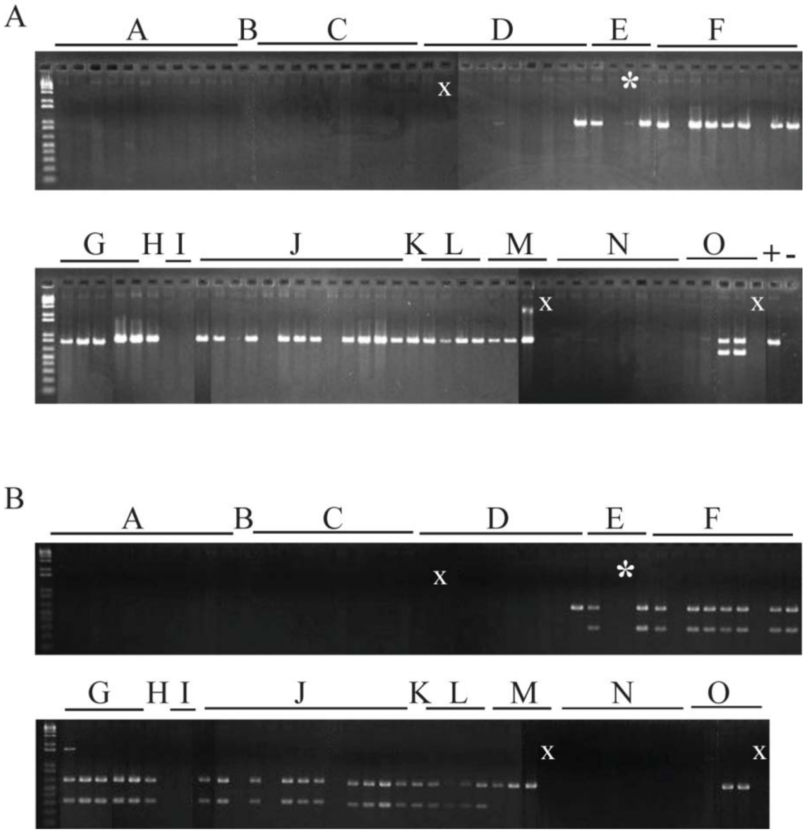

Fig. S1: Amplification of endophyte DNA from seed of Greek tall fescue lines. Total DNA extracted from 3 seeds of each tall fescue line was amplified using endophyte-specific primers designed to A) *tefA* and B) *perA*-A2. Amplicon detection indicates the presence of fungal biomass within the seeds. Tall fescue lines are provided in numerical order. The letters above indicate the collection locations marked in Fig. 1. The asterisk (*) denotes accession 45081, which had a low incidence of endophyte infection. Tall fescue lines with no seed available are represented by an X. + = positive control, - = negative control.

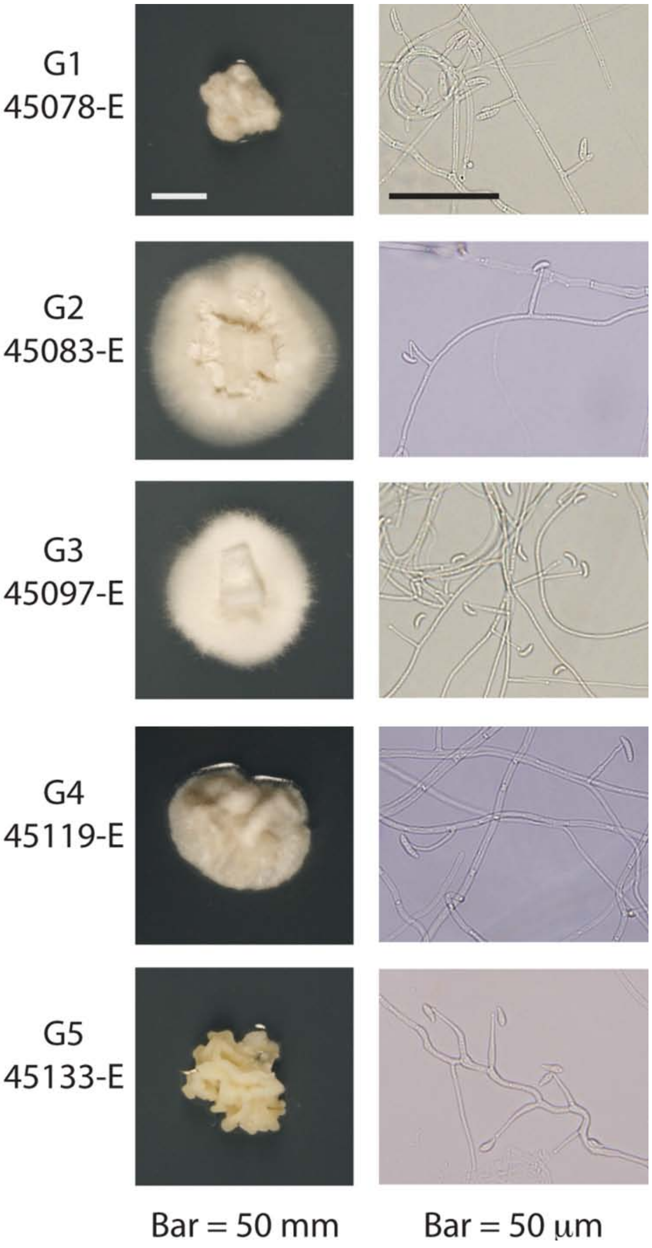

Fig. S2: Colony and conidia morphology of Greek isolates representative of the five unique morphotypes/genotypes G1-G5. Colony morphology was photographed after 21 days of growth at 22°C. Isolate designations are listed in the figure. Bar = 50 mm and 50 μm for colony and conidial photographs, respectively.

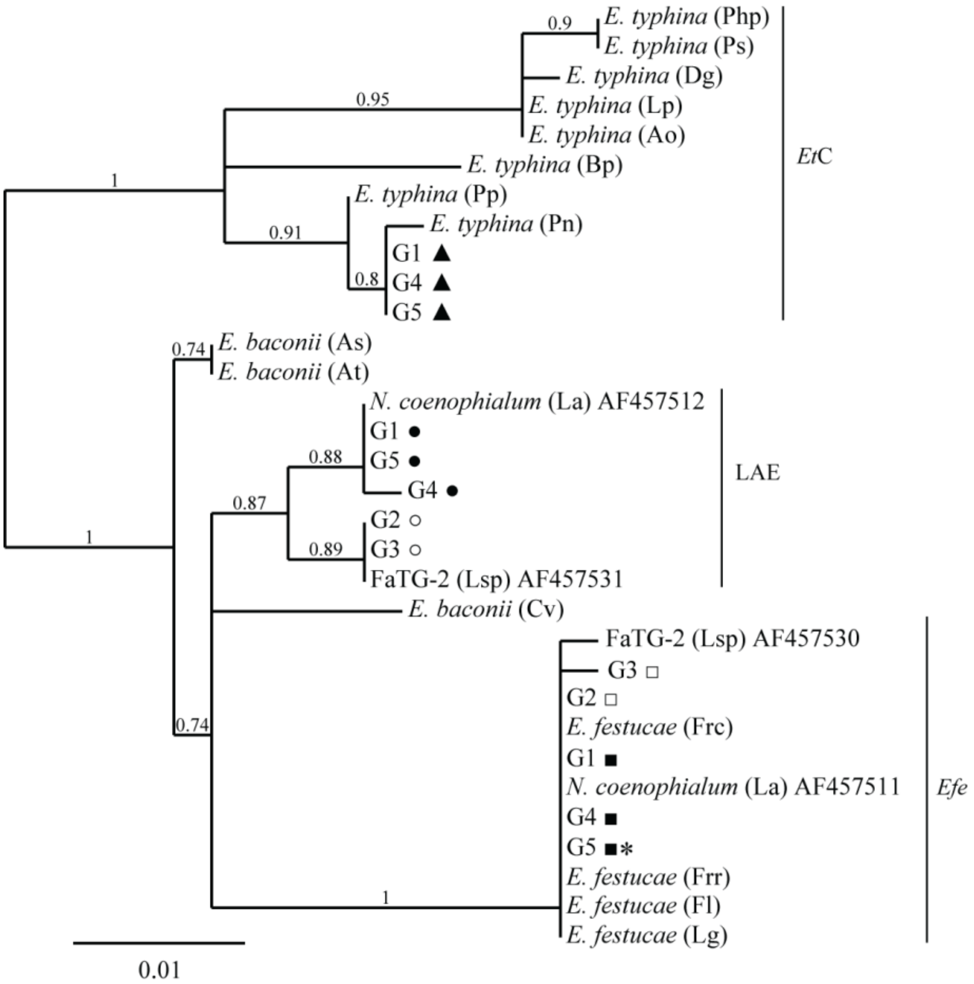

Fig. S3: Phylogeny derived from maximum likelihood (ML) analysis of introns 1-4 of *tefA* from representative haploid *Epichloë* species, hybrid *Neotyphodium* species, and copies obtained from the Greek tall fescue endophytes. Midpoint root is at the left edge. Numbers at branches are the branch support values; branches with less than 50% support were collapsed. Letters in parentheses after the *Epichloë* and *Neotyphodium* isolates refer to host designations as follows: Ao (*Anthoxanthum odoratum*); As (*Agrostis stolonifera*); At (*Agrostis tenuis*); Bp (*Brachypodium pinnatum*); Cv (*Calamagrostis villosa*); Fl (*Festuca longifolia*); Frc (*Festuca rubra* subsp. *commutata*); Frr (*Festuca rubra* subsp. *rubra*); La (*Lolium arundinaceum* = *Schedonorus phoenix*); Lp (*Lolium perenne* subsp *perenne*); Lsp (*Lolium* sp.); Php (*Phleum pratense*); Pn (*Poa nemoralis*); Pp (*Poa pratensis*); Ps (*Poa silvicola*). Multiple alleles from *N.*

coenophialum and FaTG-2 isolates are designated by Genbank accession number, and G1, G4, and G5 copies are designated by▲ (copy 1), ■ (copy 2), or ● (copy 3); alleles from G2 and G3 isolates are designated by□ (copy 1) or ○ (copy 2). The *tefA* allele of the G5 isolates containing a deletion is designated with an asterisk. *Et*C, *Epichloë typhina* complex; *Efe, E. festucae*; LAE, *Lolium*-associated endophyte clade.

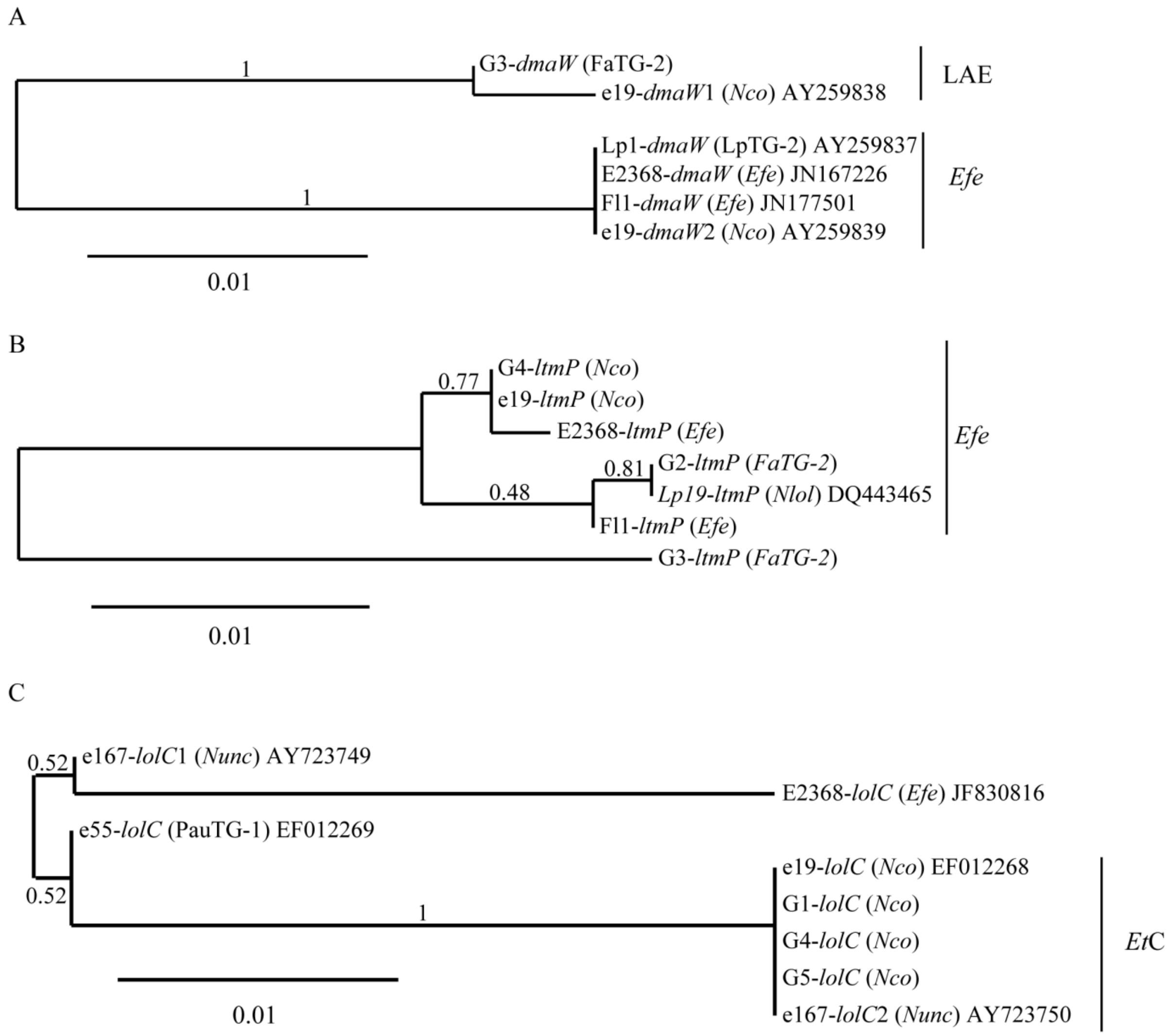

Figure S4: Phylogeny derived from maximum likelihood (ML) analysis of sequence fragments of (A) *dmaW*, (B) *ltmP*, and (C) *lolC* from *E. festucae* and *Neotyphodium* sp. Accession numbers for previously published *dmaW* gene sequences are listed to the right of isolates in the figure. Midpoint root is at the left edge. Numbers at branches are the branch support values; branches with less than 50% support were collapsed. *Et*C, *Epichloë typhina* complex; *Efe, E. festucae*; LAE, *Lolium*-associated endophyte clade.

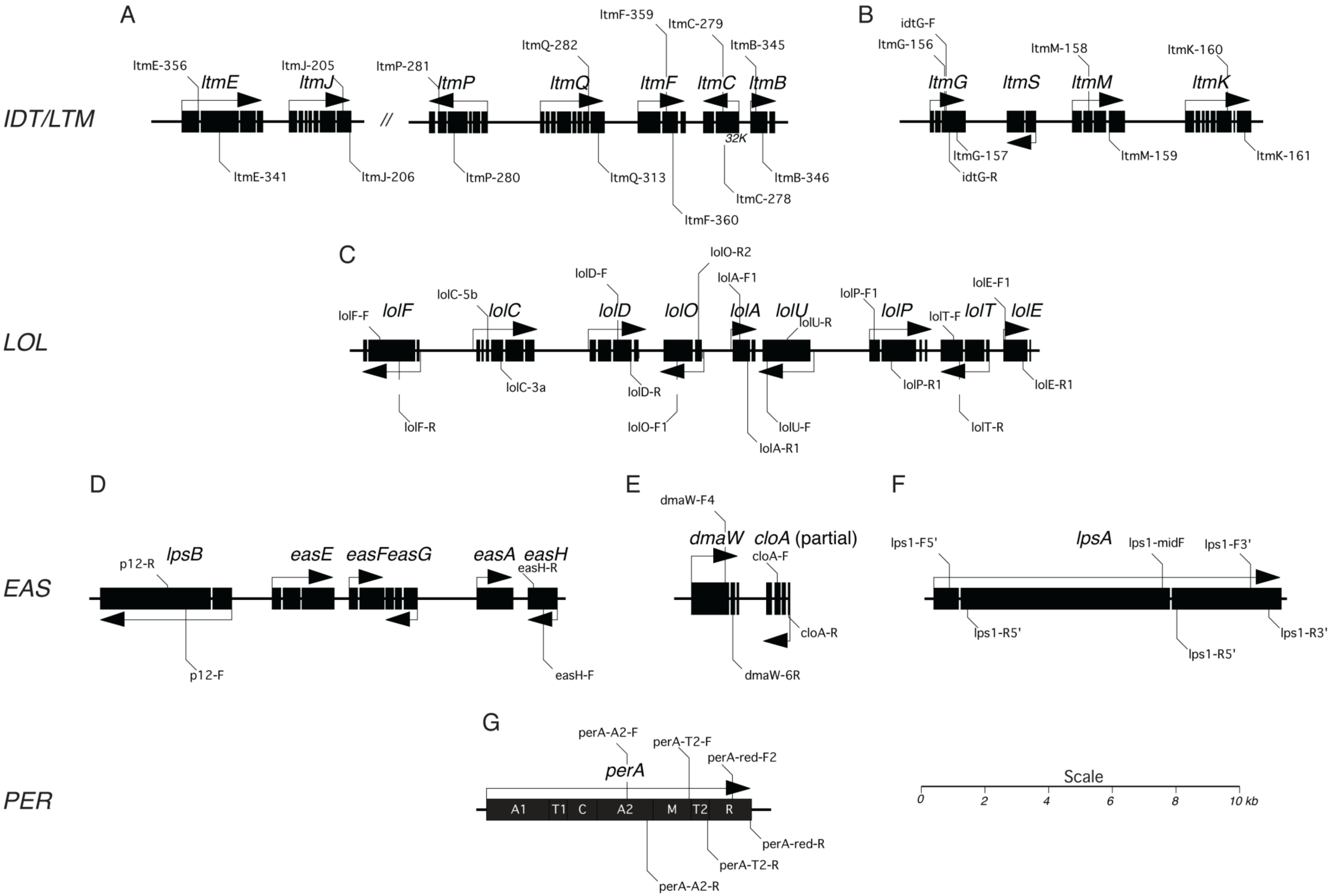
A
IDT/LTM
ltmE-356
ltmE
ltmE-341
ltmJ-205
ltmJ
ltmJ-206
//
ltmP-281
ltmP
ltmP-280
ltmQ-282
ltmQ
ltmQ-313
ltmF-359
ltmF
ltmF-360
ltmC-279
ltmC
32K
ltmC-278
ltmB-345
ltmB
ltmB-346
B
idtG-F
ltmG-156
ltmG
ltmG-157
idtG-R
ltmS
ltmM-158
ltmM
ltmM-159
ltmK-160
ltmK
ltmK-161
C
LOL
lolF-F
lolF
lolF-R
lolC-5b
lolC
lolC-3a
lolD-F
lolD
lolD-R
lolO-R2
lolO
lolO-F1
lolA-F1
lolA
lolA-R1
lolU
lolU-R
lolU-F
lolP-F1
lolP
lolP-R1
lolT-F
lolT
lolT-R
lolE-F1
lolE
lolE-R1
D
EAS
p12-R
lpsB
p12-F
easE
easFeasG
easA
easH
easH-R
easH-F
E
dmaW-F4
dmaW
dmaW-6R
cloA (partial)
cloA-F
cloA-R
F
lps1-F5'
lpsA
lps1-midF
lps1-F3'
lps1-R5'
lps1-R5'
lps1-R3'
G
PER
perA-A2-F
perA-T2-F
perA-red-F2
perA
A1
T1
C
A2
M
T2
R
perA-red-R
perA-T2-R
perA-A2-R
Scale
0
2
4
6
8
10 kb

Figure S5: Alkaloid biosynthesis gene clusters and primer locations for the alkaloid genes referenced within this study. A and B: Indole-diterpene (*IDT*/*LTM*) locus from *N. lolii*, DQ443465 and AY742903, respectively. C: Loline locus *LOL*1 of *N. uncinatum*, AY723749. D, E, and F: Ergot alkaloid (*EAS*) loci from *N. lolii* (D, EF125025), *N. coenophialum* (E, AY259839), and *N.* sp. LpTG-2 (F, AF368420). G: Peramine (*PER*) locus from *E. festucae* (AB205145).

Table S1: Primers used in this study

Gene	Forward	Primer sequence (5'→3')	Reverse	Primer sequence (5'→3')
tefA[a]	Tef1-exon1d-1	GGGTAAGGACGAAAAGACTCA	Tef1-exon6u-1	CGGCAGCGATAATCAGGATAG
tubB[b]	T1.1	GAGAAAATGCGTGAGATTGT	T1.2	CTGGTCAACCAGCTCAGCAC
matA[c]	mtAC-F2	GAGAACTGAGGAAAGACCACTCAG	mtAC-R	CGGTCTCATTCTTCCAGAGAGAGG
matB[c]	mtBA-F	TCTACCGCAAGGAACGACACAATACCG	mtBA-R	GCTTTTCCAGCAAGGCTTGCTTGACTC
ltmG[d]	ltmG-156	GCACAAACAATAAATTCGGCCAA	ltmG-157	AATTTGCCCTCTGTTAAATCCTC
ltmG	idtG-F	GAGCTTGAGAAGCTTACGAATCC	idtG-R	GGGCAATGGAGCGATTCTCTC
ltmM[d]	ltmM-158	GTGATCGGTGCTGACGGGGTCCA	ltmM-159	TATCGCCATATTTGCTCCTTGCCC
ltmK[d]	ltmK-160	ATATTGAATTGCTGCGTGAGGAG	ltmK-161	AGAGGCCAAGAAGCGGCCTGGACA
ltmB[d]	ltmB-345	AACATCGCCTGGGAGCTCGTATA	ltmB-346	CGCAGGTCCTATTTCCATCGC
ltmC[d]	ltmC-278	GAAACTGCCAATCGAGCATA	ltmC-279	TTCTTGCAATCATTTTGCAATTG
ltmF[d]	ltmF-359	GAATTATGTTACTCTTGGGG	ltmF-360	AAGTTGGCACATAGGTCTTC
ltmQ[d]	ltmQ-313	CTACCAGGACAGGCGTGACGTCC	ltmQ-282	CAGAGGTTTAACCCTCTTGACGC
ltmP[d]	ltmP-280	ATGGCTGTCATTCATACAACAGCTATG	ltmP-281	AGCGTCCCGGACAGGCATATCTCCCA
ltmP	ltmP-F1	ACATGGATCTGATTGTCATTCC	ltmP-R1	TACGATGCGCAAGTGCTTCTGG
ltmJ[d]	ltmJ-205	CCAAGCATCGATTTGTCACC	ltmJ-206	AATCTGATCGCCATCTTTGC
ltmE[d]	ltmE-356	CCGAGTTTGATGACCTGCTG	ltmE-341	TTCCGCTTCCGAGTAGACTC
lolC[e]	lolC-3a	GGTCTAGTATTACGTTGCCAGGG	lolC-5b	TCTAAACTTGACGCAGTTCGGC
lolA	lolA-F1	GAGACACTAGAGAAATGGCAGCTGC	lolA-R1	GGCATCCATGGTGGCGAAGATGTG
lolF	lolF-F	CTCTGATATGAAGACTCCTGAGC	lolF-R	GCCAAGCGGAGTTCAGATCATCC
lolD	lolD-F	CTCGACGTTTCAACAGATTGCAG	lolD-R	GTCTTTGAAGACAAGCCAGTCC
lolU	lolU-F	CGATGGTTGGATCAGTCGTTGC	lolU-R	GAGCTGATGCGGCATTGGCATC
lolT	lolT-F	CACTGACCTCCAAGTATACTTGC	lolT-R	CGTCATCCCGACCTCTTTCGGAT

lolE	lolE-F1	ACCAAGCCAACGGATATCTTCGC	lolE-R1	ACGTCTTTGGTCCGTCTTGTTAG
lolP	lolP-F1	GTTCTAAACATCGTGACTGGGC	lolP-R1	GGTAGGTCAGCATCTTGTCAACG
lolO	lolO-F1	GTGAACTGGCAGTAGTCCGTATG	lolO-R2	AATCCATGCCAGTGTCGGGAATG
perA	perA A2-F	CGTCGTGGTAACGCACGCAAACG	perA A2-R	CAGTCTGCCTTGCCGACCGGGGT
perA	perA T2-F	TCTTCAGGCATCGCAGGAAC	perA T2-R	TCGGCCACCTCCAGCCTGATG
perA	perA red-F2	GAGATCAGTTCGCAGTTGTCAG	perA red-R	CTAGCCTCCAGATCTTGTGAAAG
dmaW	dmaW-F4	GTGTACTTTACTGTGTTCGGCATG	dmaW-6R	GTGGAGATACACACTTAAATATGGC
cloA	cloA-F	GGATGAACTTGTCAGGTGACGAG	cloA-R	GTGATCAGGGATACCTTTGATTAC
easH	easH-F	AGATATGGCATCGTGACCAGCC	easH-R	GGCATGTAGCATCAAATGGTGTC
lpsA	lps1-F5'	ATGTCCGTGGATGGTGAGCAAAC	lps1-R5'	ATGCAATGGGTGCCTGTCCGTTC
lpsA	lps1-midF	CGCTGCTCTCTGTCATGCAGAAG	lps1-midR	GTTCCTCATCCAACATCTCGATC
lpsA	lps1-F3'	CGATCACCCAACTTTACATGATAC	lps1-R3'	ACAAGTCATGGTCCGGATGTGTTG
lpsB[f, g]	p12-R	TACCCACTGCCTCGAACTTG	p12-F	CCGTCTTCCCGTATACCGAA
SSR[h]	B10.1	CGCTCAGGGCTACATACACCATGG	B10.2	CTCATCGAGTAACGCAGGCGACG
SSR[h]	B11.1	CATGGATGGACAAGAGATTGCACG	B11.2	TTCACTGCTACAATTCTGTCCAGC
SSR[i]	A1AG12-F	CCCCGACAATCTAGCATACCTTCG	A1AG12-R	CGGTGATGATGACAAGCCTTGGAC

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[a] Craven KD, Hsiau PTW, Leuchtmann A, Hollin W, Schardl CL. 2001. Multigene phylogeny of *Epichloë* species, fungal symbionts of grasses. Ann. Mo. Bot. Gard. **88:**14-34.

[b] Young CA, Bryant MK, Christensen MJ, Tapper BA, Bryan GT, Scott B. 2005. Molecular cloning and genetic analysis of a symbiosis-expressed gene cluster for lolitrem biosynthesis from a mutualistic endophyte of perennial ryegrass. Mol. Gen. Genomics **274:**13–29.

[c] Primers designed from the following accession numbers HQ680587 (*mtAC*) and HQ680590 (*mtBA*)

[d] Young, CA, Tapper BA, May K, Moon CD, Schardl CL, Scott B. 2009. Indole-diterpene biosynthetic capability of *Epichloë* endophytes as predicted by *ltm* gene analysis. Appl. Environ. Microbiol. **75:**2200–2211.

[e] Spiering MJ, Wilkinson HH, Blankenship JD, Schardl CL. 2002. Expressed sequence tags and genes associated with loline alkaloid expression by the fungal endophyte *Neotyphodium uncinatum*. Fungal Genet. Biol. **36:**242–254.

[f] Tanaka A, Tapper BA, Popay A, Parker EJ, Scott B. 2005. A symbiosis expressed non-ribosomal peptide synthetase from a mutualistic fungal endophyte of perennial ryegrass confers protection to the symbiotum from insect herbivory. Mol. Microbiol. **57:**1036–1050.

[g] Johnson R, Voisey C, Johnson L, Pratt J, Fleetwood D, Khan A, Bryan G. 2007. Distribution of NRPS gene families within the *Neotyphodium*/*Epichloë* complex. Fungal Genet Biol 44:1180–1190.

[h] Moon CD, Tapper BA, Scott B. 1999. Identification of *Epichloë* endophytes in planta by a microsatellite-based PCR fingerprinting assay with automated analysis. Appl. Environ. Microbiol. **65:**1268-1279.

[i] van Zijll de Jong E, Guthridge KM, Spangenberg GC, Forster JW. 2003. Development and characterization of EST-derived simple sequence repeat (SSR) markers for pasture grass endophytes. Genome. **46:**277-290.

Table S2: Genbank accessions of *tefA* and *tubB* sequences from *Epichloë* and *Neotyphodium* species used for phylogenetic analyses in this study

		GenBank Accession number	
Endophyte	Isolate	*tubB*	*tefA*
Neotyphodium coenophialum	Greek-type 1 (G1)	JX028244	JX028257
		JX028245	JX028258
		JX028246	JX028259
N. coenophialum	(G4)	JX028247	JX028260
		JX028248	JX028261
		JX028249	JX028262
N. coenophialum	(G5)	JX028250	JX028263
		JX028251	JX028264
		JX028252	JX028265
FaTG-2 (*Neotyphodium* sp.)	(G2)	JX028253	JX028266
		JX028254	JX028267
FaTG-2 (*Neotyphodium* sp.)	(G3)	JX028255	JX028268
		JX028256	JX028269
Epichloë amarillans	E273	AF457466	AF457505
E. baconii	ATCC 76552	L06961	AF231193
E. baconii	ATCC 90167	L78279	AF231194
E. baconii	ATCC 200745	L78270	AF231196
E. festucae	ATCC 90660	AF250746	AF231214
E. festucae	ATCC 90661	L06955	AF231210
E. festucae	E28	L06956	AF231213
E. festucae	E434	L78286	AF231212
E. typhina	ATCC 200738	L78288	AF231222
E. typhina	ATCC 200739	L78292	AF231223
E. typhina	ATCC 200740	L78274	AF231225
E. typhina	ATCC 200736	X52616	AF231220
E. typhina	ATCC 200851	L78280	AF231226
E. typhina	ATCC 201667	AF062429	AF231229
E. typhina	ATCC 201669	L78284	AF231231
E. typhina	ATCC 201666	L78285	AF231228
N. coenophialum	ATCC 90664	L06964	AF457511
		L06951	AF457512
		X56847	—
FaTG-2 (*Neotyphodium* sp.)	4078=Tf14	L06963	AF457531
		L06952	AF457532