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Chemotypic diversity of epichloae, fungal symbionts of grasses

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ABSTRACT

The epichloid fungi – comprising sexual *Epichloë* species and asexual *Neotyphodium* species – are symbionts of cool-season grasses (subfamily Poöideae), mostly vertically transmissible (seedborne), and well known for production of anti-herbivore alkaloids. Four classes of alkaloids are known to be produced by epichloae: lolines (saturated aminopyrrolizidines), indole–diterpenes, ergot alkaloids, and peramine. There is a wide range of chemotypic diversity among and even within epichloid species. At the molecular level, this diversity may in part reflect the telomeric association of two of the four alkaloid biosynthesis gene clusters. Ecologically, the chemotypic diversity within species may reflect frequency-dependent selection for the alkaloids, which provide defences against insects and, in some cases, vertebrates, but can be expensive to produce. Interspecific hybridization, common among asexual epichloae, can pyramid the alkaloid biosynthesis genes. Compared to sexual epichloae, many asexual epichloae produce high levels of alkaloids – particularly lolines – suggesting that strict vertical transmission selects for enhanced capability of host protection.

Introduction: the epichloae

The epichloae are a group of fungal symbionts (endophytes) commonly found in cool season grasses (Poaceae, subfamily Poöideae), and best known for the protective effects on their hosts. The biology and effects of these symbionts, which are reviewed in Schardl *et al.* (2004), can range from highly beneficial to highly pathogenic depending in part on their associations with the flowering tillers. They may colonize embryos without causing disease, enabling them to transmit vertically; or, instead, they may suppress maturation of the tiller (choke disease) while developing into a stroma from which contagious spores mediate horizontal transmission. The epichloae constitute a monophyletic group in the family Clavicipitaceae that are placed in the genus *Epichloë* if the teleomorph (sexual state) is known, and otherwise in the genus *Neotyphodium* (anamorph, asexual) (Kuldau *et al.* 1997). Those that produce no stromata are asexual, and primarily or exclusively transmitted vertically. Many of the asexual species are interspecific hybrids derived from the sexual *Epichloë* species (Tsai *et al.* 1994; Moon *et al.* 2004).

The documented beneficial effects of epichloid fungi vary among the systems studied, and include enhancement of

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drought resistance, increased biomass production, increased tillering, and protection from vertebrate or invertebrate herbivores (Malinowski & Belesky 2000; Schardl *et al.* 2004). The anti-herbivore effects are partly attributable to several classes of alkaloids produced by some epichloid strains; namely, ergot alkaloids, indole–diterpenes, lolines and peramine. Genes and gene clusters for all four of these alkaloids have been identified and sequenced (Fig 1). The subject of this review is the considerable diversity of alkaloid profiles among the epichloae. We will begin by briefly reviewing these four alkaloid classes and their bioactivities. Then we will discuss the *Epichloë* and *Neotyphodium* species and the patterns underlying their chemotypic diversity. Finally, we will present some ideas about the ecological and evolutionary basis for this diversity.

Alkaloid structures and activities

Lolines

Loline alkaloids are a group of saturated *exo*-1-amino pyrrolizidines with an ether bridge between bridgehead carbons C(2) and C(7), constituting a very unusual structure for biogenic compounds. Variations in the R groups linked to N(1) distinguish different lolines (Petroski *et al.* 1989) (Fig 2). These alkaloids were first discovered in the late 19th to middle 20th centuries in investigations of the cause of livestock toxicosis associated with *Lolium temulentum* (Antze 1891; Batirov *et al.* 1977). Interestingly, there has been no definitive evidence of toxicity due to any particular metabolite, including loline alkaloids, in *L. temulentum*. In fact, early reports of toxicity associated with lolines from *Lolium arundinaceum* were probably due to ergot alkaloids in the alkaloid preparations (Yates *et al.* 1990). Lolines have broad spectrum insecticidal activity comparable to nicotine (Riedell *et al.* 1991), and possibly also contribute to anti-nematode effects of the endophytes (Bacetty *et al.* 2009). A genetic test has shown that loline alkaloid production by an *Epichloë festucae* isolate from *Lolium giganteum* is strictly associated with activity against the aphids, *Rhopalosiphum padi* and *Schizaphis graminum* (Wilkinson *et al.* 2000). Though this effect was observed at concentrations as low as 300 $\mu g g^{-1}$ dry mass, lolines can reach 10–20 $mg g^{-1}$ dry mass in some plant-fungus symbiota (Craven *et al.* 2001; Zhang *et al.* 2009).

Genetic analysis of *E. festucae* showed that loline production strictly obeys Mendel's laws, suggesting that the biosynthesis genes are encoded in a single locus, designated *LOL* (Wilkinson *et al.* 2000). Subsequently, Spiering *et al.* (2002) found two genes, designated *lolA* and *lolC*, that were up-regulated during production of lolines in cultures of *Neotyphodium uncinatum*, and were only found in those epichloid species that produce lolines. RNA interference (RNAi) of *lolC* resulted in a significant decrease in loline-alkaloid production in *N. uncinatum* cultures, confirming involvement of LolC in loline alkaloid biosynthesis (Spiering *et al.* 2005). The *lol* genes were identified in two homologous gene clusters (*LOL1* and *LOL2*) in *N. uncinatum* (Spiering *et al.* 2005), and similar gene clusters were identified in other loline alkaloid-producing epichloae (Kutil *et al.* 2007). With completion of the *E. festucae* E2368 genome sequence the *LOL* cluster has been characterized as containing 11 genes in the order *lolF*, *lolC*, *lolD*, *lolO*, *lolA*, *lolU*, *lolP*, *lolT*, *lolE*, *lolN* and *lolM* (Spiering *et al.* 2005; J. Pan and C.L. Schardl, unpublished) (Fig 1). Bioinformatic analysis suggests that most or all of these genes encode enzymes or regulatory proteins that might be involved in LA biosynthesis (Spiering *et al.* 2005; J. Pan and C.L. Schardl, unpublished).

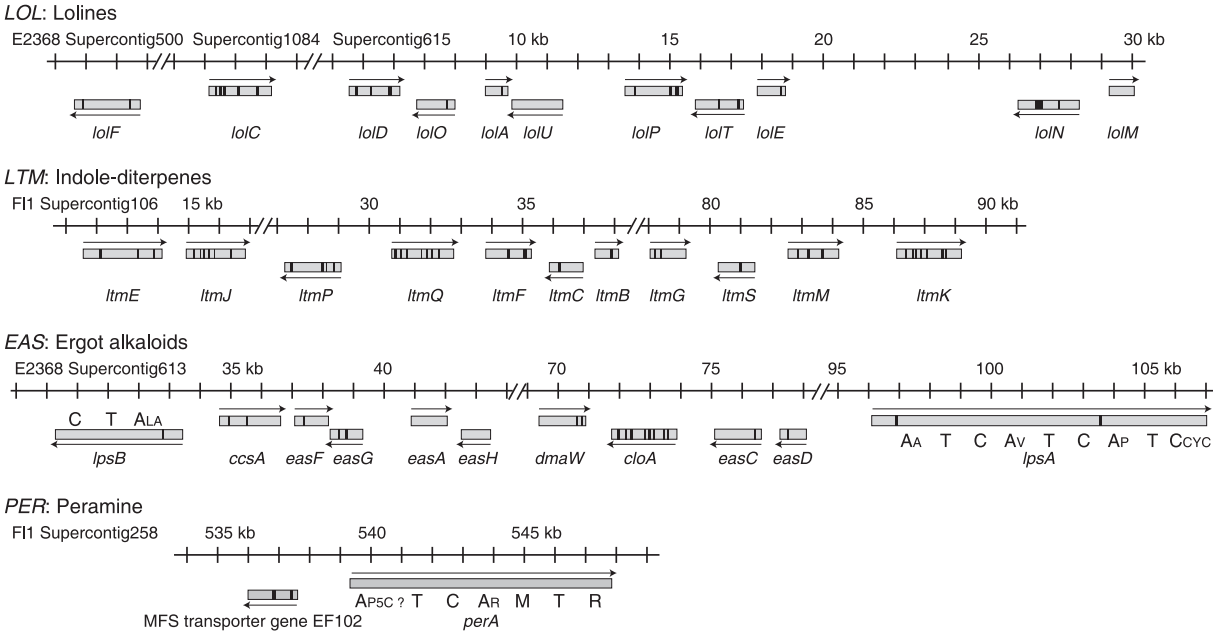

Fig 1 – Gene clusters for biosynthesis of the epichloid alkaloids. Coordinates of supercontigs in the assembled *Epichloë festucae* E2369 and Fl1 genomes are indicated. Nonribosomal peptide synthetase domains are designated as follows: A = adenylation, with subscripts indicating specificity for lysergic acid (A_{LA}), L-alanine (A_A), L-valine (A_V), L-proline (A_P), Δ1-pyrridoxal-5-carboxylate (A_{P5C}), or arginine (A_R); T = thiolation; C = condensation; C_{CYC} = condensation and cyclization; M = methylation; R = reduction.