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GENETIC IDENTIFICATION AND EVOLUTION OF EPICHLOË ENDOPHYTES

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1N

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ABSTRACT

The Epichloë endophytes are a group of filamentous fungi that include both sexual (*Epichloë*) and asexual (*Neotyphodium*) species. As a group, they are genetically diverse and form mutualistic to antagonistic symbiotic associations with temperate grasses (subfamily Pooideae). In this study, a multi-locus microsatellite-based PCR fingerprinting assay was developed for the genetic identification of Epichloë endophytes, both in culture and in planta. Microsatellites were isolated from endophyte partial genomic libraries, or identified from existing endophyte DNA sequences, and PCR assays that amplify these loci were developed. Multiplex assays were optimised, and fluorescently labelled primers were employed to allow precise sizing and automatic analysis of the PCR products with a laser scanner and the appropriate software. A reference database of allele sizes has been established for the panel of endophytes examined, and it has been shown that this assay is able to resolve endophyte groupings to the level of known isozyme phenotype groups. In a blind test the assay was used successfully to identify a set of endophytes in planta. The segregation of microsatellite alleles from an E. festucae sexual mating was also examined.

This microsatellite assay, in addition to β-tubulin and ribosomal RNA gene sequence analysis, was used to genetically characterise *Neotyphodium*-like endophytes from annual *Lolium* ryegrasses and *Hordeum* grasses. The endophytes examined were indeed found to be *Neotyphodium* with unique evolutionary origins. The common endophyte of annual ryegrasses was found to have a unique hybrid origin involving *E. baconii* and *E. bromicola* ancestry, and it is proposed that this taxonomic group is named LmTG-1. A second previously undescribed *Neotyphodium* endophyte was found in *L. canariense*, and this was shown to be an asexual derivative of *E. typhina*. The name proposed for this taxonomic group of endophytes is LcTG-1. The two *Hordeum* endophyte isolates, HaB and Hd1, were also both shown to have unique hybrid origins. HaB has *E. elymi* and *E. amarillans* ancestry, and Hd1 has *E. typhina* and *E. bromicola* ancestry. The proposed names for the *Neotyphodium* taxonomic groups that contain these isolates are HdTG-1 and HdTG-2 respectively. The revelation of interspecific hybrid origins for three more *Neotyphodium* endophytes illustrates the prevalence of hybridisation in the evolution of the Epichloë endophytes, and further contributes to our understanding of the evolution of this group.

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