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HOMOGENISATION IN THE RIBOSOMAL RNA GENES OF AN *EPICHLOË* ENDOPHYTE HYBRID

A thesis presented in partial fulfilment of the requirements for the degree of

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For Denis and Phil Ganley
ABSTRACT

Homogenisation mechanisms in the ribosomal RNA genes were investigated using *Epichloë* fungal endophyte interspecific hybrid isolate, Lp1. The two progenitor isolates, *Neotyphodium lolii* Lp5 and *E. typhina* E8, were used for comparison. Three areas of homogenisation were examined.

The first area involved characterisation of extraordinary length heterogeneity in the rDNA of Lp1. This was shown by Southern analyses on single-spore isolates to be present intragenomically and localised to the intergenic spacer (IGS). Length heterogeneity is not a feature of either progenitor, suggesting it is a consequence of the hybridisation. The length heterogeneity was shown to result from copy number variation of sub-repeats in the IGS, which is consistent with unequal crossing over occurring in the rDNA, suggesting that unequal crossing over plays a role in homogenisation. Multi-variant repeat PCR mapping of the sub-repeat array revealed that the ends of the array behave differently, and biased initiation of recombination is discussed. Several results are not consistent with homogenisation by unequal crossing over and the potential roles of gene conversion and extrachromosomal rDNA circles in homogenisation are discussed. Finally, evidence is presented that suggests the rate of homogenisation is very rapid. A group I intron is present in the 28S *rrn* gene of Lp1, and is widespread in the *Epichloë* endophytes. Closely-related introns in other fungal 18S *rrn* genes provide evidence for intron transposition.

The second area involved testing the hypothesis that the presence of one type of rDNA sequence in Lp1 is the result of interlocus homogenisation. CHEF gel electrophoresis revealed that Lp1 and Lp5 have at least five rDNA arrays organised as major and minor loci, an unusual situation in fungi. The organisation in E8 could not be determined. One potential rDNA-DNA junction was cloned but has not been analysed.

The final area initially involved testing the hypothesis that interlocus homogenisation of 5S rRNA gene arrays occurs more slowly than that of rDNA arrays in hybrids. However the 5S rRNA genes in the *Epichloë* endophytes were shown to be organised as dispersed copies, not in tandem arrays. Shared polymorphisms between Lp1, Lp5 and E8 may indicate the homogenisation rate of these dispersed repeats is slower, and gene conversion as a homogenisation mechanism is discussed. The 5S rRNA genes are located on the same chromosomal bands as the rDNA in Lp1 and Lp5, and therefore are markers that demonstrate the rDNA-containing Lp5 chromosomes are present in Lp1. This and the CHEF results provide evidence for interlocus homogenisation of the rDNA having occurred in Lp1, and extends observation of this phenomenon to fungi.
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