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Revealing the past: New insights into plant evolution using a novel snoRNA marker system

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Due to the limitations of both ITS and organellar sequences, and the need to incorporate information on hybridisation and polyploidisation events, it would be of extreme value to identify other gene loci, and in particular nuclear genes, for use in plant evolutionary biology. The characterization of plant small nucleolar RNA (snoRNA) genes and their organisation in multigene clusters provides a potential nuclear marker system which could help to discover the phylogenetic history in plants. SnoRNA genes encode small RNAs involved in ribosome biogenesis. They contain sequences which base-pair with ribosomal RNA, thereby determining sites of nucleotide modification (2'-O-methylation and pseudouridylation). Many snoRNA "guide" sequences are highly conserved across eukaryotes (from yeast to animals). In plants, snoRNA genes have a unique organization where the genes are arranged in closely linked clusters. This provides the opportunity to design universal primers to the conserved internal guide sequences and amplify gene and intergenic sequences. As the gene sequence does not code for proteins, sequence variation and indels are tolerated giving low to moderate allelic variation. An analysis of snoRNAs in plant sequence databases has identified over 250 genes in 17 different species including gymnosperms and mosses. On the basis of conserved gene order, universal primer sets have been designed. We have been concentrating on around 20 snoRNA primer pairs using the well-known species-group around Senecio squalidus. PCR amplification of different accessions were examined by fragment analysis and DNA-sequencing. The fragment analysis using different primer pairs was able to cluster species with different levels of success – some were informative, some split off species groups. The DNA sequences of the primers examined to date can be complicated by more than one copy of the gene pair in the diploids. We are currently selecting primer pairs which generate single bands to examine the variation in DNA sequence. After the establishment of a "SnoRNA-marker system", the taxonomy and evolution of more problematic groups (e.g. Mediterranean and South African group) within Senecio will be

examined. It is hoped that the snoRNA-marker will provide information on putative speciation/hybridization events and migration routes of the species of these groups.

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