

MOLECULAR EVOLUTIONARY AND PHYLOGENETIC IMPLICATIONS OF LINEAGE-SPECIFIC GROUP II INTRON GAINS AND LOSSES IN MITOCHONDRIA OF GYMNOSPERMS

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The rapid increase of complete mitochondrial (mt) genomic sequences in the public databases has revealed the uniqueness of their architecture, organization, expression and evolutionary history in plants. Huge size, additional genes, the presence of both Group I and Group II introns, the incorporation of foreign DNA from the chloroplast and the nucleus, high rate of recombination, and an ongoing functional gene transfer from the mitochondrion to the nucleus are typical features of the plant mt genomes.

Group II introns have been found in all land plants and algae mt genomes examined to date; their composition, position and distribution differs strikingly between vascular and non-vascular plant confirming the hypothesis that many independent intron gains and losses have occurred during evolution.

To get further insights into the pattern of group II introns allocation and conservation we came across the mt rps3 gene of a wide range of representatives of all the extant gymnosperms. According to a PCR assay and automated sequencing data, we now report the shared presence of two rps3 introns, namely the rps3i1 and the rps3i2 (Regina et al., 2005), in the mitochondria of the surveyed gymnosperms and unveil several remarkable exceptions among closely related species. On the whole our results provide compelling evidence that the distribution pattern of the rps3 introns is able to discriminate among divergent lineages of living gymnosperms and assist inferences of the evolutionary affiliations of particularly problematical representatives of this group of land plants.

Upon comparison of genomic and partial cDNA sequences, we also confirmed that both rps3i1 and the rps3i2 are accurately and efficiently excised in vivo. In addition, the comparative analysis of the novel rps3i2 orthologous sequences of *Cycas*, *Ginkgo*, *Gnetum* and *Pinus* allowed us to derive a secondary group IIA structural model.

These vascular plants constitute one of the major, but understudied plant group and occupy a pivotal position in evolution particularly with respect to the study of the seed plant origins and early divergence. Nonetheless, the relationships within the extant gymnosperm orders and families remain as yet questionable because of the diverse set of conflicting hypotheses generated in the past decade by morphological and molecular phylogenetic data.

To explore the phylogenetic utility of the mt intron sequences, we generated new mt molecular data sets combining sequence information from the rps3 locus and other mt sequences as well as information from all the three plant genomes of the same gymnosperm exemplar taxa. Our multigene and/or multigenome maximum parsimony and likelihood analyses support a phylogeny congruent with the conclusions reached by other molecular phylogenetic studies.

We propose, thus, the mt rps3i2 as a suitable informative character to highlight new mt genomic endeavours and diverse innovations characterizing the plant molecular biodiversity as well as to reinterpret the inter- and intrafamilial phylogenetic relationships among gymnosperms and to resolve the controversial position of Gnetales within seed plants.

Regina et al. (2005) *J Mol Evol* 60: 196-206