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Evaluating Mechanisms of RNA Editing in Plants

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Abstract

RNA editing is one of several post-transcriptional RNA processes. This process generates RNA and protein diversity in eukaryotes and results in specific amino acid substitutions, deletions, and changes in gene expression levels. It occurs in both plastids and mitochondria and typically involves the changing of specific C to U (cytosine to uracil). *Welwitschia* belongs to the gymnosperms (a group of seed-producing plants that includes conifers, cycads, *Ginkgo*, and Gnetales). It has already been substantiated that *Welwitschia mirabilis* has a major loss of *cis*-spliced introns and unusual *trans*-splicing introns. Research in the Mower lab has already proven that ancestral gymnosperm has high editing sites, from examining *Ginkgo* and *Cycas*. Knowing these high editing sites in other Gymnosperms, a prediction was made in *Welwitschia mirabilis* for a major loss of editing. In this study, we wished to evaluate the accuracy of this prediction.

Materials and Methods

- *Welwitschia* was grown in the Beadle Center green house at the University of Nebraska-Lincoln. Fresh tissue was collected for RNA extraction.
- RNA was isolated using a TRIzol reagent (Life technologies), and to remove genomic DNA, the isolated RNA was incubated with RNase-free DNase I (Thermo Fisher Scientific Inc.) and then the reaction was terminated with EDTA.
- First strand cDNA was made from the isolated RNA by reverse transcription using random hexamers and M-MLV reverse transcriptase. A negative control was also prepared to test for contamination.
- Reverse-transcriptase PCR were made using the cDNA and degenerate primers. RT-PCR primers were designed to amplify the protein coding genes in *Welwitschia*. Additional primers were made to assist in sequencing.
- RT-PCR products were sequenced at GenScript, and assembled with CodonCode Aligner. RNA editing sites were determined by comparing the aligned cDNA sequences with the DNA sequences (Hepburn et al. 2012, Rice et al. 2013, Richardson et al. 2013)

Images and Graphs

Figure 1 First Strand cDNA synthesis and RT-PCR

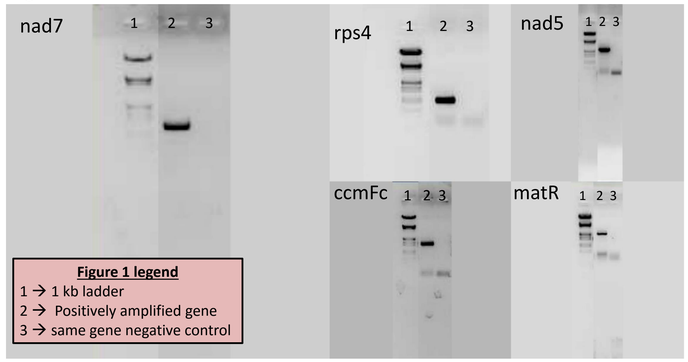

Figure 1. First strand cDNA synthesis and RT-PCR results for selected mitogenes. Gene name is labeled at top left on each gel picture. Lane 1 is the 1 kb marker, lane 2 is the positively amplified gene and lane 3 is the negative control for the same gene.

Table 2 Comparing predicted and observed edit sites in gymnosperms

	Gene	atp1	atp8	atp9	ccmFc	cob	cox1	cox3	matR	nad2	nad4	nad4L	nad5	nad7	rpl10	rps4	sdh4	Total
Welwitschia	Predicted	2	6	0	6	3	2	1	17	2	1	4	4	8	6	9	2	73
	Observed	0	0	0	3	0	0	0	5	0	0	0	1	7	0	4	0	20
Cycas	Predicted	15	14	12	-	53	9	29	-	-	-	16	-	-	-	-	-	148
	Observed	21	15	14	-	54	11	29	-	-	-	17	-	-	-	-	-	161
Ginkgo	Predicted	7	-	-	-	-	3	40	-	66	84	-	67	44	-	-	-	311
	Observed	7	-	-	-	-	1	40	-	79	95	-	74	55	-	-	-	351

Table 1 Comparing the predicted and observed edit sites in *Welwitschia*

Gene	Predicted*	Observed	O-P
atp1	2	0	-2
atp8	6	0	-6
atp9	0	0	0
ccmFc	6	3	-3
cob	3	0	-3
cox1	2	0	-2
cox3	1	0	-1
matR	17	5	-12
nad2	2	0	-2
nad4	1	0	-1
nad4L	4	0	-4
nad5	4	1	-3
nad7	8	7	-1
rpl10	6	0	-6
rps4	9	4	-5
sdh4	2	0	-2
Total	73	20	

* Predicted counts were taken from the regions that were amplified for empirical analysis

Figure 2 Loss of RNA editing sites and introns in selected *Welwitschia* genes compared with shared edits sites and introns in Ginkgo.

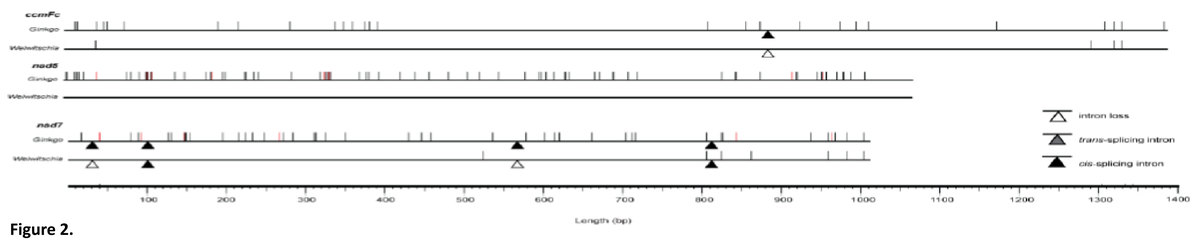

Figure 2.
Loss of RNA editing sites and introns in selected *Welwitschia* genes are compared with shared edits sites and introns in Ginkgo. Vertical lines indicate RNA editing sites. The selected gene names are shown on the left. For each gene only the amplified shared region was displayed and the length bar shown at the bottom.

Literature Cited

1. Hepburn NJ, Schmidt DW, Mower JP. 2012. Loss of two introns from the Magnolia tripetala mitochondrial cox2 gene implicates horizontal gene transfer and gene conversion as a novel mechanism of intron loss. Mol Biol Evol 29: 3111-3120.
2. Richardson, Rice DW, Young GJ, Alverson AJ, Palmer JD. 2013. The "fossilized" mitochondrial genome of Liriodendron tulipifera: ancestral gene content and order, ancestral editing sites, and extraordinarily low mutation rate. BMC Biol 11: 29.
3. Mower JP. 2009. The PREP suite: predictive RNA editors for plant mitochondrial genes, chloroplast genes and user-defined alignments. Nucleic Acids Res 37: W253-W259.
4. Guo W, Grewe F, Fan W, Young GJ, Knoop V, Palmer JD, Mower JP. 2016. Ginkgo and Welwitschia Mitogenomes Reveal Extreme Contrasts in Gymnosperm Mitochondrial Evolution. Mol Biol Evol 33: 1448-1460.
5. Rice DW, et al. 2013. Horizontal transfer of entire genomes via mitochondrial fusion in the angiosperm Amborella. Science 342: 1468-1473.

Results

- Successfully amplified several mitogenes based on first strand cDNA synthesis products. No bands show in the negative control confirmed that the amplified regions are from the RNA sequences (Figure 1).
- After amplifying the cDNA with designed primers specific to protein-coding genes we compared the cDNA and genomic sequences
- A total number of 20 C-to-U RNA editing sites were identified in five genes out of the selected 16 protein-coding genes in the *Welwitschia* mitogenome (Table 1). Prediction of C-to-U editing sites was conducted using PREP-Mt online tool (Mower 2009) with a cutoff of 0.2. Surprisingly, the number of editing sites determined from experimental data was much less than from the prediction (Table 1).
- The editing events were located in *rps4*, *nad5*, *nad7*, *ccmFc*, and *matR* genes in *Welwitschia*.
- 73 editing sites were located in the regions empirically examined by amplified RT-PCR products, whereas only 20 sited were experimentally identified
- *Welwitschia* lost many introns, and the surrounding editing sites are also lost. There maybe some relationships between the intron loss and RNA editing sites loss (Figure 2).

Discussion

- A previous study on the mitogenomes of gymnosperms, in the Mower lab, indicated a dramatic loss of RNA editing sites in *Welwitschia* relative to the ancestral high level of editing in gymnosperms (Table 2). When compared with the other selected/reported gymnosperm mitogenomes, *Welwitschia* is very low in editing sites (Figure 2).
- In this study, data confirmed that RNA editing is very low in *Welwitschia*, and surprisingly, even lower than the predicted number. Within the 16 examined functional protein-coding genes in *Welwitschia* mitogenome, RNA editing sites were detected from only 5 of them.
- In *Welwitschia*, there are two genes (*ccmFc* and *nad7*) that showed a significantly nonrandom distribution of edit sites (Figure 2), consistent with retroprocessing.
- In addition, there are three genes, *ccmFc*, *nad5* and *nad7*, that have lost introns and surrounding edit sites, which could be best explained by retroprocessing (Figure 2).