

# Legends to supplemental material

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## High level of conservation of mitochondrial RNA editing sites among four *Populus* species

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### Figure S1. Overview over the potential RNA editing sites in 29 mitochondrial CDS of three *Populus* species

All potential RNA editing sites identified in combined RNA-seq data sets of three *Populus* species (Table 1) were plotted to the nucleotide positions (Base) of the related CDS annotated in *P. tremula* W52 (Genbank accession KT337313) (Kersten *et al.* 2016). Bars in red indicate edited bases (editing sites), their height shows the editing extent in percent. Blue lines show the coverage at each base as long as it is 100 or below. All 29 CDS that are potentially affected by RNA editing in at least one of the four *Populus* species investigated are shown in individual rows. The mitochondrial RNA editing sites in the fourth species investigated, *P. tremula*, are shown in Figure 1 in the main text.

### Figure S2. RNA editing in mitochondrial genes of four *Populus* species

The 29 mitochondrial CDS that are potentially affected by RNA editing in at least one species are shown, one CDS on each page. Each line shows the editing extent of 100 bases. Bars indicate edited bases, their height shows the editing extent in percent. Lines show the coverage at each base as long as it is 100 or below. The four *Populus* species are color-coded in both bars and lines.

### Figure S3. Graphical representation of the mitochondrial genome of *Populus alba*

The outer circle represents the gene map. The grey arrows indicate the direction of transcription of the CDS drawn on the inner or outer side of the circle, respectively. A GC content graph is depicted within the inner circle. The circle inside the GC content graph marks the 50% threshold.

### Figure S4. Replacement of an editable cytidine by thymidine at the genomic level in the CDS of *nad6* at position 146 in *P. alba*.

In other *Populus* species (*P. tremula* and *P. davidiana*), the cytidine is edited at the RNA level (C-to-U; Table S4). For *P. trichocarpa*, a related genomic reference sequence is missing. The *nad6* CDS of *P. alba* var. *pyramidalis* was extracted from scaffold GWHAAEP00000188 (105625-106254 bp) of a recent whole genome assembly (Ma *et al.* 2018) and of *P. tremula* Asp201 from the scaffold Potra197846 (19887-20516 bp) of the *P. tremula* v1.1 whole genome assembly at PopGenIE (<http://popgenie.org/>) (Sundell *et al.* 2015). An excerpt of this alignment highlighting the polymorphism replacing the edited cytidine is shown in Figure 4 in the main text.

**File S1. R script to evaluate and graphically display bioinformatically identified potential editing sites**

**File S2. Varianttools software modified for the purpose of assessing genome-wide RNA editing**

**File S3. Reference sequences used to map RNA-seq data for the purpose of bioinformatically identifying potential RNA editing sites**

The file is in FASTA format.

**Table S1. Information about the NGS data used in this study**

- a. Metadata of the NGS data downloaded from SRA
- b. Metadata of the NGS data of the Thünen Institute of Forest Genetics

**Table S2. Detailed settings of the trimming, mapping, and variant detection tools of CLC Workbench Version 11.0**

**Table S3. Evaluation tables of potential RNA editing sites**

- a. List of all potential RNA editing sites bioinformatically identified by the procedure described in Materials and Methods. In the first columns, the editing extents ("frequency") in each of the four species are listed, followed by the editing extent quantified by Edera *et al.* (2018). The result of the manual evaluation of the sites not found by Edera *et al.* (2018) is also indicated. In the further columns, the raw data are listed.
- b. List of the RNA editing sites remaining after manual evaluation. In addition to the columns listed in Table S3a, there is the following information listed: The codon position, the genomic codon, the edited codon, the genomically encoded amino acid, and the amino acid encoded by the edited codon.
- c. Density calculation of RNA editing sites for the individual mitochondrial CDS.
- d. Comparison of RNA editing sites between the four *Populus* species investigated. The values for the editing extent are highlighted to help identify sites that are not detected as being edited in some species.
- e. List of all RNA editing sites that show editing in all four *Populus* species investigated.

**Table S4. List of SNPs between the mitochondrial genomes of *P. davidiana* and *P. alba*.**