

# PHYSICAL LOCATION OF 18S-28S AND 5S RIBOSOMAL RNA GENES IN CHINESE CHESTNUT (*CASTANEA MOLLISSIMA*)

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For the current analysis we report on the distribution of the ribosomal rRNA genes (i.e., rDNA loci) in Chinese chestnut. Two sites of 18S-28S rDNA (one major and the other minor) and one site of 5S rDNA were identified, and each is located on a different pair of homologous chromosomes. Earlier we reported similar results for these rDNA loci in American chestnut. However, we note here that the chromosome pair with the major 18S-28S rDNA locus contains a much larger satellite (i.e., chromosome region between major 18S-28S locus and the telomere) in Chinese chestnut than in American chestnut.

## Introduction

The American chestnut (*Castanea dentata*,  $2n=2x=24$ ), once known as the “King of the Appalachian Forest”, was nearly destroyed by *Cryphonectria parasitica* (causing chestnut blight disease) a fungal pathogen accidentally introduced from Asia in the late 1800s (Hepting, 1974). In contrast to American chestnut, Chinese chestnut (*C. mollissima*,  $2n=2x=24$ ) is resistant to *C. parasitica*. Utilizing American chestnut trees (mostly stump sprouts) still found in the wild, The American Chestnut Foundation (TACF) is transferring resistance from Chinese chestnut into American chestnut through backcross breeding. To facilitate this work our lab has teamed up with TACF to carryout cytogenetics research. We have been successful in preparing chestnut chromosome spreads from somatic tissue (root-tip meristems) and reproductive tissue (pollen mother cells) and applying fluorescent *in situ* hybridization (FISH) techniques to localize genetic elements on particular chromosomes. FISH analysis along with traditional cytogenetics is revealing important details of the structural organization of the chestnut genomes. Ribosomal RNA gene families (18S-28S rDNA and 5S rDNA) provide valuable cytological landmarks for karyotyping and studying the relationships between species and genera (Heslop-Harrison et al., 1992). Earlier we reported that American chestnut has two 18S-28S rDNA sites, one major and the other is minor, and each is located on a different pair of homologous chromosomes (Islam-Faridi et al., 2009). We also reported that the 5S rRNA gene is located interstitially on a third chromosome pair. Here we provide a preliminary report on the physical location of the rRNA genes in Chinese chestnut.

## Materials and Methods

Germinating seedlings of the Chinese chestnut cultivar ‘Veselicky’ were grown in MetroMix potting soils (SunGrow SB-650) in a greenhouse in College Station, TX. Actively growing root tips, about 1 cm long and appearing milky white and transparent, were harvested into either 2.5 mM hydroxyquinoline solution or 0.8%  $\alpha$ -bromonaphthalene aqueous solution, pre-treated for 2

h in the dark to accumulate metaphases, and then fixed in 4:1 95% EtOH:GAA. The root-tips were then enzymatically processed to prepare chromosome spreads as described elsewhere (Jewell and Islam-Faridi 1994), except that the enzyme solution was modified to the following: 40% (v/v) Cellulase (C2730, Sigma), 20% (v/v) Pectinase (P2611, Sigma), 40% (v/v) 0.01 M Citrate buffer, 2% (w/v) Cellulase RS (SERVA Electrophoresis GmbH), 3% Cellulase R10 (Yakult Pharmaceutical, Japan), 1% Macerozyme (Yakult Pharmaceutical, Japan) and 1.5% Pectolyase Y23 (Kyowa Chemical, Japan).

Whole plasmid DNA with 18S-28S rDNA insert of maize or 5S rDNA insert of sugar beet including the spacer region were labeled with biotin-16-dUTP (Biotin-Nick Translation Mix, Roche, Germany) or digoxigenin-11-dUTP (Dig-Nick-Translation Mix, Roche, Germany) following the manufacturer's instructions. Agarose gel electrophoresis was used to check the fragment sizes of the probe DNA and labeled nucleotide incorporation was verified by dot-blotting.

Standard FISH technique was utilized as previously reported (Hanson et al., 1996; Islam-Faridi et al., 2002). Sites of biotin-labeled probe hybridization were detected using Cy3-conjugated streptavidin (Jackson Immuno Research Laboratories, USA). Sites of digoxigenin-labeled probe binding were visualized using fluorescein-conjugated sheep anti-digoxigenin (Roche, Germany). Slides were counter stained with DAPI (4 ug/ml) in McIlvaine buffer (pH 7.0) including a small drop (10 ul) of Vectashield (Vector Laboratories, USA) to prevent photo-bleaching the fluorochromes.

Chromosome spreads were viewed under a 63X plan apo-chromatic objective and digital images were recorded using an epi-fluorescence microscope (AxioImager M2, Carl Zeiss, Germany) with suitable filter sets (Chroma Technology, USA) and a CoolCube 1 high performance CCD camera. Images were pre-processed with Ikaros and ISIS v5.1 (MetaSystems Inc., USA) and then further processed with Adobe Photoshop CS v8 (Adobe Systems, USA).

## **Results and Discussion**

Our data show that we were successful at preparing chestnut somatic chromosome spreads from root-tips meristems on ethanol-cleaned glass slides. The chromosomes are sharp and distinct and all are observed to be in unifocal position (e.g., see Fig. 1). All 24 chromosomes of Chinese chestnut can easily be separated from each other with essentially no overlap. We occasionally see 26 chromosomes, mainly from prophase to early metaphase. The extra two apparent chromosomes are actually detached satellite regions (i.e., region distal of the major 18S-28S rDNA locus) from the homologous pair of satellited chromosomes. FISH with 18S-28S rDNA probe showed that the satellite region is connected with the nucleolus organizer region (NOR), the site of the rRNA gene, as revealed by the 18S-28S rDNA signal (Fig. 1).

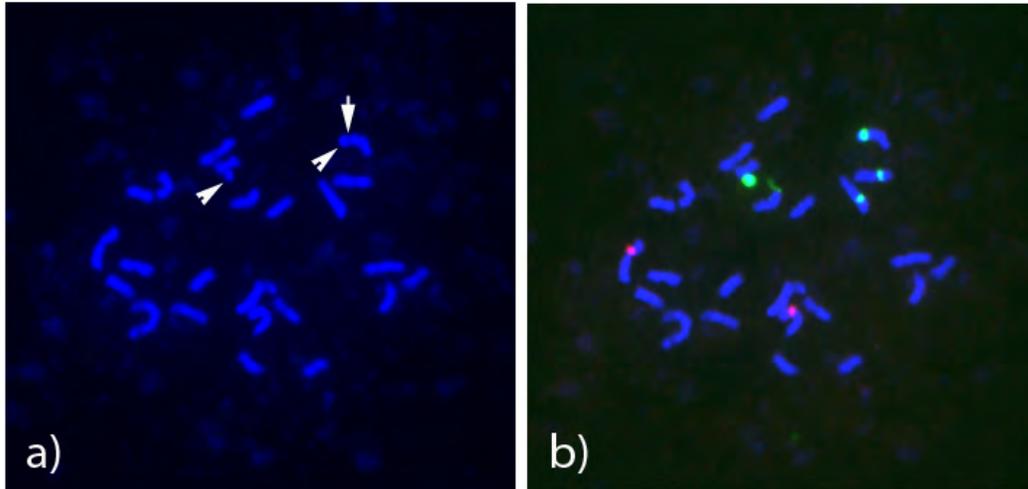


Figure 1. Images of FISH using rDNA probes (18S-28S and 5S) on somatic chromosome spread of Chinese chestnut cultivar ‘Veselicky’: (a) DAPI image of metaphase chromosomes, arrow shows the NOR (the site of 18S-28S rDNA, green signal in 1b) and arrowheads show the satellite regions; (b) the same chromosomes as in 1a with 18S-28S and 5S rDNA FISH signals (green and red, respectively).

We have observed two sites of 18S-28S rDNA (one major and one minor) and one site of 5S rDNA in Chinese chestnut cultivar ‘Veselicky’ (Fig. 1b). Similar results were also reported in American chestnut (Islam-Faridi et al., 2009). The satellite region in Chinese chestnut is clearly larger than its counterpart in American chestnut. The larger satellite region in Chinese chestnut might have resulted from an unequal cross-over. In addition, our results suggest that there might be a second 5S rDNA locus in Chinese chestnut. Additional research involving other Chinese chestnut cultivars is being carried out to confirm the second 5S rDNA locus and to evaluate size of the satellited region and other possible variations in the major 18S-28S rDNA locus.

Based on the above results, we conclude that these two species are structurally different from each other with respect to their satellited chromosome. This structural difference can serve as a marker indicating species origin of this chromosome in any individual tree, which can be especially useful in interspecies hybrid backcross breeding programs. It remains to be determined whether species heterozygosity at this rDNA locus will be beneficial or not, or if any important QTLs or genes are linked to the locus.

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