Chromosomal Distribution of Repetitive DNA Sequences in Pine using Fluorescent *In Situ* Hybridization (FISH)

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The genus *Pinus* (pines, $2n = 2x = 24$) includes many economically and ecologically important species worldwide. Pine genomes are exceptionally large with 1C (i.e., haploid DNA) contents ranging from 20 to 30 billion base pairs (Wakamiya et al. 1993; Bogunic et al. 2003). Much of this DNA content consists of high numbers of *copia*- and *gypsy*-like long terminal repeat (LTR) retrotransposons. A few of these retrotransposons have been formally described in conifers (Kamm et al. 1996; Kossack and Kinlaw 1999; Friesen et al. 2001; Rocheta et al. 2007; Morse et al. 2009), although only one (G*ymny*, Morse et al. 2009) has been characterized using fluorescent *in situ* hybridization (FISH). Here we present initial FISH-based characterization of 32 newly isolated repetitive pine DNA sequences.

MATERIALS AND METHODS

Plant Material and Slide Preparation: Actively growing root tips from loblolly and slash pines (*P. taeda* and *P. elliottii*, respectively), about 1.5 cm long, were excised and pre-treated in the dark with 0.15% w/v colchicine solution (Sigma, USA). Following pre-treatment, the root tips were fixed in 2:1:1 95% ethanol:glacial acetid acid:double distilled water. Chromosome spreads were prepared as described previously (Islam-Faridi et al. 2007).

Probes: Loblolly pine isolated Cot fractionated DNA components and random genomic DNA fragments were cloned and sequenced using Sanger/capillary sequencing as described previously (Peterson et al. 2002). The *Sequence Read Classification Pipeline* (SRCP) of Chouvarine et al. (2008) was used to identify sequences that either exhibited homology to known repeats or were shown to be repetitive based upon their relative frequencies in the loblolly pine data sets.

Probe DNA and Nick Translation: The probe DNAs were isolated as described by Childs et al. (2001) and labeled either with biotin-16-dUTP (Biotin-Nick Translation Mix, Roche, Germany) or digoxigenin-11-dUTP (Dig-Nick-Translation Mix, Roche, Germany) following instructions provided by the manufacturer.

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Fluorescent *In Situ* Hybridization: Hybridization was performed according to a standard protocol (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 µg/ml) and place a small drop (10µl) of Vectashield (Vector Laboratories, USA) to prevent fluorochrome (Cy3 and fluorescein) photo-bleaching.

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

RESULTS AND DISCUSSION

To date about half of the 32 repetitive DNA clones have been studied in FISH experiments. In most experiments an 18S-28S rDNA probe was used as a control. Most of the repetitive clones show sparse to dense hybridization signals scattered throughout the genome except clones PT-7G-2G21 (GenBank Accession ET182153.1) which was classified as a repeat based on *de novo* (within loblolly pine) sequence analysis and PT-7G-2H13 (ET182162.1) which shows significant homology (S' = 464) to the *Prunus x yedoensis gypsy*-like retrotransposon PIRE1. Clone PT-7G-2G21 was found to be distributed densely in the distal half of each chromosome arm with only sparse hybridization observed in the pericentromeric region of each chromosome and essentially no hybridization in the centromere regions (Figure 1). Clone PT-7G-2H13 hybridized to the end of a single pair of homologous chromosomes and was positioned distal to an 18S-28S rDNA locus (Figure 2), a finding somewhat surprising based upon its high sequence similarity to PIRE1.

Fluorescent *in situ* hybridization combined with molecular characterization of various retrotransposons promises to shed light on the evolution of pine genome size and complexity (Morse et al. 2009). Uniquely distributed, repetitive DNA sequences are useful as "genomic landmarks or signatures" and they will likely play an important role in chromosome identification, physical mapping and genome sequencing.

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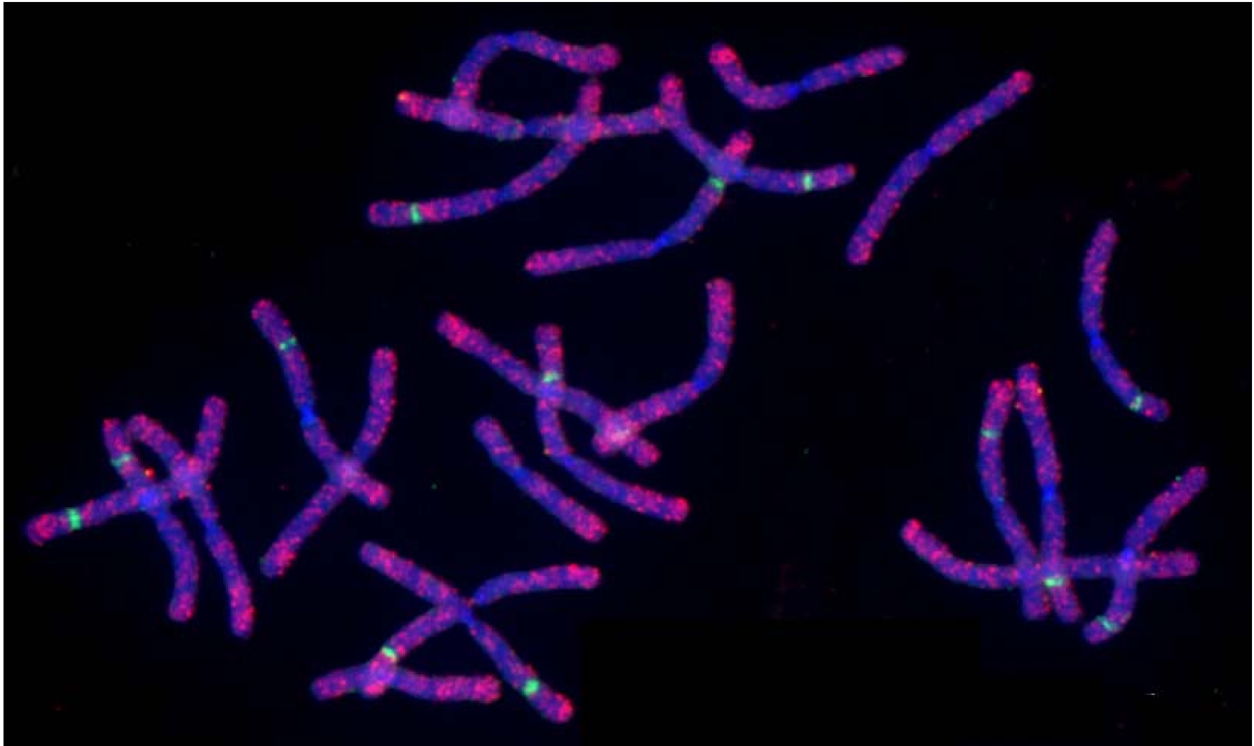

Figure 1. FISH of loblolly pine chromosomes probed with 18S-28S rDNA (green signals) and PT-7G-2G21 (red signals).

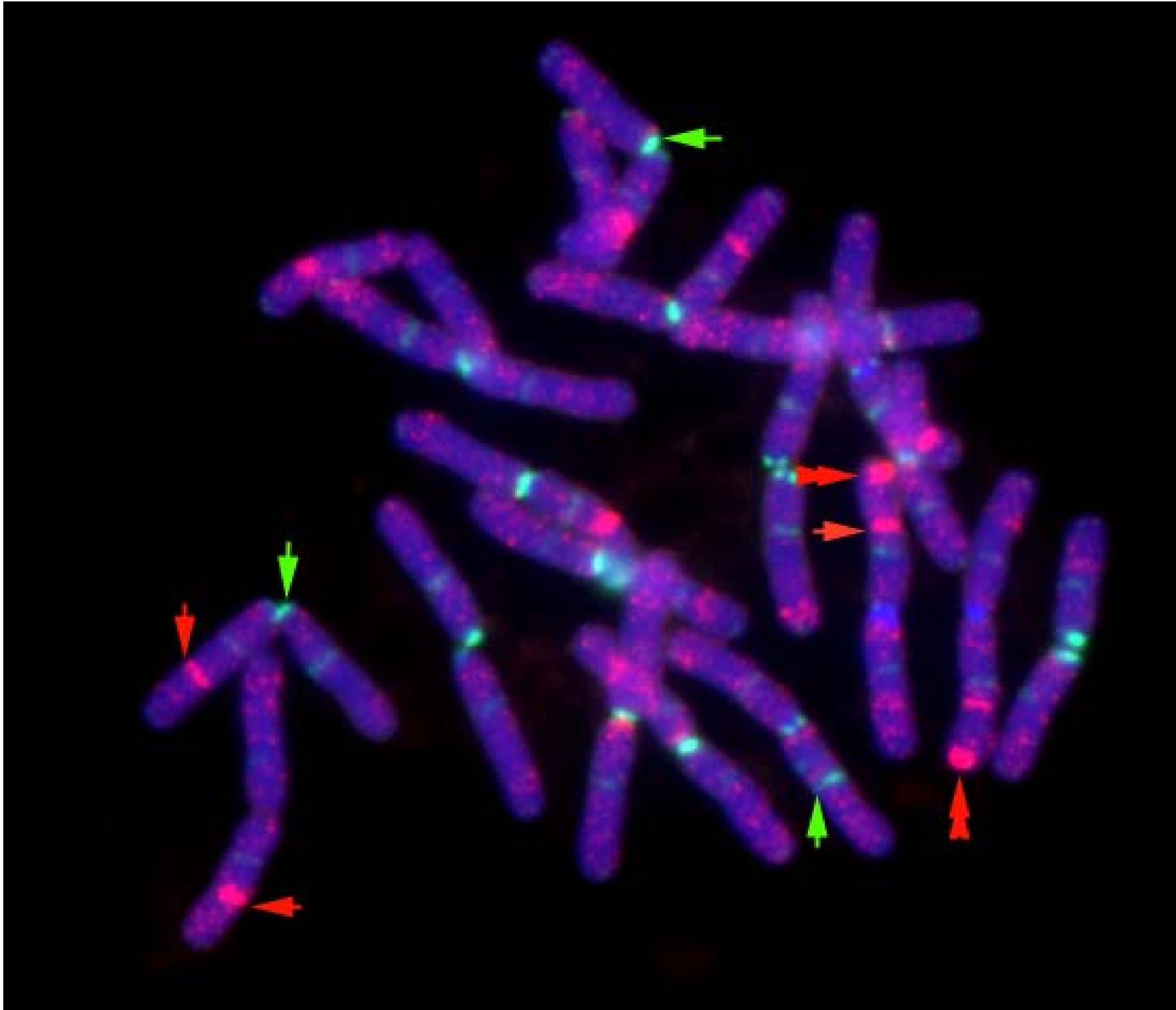

Figure 2. FISH of slash pine chromosomes probed with 18S rDNA (red signals appearing as blocks, red arrows), Arabidopsis-type telomere repeat DNA (green signals, green arrows) and PT-7G-2H13 (red signals). Probe PT-7G-2H13 is sparsely dispersed on every chromosome and densely located near the ends of a pair of homologous chromosomes (red double arrow heads).