# CHROMOSOME STRUCTURE AND MOLECULAR CYTOGENETICS OF SOUTHERN PINES

Mary E. Mason and Robert L. Doudrick

Biologist and Project Leader, Southern Institute of Forest Genetics,
USDA Forest Service, Harrison Experimental Forest, 23332 Hwy 67, Saucier, MS 39574.

Keywords: *Pinus elliottii, Pinus palustris, Pinus strobus,* molecular cytogenetics

Molecular cytogenetics is the application of the techniques of molecular biology to the study of chromosome structure and function. Molecular cytogenetics principally involves the application of fluorescent *in situ* hybridization or FISH, a technique that allows the localization of specific sequences on metaphase chromosomes and interphase nuclei. Other techniques, such as primed *in situ* amplification, *in situ* polymerase chain reaction, and total genomic *in situ* hybridization are also important. These techniques permit the correlation of genetic linkage groups and physical chromosomes. The techniques also allow the localization of repetitive sequences, and the localization of genes in gene families which often have greatly different copy numbers at each locus and intra locus polymorphism, making them difficult to map using other mapping techniques.

Physical mapping using fluorescent *in situ* hybridization (FISH) involves the preparation of spreads of intact chromosomes, pretreatment of these spreads to remove cytoplasmic materials, labeling and hybridization of probe DNA, and finally detection and visualization of sites of hybridization using fluorescent microscopy. Successful FISH experiments require preparation of slides with adequate numbers of metaphase chromosomes from diploid cells, well spread but with the complete complement, and without overlaying cytoplasm that blocks hybridizations. For preparation of pine chromosome spreads, radicles are collected from germinating seedlings and treated with colchicine to increase the number of metaphase cells. Root tips are then partially digested in a cell wall degrading enzyme mix (cellulase, hemicellulase, pectolyase, and pectinase) and the meristem dissected into a pool of acetic acid on a clean microscope slide. The dispersed meristem cells are then squashed onto the slide by standing on a glass coverslip placed over the slide. Before hybridization, chromosome spreads are treated with RNase and pepsin (a protease) to remove cytoplasmic material covering the chromosomes. Commercially available nucleotides labeled with fluorescent molecules (or molecules to which antibodies with fluorescent labels attached are available) are used to prepare DNA to be used as probe in FISH experiments. Labeled nucleotides can be incorporated into DNA using standard molecular biological techniques. This DNA and the chromosome spreads are heat denatured to separate the complementary strands of DNA. The probe solution is added to the slides so that during re-annealing the labeled DNA will base pair with the chromosomal DNA forming a hybrid strand. When viewed under a microscope with the appropriate filter sets, chromosomes, nuclei, and sites of hybridization can be clearly observed. Fluorescent dyes which stain nucleic acids, such as DAPI (4',6-diamidino-2-phenylindole, which stains A-T rich regions) and CMA

(chromomycin-$A_3$, which stains G-C rich regions)), are also used. These dyes provide information about overall base composition along the chromosomes and serve to counterstain the chromosomes, which is useful during analysis. Images of hybridization to chromosomes are captured on color print film. The negatives are then scanned and digitized, allowing computer analysis of pixel intensity along the chromosome median axis. Multiple chromosome spreads are analyzed and the data pooled to create ideograms, or diagrams of gene locations along chromosomes.

Applications of molecular cytogenetics techniques to pine is at an early stage of development. Patterns of fluorescent *in situ* hybridization to genes for the large and small rDNA subunits and fluorochrome banding patterns using CMA and DAPI have allowed all twelve pairs of chromosomes of slash pine to be identified and a standard karyotype proposed for pine (Doudrick et al. 1995). We are currently extending this karyotype to other pine species, including longleaf pine and white pine, allowing comparative analyses of patterns of hybridization. Preliminary results indicate that the 18-25S rDNA sites are highly conserved among the three species, with both intercalary and centromeric sites being observed in both longleaf and white pine, as in slash pine. Further analysis will be necessary to determine the conservation of exact distances along the chromosomes. The DAPI banding pattern of slash pine appears to be conserved in longleaf pine, but different in white pine as has been previously reported (MacPherson and Fillion 1981). DAPI negative bands have been observed in white pine but not DAPI positive bands. Both DAPI positive and negative bands are seen in longleaf pine, much as in slash pine. We are currently working to quantify these similarities and differences and to extend these results to other species.

## Literature Cited

Doudrick, RL, JS Heslop-Harrison, CD Nelson, T Schmidt, WL Nance, and T Schwarzacher, 1995 Karyotype of Slash Pine *(Pinus elliottii* var. *elliottii)* Using Patterns of Fluorescent *in* situ Hybridization and Fluorochrome Banding. Journal of Heredity 86: 289-296.

MacPherson P and WG Fillion, 1981. Karyotype analysis and the distribution of constitutive heterochromatin in five species of *Pinus*. Journal of Heredity 72: 193-198.