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Mapped DNA probes from loblolly pine can be used for restriction fragment length polymorphism mapping in other conifers

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Abstract A high-density genetic map based on restriction fragment length polymorphisms (RFLPs) is being constructed for loblolly pine (*Pinus taeda* L.). Consequently, a large number of DNA probes from loblolly pine are potentially available for use in other species. We have used some of these DNA probes to detect RFLPs in 12 conifers and an angiosperm. Thirty complementary DNA and two genomic DNA probes from loblolly pine were hybridized to Southern blots containing DNA from five species of *Pinus* (*P. elliottii*, *P. lambertiana*, *P. radiata*, *P. sylvestris*, and *P. taeda*), one species from each of four other genera of Pinaceae (*Abies concolor*, *Larix laricina*, *Picea abies*, and *Pseudotsuga menziesii*), one species from each of three other families of Coniferales [*Sequoia sempervirens* (Taxodiaceae), *Torreya californica* (Taxaceae) and *Calocedrus decurrens* (Cupressaceae)], and to one angiosperm species (*Populus nigra*). Results showed that mapped DNA probes from loblolly pine will cross-hybridize to genomic DNA of other species of *Pinus* and some other genera of the Pinaceae. Only a small proportion of the probes hybridized to genomic DNA from three other families of the Coniferales and the one angiosperm examined. This study demonstrates that mapped DNA probes from loblolly pine can be used to construct RFLP maps for related species, thus enabling the opportunity for comparative genome mapping in conifers.

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Introduction

We are constructing genetic maps for loblolly pine (*Pinus taeda* L.) based on restriction fragment length polymorphisms (RFLPs). We have mapped a large number of loci using complementary DNA (cDNA) and genomic DNA probes from loblolly pine (Devey et al. 1991; Neale and Williams 1991; Neale et al. 1992). The purpose of the present study was to determine whether mapped DNA probes from loblolly pine would cross-hybridize to the genomic DNA of other pines and conifers and, if so, whether such probes could be useful for mapping in other species. Screening cDNA and genomic DNA libraries for DNA probes that reveal interpretable RFLP patterns is very time-consuming. Much additional effort is required to obtain segregation data and map such probes. Thus mapped RFLP probes are a valuable resource for mapping projects in other loblolly pine crosses or for other conifers. A more important reason for using common DNA probes across species is the opportunity to directly compare genetic maps and other genetic information. A wealth of information on conifer chromosome evolution can be gained if common probes are used among species. Our data suggest that nearly all loblolly pine probes cross-hybridize to DNA from species within the genus *Pinus* and a large proportion of these probes can be used for other genera of Pinaceae.

Materials and methods

Plant species

A sample of 12 conifer and one angiosperm species was chosen for this study. Species were selected to obtain a broad taxonomic representa-

tion and to include species likely to be the subject of genetic mapping projects. We chose five species from the genus *Pinus* (*P. taeda* L., *P. elliottii* Engelm., *P. radiata* D. Don, *P. sylvestris* L., and *P. lambertiana* Dougl.), one species from each of four other genera of Pinaceae [*Abies concolor* (Gord. and Glend.) Lindl., *Larix laricina* (Du Roi) K. Koch, *Picea abies* L. Karst, and *Pseudotsuga menziesii* (Mirb.) Franco], one species of one genus of three families of the Coniferales [*Sequoia sempervirens* D. Don. Endl. (Taxodiaceae), *Torreya californica* Torr. (Taxaceae), and *Calocedrus decurrens* (Torr.) Florin (Cupressaceae)], and one angiosperm, *Populus nigra* L. (Salicaceae). Plant material was collected from the Eddy Arboretum at the Institute of Forest Genetics, Placerville, Calif., and the Botanical Garden of the University of California, Berkeley, except for *Pinus elliottii*, which was obtained from Oklahoma State University.

DNA isolation and RFLP analyses

Genomic DNA was isolated from the 12 conifers and one *Populus* species by a slight modification of the method of Devey et al. (1991). Needle/leaf tissue was cut into small pieces, then ground in liquid nitrogen in a steel blender and stored at −80 °C. Ten grams fresh weight of ground tissue was homogenized with a Brinkmann polytron at 4 °C in 150 ml of extraction buffer [50 mM TRIS (pH 8.0), 5 mM EDTA, 0.35 M sorbitol, 0.1% BSA, 0.1% mercaptoethanol, 10% (wt/vol) polyvinyl pyrrolidine (M.Wt. 40,000)]. The homogenate was filtered through two layers of cheesecloth and one layer of Miracloth (Calbiochem) and then centrifuged at 3 000 rpm (Sorvall, GSA rotor), at 4 °C for 10 min. The pellet was resuspended in 15 ml of buffer [50 mM TRIS (pH8.0), 25 mM EDTA, 0.35 M sorbitol, 0.1% mercaptoethanol] in a 50-ml tube. The suspension was brought to room temperature, and N-laurylsacrosine was added to a concentration of 1% (w/v); after 15 min the sample was brought to a final concentration of 0.7 M sodium chloride and then to 1% (w/v) with hexadecyltrimethylammonium bromide (CTAB) and incubated at 60 °C for 30 min. An organic extraction was then performed on the DNA sample using an equal amount of a choloroform: octanol mixture (24:1). The sample was centrifuged at high speed, and the aqueous layer precipitated with 2/3 vol isopropanol, 0.03 M ammonium acetate. The DNA pellet was removed and resuspended in 1–2 ml of double distilled water, to which calcium chloride was added to bring the concentration to 5 mM, and centrifuged at 9 000 rpm (Sorvall, rotor SS-34), at 4 °C for 10 min. The supernatant was transferred to another tube, and 1/4 vol 10 M ammonium acetate, and then 2/3 vol isopropanol alcohol was added to precipitate the DNA. The DNA was transferred to 50% isopropanol, 0.3 M ammonium acetate for 30 min to overnight. The DNA was dissolved in a small amount (0.2–0.5 ml) of TRIS-EDTA, pH 8.0. The concentration of DNA was read on a fluorometer.

Genomic DNA was digested with the restriction enzyme *Hin*dIII using 1 unit of enzyme per microgram of DNA for an incubation period of at least 4 h. Ten micrograms of loblolly pine DNA was used as a standard and the DNA amounts of the other species were adjusted to equal genomic equivalents using the genome size estimates of Dhillon (1987) and Kriebel (1993).

Southern blotting, probe preparation, and probe hybridization were carried out as described by Devey et al. (1991). Blots were hybridized with 30 cDNA (pPtIFG86,147, 149, 602, 605, 606, 624, 653, 658, 660, 669, 670, 975, 1154, 1584, 1635, 1889, 1918, 1919, 1940, 2006, 2022, 2150, 2220, 2274, 2421, 2553, 2568, 2610, and 2738) and two genomic DNA (pPtIFG1A2 and pPtIFG1D9) mapped probes from loblolly pine. The final wash of all hybridizations was done under high-stringency conditions, e.g., 65 °C, 0.1 × SSPE. These probes were selected on the basis of the relatively simple RFLP band patterns which they produced in loblolly pine and also to include at least two probes per linkage group.

The 32 autoradiograms produced by this study have been submitted to the forest tree genome database, Dendrome. The images can be accessed and viewed using gopher software through Internet. Inquiries regarding electronic retrieval of the images can be sent to info@s27w007.pswfs.gov or by contacting DBN directly.

Table 1 The number of loblolly pine RFLP probes hybridizing to genomic DNA of 12 conifers and one angiosperm from a total of 32 probes tested

Species	No. probes hybridizing	Percent
Pinus taeda	32	100%
Pinus elliotti	32	100%
Pinus lambertina	32	100%
Pinus radiata	32	100%
Pinus sylvestris	32	100%
Picea abies	31	97%
Pseudotsuga menziesii	27	85%
Larix laricina	28	88%
Abies concolor	25	78%
Sequoia sempervirens	2	38%
Calocedrus decurrens	7	22%
Torreya californica	4	13%
Populus nigra	6	19%

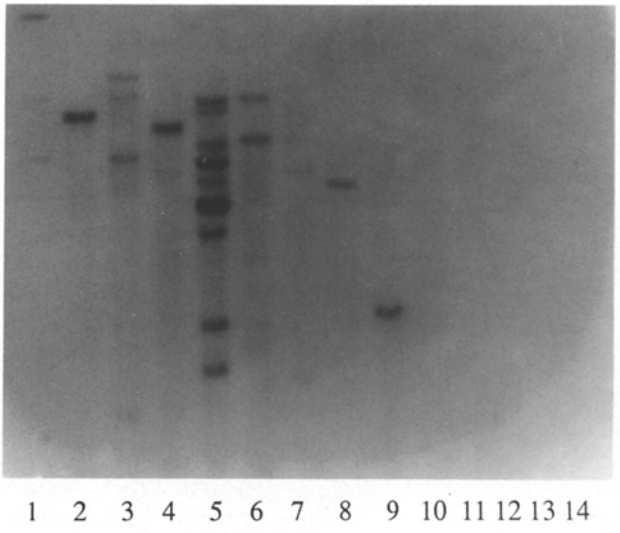

Fig. 1 Hybridization of loblolly pine cDNA probe 624 to genomic DNA from 12 conifer and one angiosperm species. *Lane 1 Hin*dIII lambda size marker. *Lanes 2–16: Pinus elliottii, P. lambertiana, P. radiata, P. sylvestris, P. taeda, Abies concolor, Larix laricina, Picea abies, Pseudotsuga menziesii, Calocedrus decurrens, Populus nigra, Sequoia sempervirens, Torreya californica*, respectively

Results and discussion

This study demonstrates that mapped RFLP probes from loblolly pine will cross-hybridize to DNA from other pines and occassionally to even more distantly related conifers. Thirty-two mapped RFLP probes from loblolly pine were cross-hybridized to Southern blots containing DNA from 12 conifers and one angiosperm (Table 1). All 32 probes hybridized to all five pine species and thus it seems reasonable to expect that nearly all of the mapped RFLP probes would hybridize to any species of pine. This is an important result because it suggests that genome maps in pines can be constructed

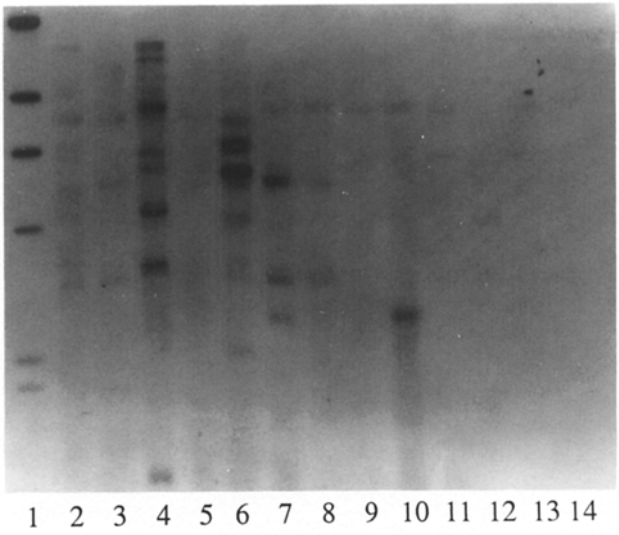

Fig. 2 Hybridization of loblolly pine cDNA probe 658 to genomic DNA from 12 conifer and one angiosperm species. Lane identifications are the same as in Fig. 1

with a common or shared set of DNA probes, which would ultimately lead to new and fundamental insights into pine chromosome evolution. Additionally, DNA marker information gained in one species might be directly applied to a second species. For example, RFLP loci linked to a quantitative trait locus (QTL) in one species might in fact be linked to a similar QTL in another species. The potential for integration of genetic information across species from common genetic markers seems limitless.

The loblolly pine RFLP probes also cross-hybridized to conifers other than pines (Table 1). Five probes (658, 1918, 1919, 2006, and 2274) cross-hybridized to all conifers as well as to poplar. It is likely that these cDNA clones are from very conserved plant genes. We plan to determine the nucleotide sequence of these and all other probes and will subsequently be able to test this hypothesis. Five probes (86, 1889, 2220, 2568, and 2738) hybridized to at least one of the other families of Pinaceae, and an additional 16 probes (1A2, 1D9, 149, 602, 605, 606, 624, 653, 669, 975, 1154, 1635, 2022, 2421, 2553, and 2610) hybridized to all the examined members of the Pinaceae. These cDNAs presumably represent a more evolutionarily diverged set of genes, but could nevertheless be useful for comparing genetic maps from different genera of Pinaceae that were made with genus-specific probes. Finally, six probes (147, 660, 670, 1584, 1940, and 2150) hybridized to only the pines and to one or two other genera of Pinaceae. These cDNAs are the most evolutionarily divergent and are useful only for mapping within the pines.

A crude approximation of gene-family size can be made by counting fragments on autoradiograms. Some probes, such as 605, hybridize to as few as 2–5 bands, whereas others, such as 2274 and 1919, hybridize to more than ten bands. It is clear from these data that gene-family sizes in conifers are universally quite large. There are, however, some very interesting exceptions. Three probes (1154, 1584, and 2022) showed large differences among the pines in respect of the number of fragments. The most striking example was probe 2022 which revealed three fragments in *P. taeda*, *P. elliottii*, *P. radiata*, and *P. sylvestris* but more than ten fragments in *P. lambertiana*. Differences of this type can also be seen among the other genera of Pinaceae for probes 149, 602, 975, and 2553. Probes 602 and 975 hybridized to more than ten fragments in pines but only 3–4 fragments in the other genera. This difference could have resulted from specific amplification of these gene families in pines but alternatively could be due to sequence divergence and reduced hybridization of the loblolly pine probes. More interesting hybridization patterns were revealed by probes 149 and 2553. In these cases, one or more genera of Pinaceae had many more fragments than the pines. These probes show gene-family amplification specific to just a few genera. Genes of this type would be interesting for future study.

Genome maps have now been prepared for dozens of plant species. In some cases, maps for one species were constructed with RFLP probes from a related species: for example tomato and potato (Bonierbale et al. 1988; Gebhardt et al. 1991), tomato and pepper (Tanksley et al. 1988), sorghum and related species (Hulbert et al. 1990), pea and lentil (Weeden et al. 1992), and barley and rye (Wang et al. 1992). Comparative maps within groups of related species will be valuable for understanding speciation and evolution as well as having practical importance for the map-based cloning of important genes. The current study demonstrates that such comparative mapping within the pines, and even within the Pinaceae, is possible with a common set of RFLP probes. We used loblolly pine cDNA and genomic DNA probes but would expect that cDNA probes, at least, from any species could be used. Conkle (1981) showed that some degree of synteny exists among pines on the basis of a small number of isozyme linkage relationships; however, high-density molecular maps should provide new insight into the comparative genome organization of conifers.

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