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Inheritance of restriction fragment length polymorphisms and random amplified polymorphic DNAs in coastal Douglas-fir

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Abstract A total of 225 new genetic loci [151 restriction fragment length polymorphisms (RFLP) and 74 random amplified polymorphic DNAs (RAPD)] in coastal Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*] have been identified using a three-generation outbred pedigree. The Mendelian inheritance of 16 RFLP loci and 29 RAPD loci was demonstrated based on single-locus segregation in a sample of F_2 progeny. One RFLP locus, PtIFG2025, showed segregation distortion. Probe pPtIFG2025 is a loblolly pine cDNA probe encoding for *rbcS*. The 16 RFLP loci and 23 allozyme loci were also assayed in a sample of 16 Douglas-fir seed-orchard clones. Allelism was determined at 11 of the 16 RFLP loci. RFLPs were able to detect slightly more variation (4.0 alleles per locus) than allozymes (3.1 alleles per locus). The inheritance of an additional 80 RAPD loci was determined based on haploid segregation analysis of megagametophytes from parent tree 013-1. Once 200–300 markers are identified and placed on a genetic map, quantitative trait loci affecting bud phenology will be mapped.

Key words Douglas-fir · Genetic mapping · RFLP RAPD · allozymes

Introduction

We are constructing a genetic linkage map for coastal Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*] by using restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), and allozymes. The mapping population is a three-generation outbred pedigree. Our objectives are to identify and map 200–300 genetic markers and, subsequently, to identify quantitative trait loci (QTLs) controlling bud phenology traits in Douglas-fir.

Douglas-fir is a long-lived species and has an extensive range in western North America (Silen,1978). It is monoecious and has an outcrossed mating system (Shaw and Allard 1982; Neale and Adams 1985). Douglas-fir shows a high degree of genetic variation between and among populations (Yeh and O'Malley 1980), which gives rise to a number of ecotypes (Campbell 1986; Campbell and Sorenson 1978; Rehfeldt 1979, 1989; Sorenson 1983). Estimates of the size of the Douglas-fir genome range between 25 and 34 pg per haploid nucleus (Ingle et al. 1975; Carlson et al. 1991). The genome is organized into 13 pairs of chromosomes (2n=26). This organization is an unusual and intriguing facet of *P. menziesii* as virtually all other members of the *Pinaceae* have 12 pairs of chromosomes (2n=24).

A variety of genetic marker types have been used in Douglas-fir genetic studies. Morphological markers such as yellow foliage, white cotyledons, non-white cotyledonous lethals, virescence, dwarfs, and mottled and curly needles were used in many early studies (Piesch and Stettler 1971; Sorensen 1971, 1973); these are generally single-gene recessive markers. Monoterpene genetic markers have been used to study genic diversity, pest resistance, genetic drift and migrational history of Douglas-fir (Zavarin and Snajberk 1973; von Rudloff 1975; Snajberk and Zavarin 1976; von Rudloff and Rehfeldt 1980; Critchfield 1984; Hanover 1992). Although there are numerous terpene markers, seasonal and epigenetic variation present constraints (Hanover 1992). During the last 15 years, allozymes have been used extensively for studies of genic diversity (Yeh and O'Malley 1980; Merkle and Adams 1987) mating system (Neale et al. 1984; Nakamura and Wheeler 1992), seed-orchard efficiency (Adams 1983) and linkage analysis (El Kassaby et al. 1982; Adams et al. 1990) in Douglas-fir. Allozymes are multi-allelic, co-dominant markers that are inexpensive and easy to apply. However,

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as with terpenes, only a limited number of allozyme marker loci are available for assay, and gene products can be expressed differentially across tissue types.

The recent development of DNA markers such as RFLPs and RAPDs has added an extra dimension to the potential number of genetic markers that can be examined. RFLP markers have already been used to study inheritance and variation in organellar genomes of Douglas-fir (Neale et al. 1986; Ali et al. 1991; Tsai and Strauss 1989; Marshall and Neale 1992). Nuclear ribosomal DNA gene copy number polymorphisms have been detected in natural populations of Douglas-fir (Strauss and Tsai 1988); however, this type of marker may be somatically unstable within the life-cycle of the organism (Russell and Rodland 1986). RFLPs have yet to be applied to single-copy or low-copy nuclear DNA loci in Douglas-fir. Despite the difficulty of detecting low-copy loci in the large genomes of conifers, RFLP linkage maps have been successfully constructed in loblolly pine (*Pinus taeda* L.) (Devey et al. 1991, 1994). RAPDs (Williams et al. 1990) are another genetic marker that are being widely applied in forest-tree genetic mapping (Grattapaglia et al. 1992; Tulsieram et al. 1992). Carlson et al. (1991) have already used RAPDs in Douglas-fir to demonstrate their inheritance in F_1 families.

The present paper reports the identification of 225 (151 RFLP and 74 RAPD) new genetic markers in Douglas-fir and demonstrates the single-locus inheritance of 16 RFLP loci and 29 RAPD loci based on segregation data from a three-generation pedigree. The inheritance of an additional 80 RAPD loci was determined based on segregating haploid megagametophytes of a single mother-tree. In addition, RFLP and allozyme allele frequencies were estimated and compared on a small sample of unrelated Douglas-fir seed-orchard clones.

Materials and methods

Plant materials

We are using a three-generation outbred pedigree developed by the Weyerhaeuser Company[1], Centralia, Washington (Fig. 1). This pedigree was constructed for the goal of mapping QTLs for bud phenology traits. The two parent trees were mated in 1991 and seeds from the controlled cross were imbibed, stratified for 8 weeks, and sown Dec. 1991. They produced 294 progeny seedlings. Of these, 48 will be used for our mapping population and the remaining 246 have been clonally propagated for future QTL studies. Needle tissue was sampled from the four grandparents, the two parents, and the mapping population, to isolate DNA for RFLP and RAPD analysis. Open-pollinated seed from the parents was used to obtain haploid megagametophyte DNA for RAPD and isozyme assays.

Needle tissue from 16 unrelated Douglas-fir seed-orchard clones was also obtained from the Weyerhaeuser Co. These materials were used in a preliminary study to compare RFLP and allozyme variation in a small population sample.

[1] Trade names and commercial enterprise of products are mentioned solely for identification. No endorsement by the U.S. Department of Agriculture is implied

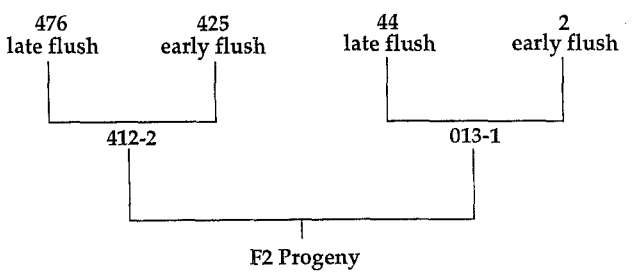

Fig. 1 Douglas-fir three-generation outbred pedigree used for genetic mapping and future mapping of QTLs controlling bud flush phenology traits

RFLPs

Screening strategy

Southern blots containing *Hin*dIII-restricted genomic DNA of each parent and grandparent were hybridized with RFLP probes for the detection of polymorphism and the determination of zygosity in the parents. Probes revealing polymorphic, informative loci in the parents were then hybridized to blots containing progeny DNAs prepared in a similar fashion to the parent DNA. Progeny genotypes were then scored and genotypic ratios tested for goodness-of-fit to expected Mendelian ratios. Probes detecting loci that segregated according to expected ratios in the progeny were also hybridized to blots containing *Hin*dIII-restricted DNA from 16 seed-orchard clones.

DNA extractions and blot preparation

DNA extractions, restriction digests and Southern-blot preparation were performed by using methods described by Devey et al. (1991).

Southern hybridization

Hybridizations and post-hybridization washes were also performed using methods described by Devey et al. (1991). Blots hybridized with heterologous probes were washed at lower stringency than blots hybridized with homologous probes. After hybridization with loblolly pine cDNA probes, blots were rinsed in 2×SSPE [0.72 M NaCl, 40 mM sodium phosphate (pH 7.4), 4 mM EDTA], washed at 65°C with 2×SSPE, 0.5% SDS (sodium dodecyl sulfate) for 20 min; washed once for 30 min at 65°C with 1×SSPE, 0.1% SDS and once for 30 min with 0.5×SSPE, 0.1% SDS. Blots were exposed to X-ray film for 3–9 days at –80°C. Blots were stripped of probe for re-use by washing in 0.1×SSPE, 1% SDS for 1–2 h at 65°C.

RFLP probes

Three sources of probes were used: (1) Douglas-fir cDNA probes, (2) Douglas-fir genomic-DNA probes and (3) loblolly pine cDNA probes. Six Douglas-fir *lhcp* cDNA probes were obtained from C. Alosi (Alosi et al. 1990). Douglas-fir genomic-DNA probes were prepared by ligation of *Pst*I/*Bam*HI-restricted genomic-DNA into pUC 19 (Bethseda Research Laboratories). *E. coli* was transformed with the ligated plasmid DNA and incubated on ampicillin plates (50 mg/ml) overnight. Liquid media cultures were then innoculated and incubated overnight in preparation for plasmid extraction. Loblolly pine cDNA probes were prepared using methods described by Devey et al. (1991).

A boiling mini-prep procedure was performed on overnight liquid-media cultures to extract plasmid DNA. Electrophoresis on low-melting-point agarose gel was used to isolate insert DNA. Insert DNA was radio-labelled according to methods described by Feinberg and Vogelstein (1983) and unincorporated isotope removed by gel filtration with sephadex-50 (Pharmacia) spin columns.

RAPDS

Screening strategy

DNA from six megagametophytes from each parent tree was screened with 142 commercially-prepared primers (Operon Technologies) to detect polymorphisms and determine zygosity. Primers revealing loci with alleles expected to segregate in either a 1:1 (Aa×aa or aa×Aa) or a 3:1 (Aa×Aa) ratio in the F_2 population were then applied to diploid DNAs extracted from needle tissue of the F_2 mapping population. Primers revealing heterozygosity in the paternal parent tree (013-1) were also applied to a sample of 71 megagametophytes from that tree.

DNA isolation and PCR methods

DNA was extracted from megagametophytes using the SDS-based extraction method of Kreike (1990). The same F_2 progeny DNA was used for RAPD and RFLP analyses. DNA preparations were treated with 20 ng of RNase/ng DNA and incubated for 3 h at 37°C (Pikaart and Villeponteau 1993). Polymerase chain reaction (PCR) conditions were as follows: 200 µM of each dNTP (Boehringer-Mannheim), 3.5 mM of $MgCl_2$ (Sigma), 1×reaction buffer (100 mM Tris pH 8.3, 500 mM KCl), 0.4 µM of random 10-mer primer (Operon Technologies), 1.0 unit of *Taq* polymerase (Perkin-Elmer) and 8.0 ng of DNA template. A working concentration of 0.5% Tween 20 was added to the RAPD reactions of megagametophyte DNA because it has been shown to counteract inhibition of polymerase activity caused by residual SDS from DNA preparations (Demeke and Adams 1992). Cycling parameters were set at 94°C for 1 min, 37°C for 1 min and 72°C for 2 min for 45 cycles. A final extension period of 10 min at 72°C completed the reaction. Products were separated on a 1.5% agarose gel by electrophoresis in 1×TBE (89 mM Tris-borate, 2 mM EDTA pH 8.0) and visualized by ultraviolet light after staining with ethidium bromide.

Allozymes

A sample of eight megagametophytes from each parent was assayed for 14 enzyme systems (6PGD, PGI, PGM, MDH, LAP, ME, ACO, UGPP, GOT, IDH, SKD, G6PD, GDH and DIA). Zygosity was determined at 19 loci. The methods used were as described by Adams et al. (1990).

Results

RFLPs

Parental screening

Five of the six Douglas-fir cDNA probes cross-hybridized to parent and grandparent genomic DNAs and each probe revealed at least one polymorphic locus (Table 1). Seventy-nine percent of the Douglas-fir genomic-DNA probes cross-hybridized to the parental blots and 32% of these probes revealed polymorphism in the parents (Fig. 2 and Table 1). More than half of the genomic-DNA probes that

Table 1 Results from screening parent and grandparent DNAs for polymorphism using Douglas-fir cDNA, Douglas-fir genomic-DNA and loblolly pine cDNA probes in Southern hybridizations

Probes	No. screened	No. cross-hybridized (%)	% Polymorphic[a]
Douglas-fir cDNA	6	5 (83.0)	100.0
Douglas-fir genomic	178	141 (79.2)	32.0
Loblolly pine cDNA	171	122 (71.3)	66.0

[a] Percent of probes which successfully cross-hybridized and were also polymorphic

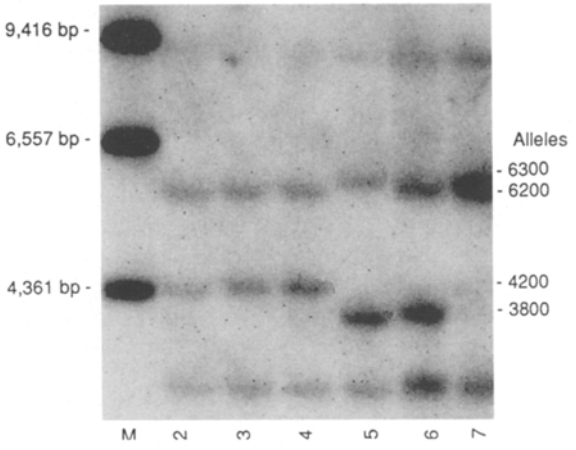

Fig. 2 Southern-blot analysis of the four grandparent and two parent (Fig. 1) DNAs hybridized with Douglas-fir genomic-DNA probe pPmIFG121. *Lane 1*, lambda/*Hin*dIII marker (*M*); *lane 2*, 476; *lane 3*, 425; *lane 4*, 412-2; *lane 5*, 013-1; *lane 6*, 44; *lane 7*, 2. Alleles are identified by visual comparison to molecular-weight standards

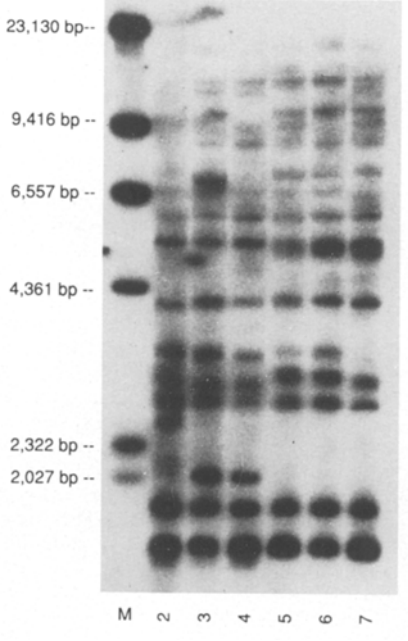

Fig. 3 Southern-blot analysis of the four grandparent and two parent (Fig. 1) DNAs hybridized with loblolly pine probe pPtIFG2356. *Lane 1*, lambda/*Hin*dIII marker (*M*); *lane 2*, 476; *lane 3*, 425; *lane 4*, 412-2; *lane 5*, 013-1; *lane 6*, 44; *lane 7*, 2

Table 2 Single-locus segregation analysis of 16 RFLP loci in F_2 progeny

Locus	Parental genotypes	Expected segregation ratio	Observed segregations	*df*	Chi-square
PtIFG1949[a]	11×23	1:1	13:19	1	1.23
PtIFG2006a	12×34	1:1:1:1	5:11:9:6	3	2.94
PtIFG2006b	13×23	1:1:1:1	9:7:5:10	3	1.90
PtIFG2025	12×11	1:1	4:16	1	7.20*
PtIFG2271	12×22	1:1	5:5	1	0.00
PtIFG2356	12×22	1:1	4:6	1	0.40
PtIFG2553	12×22	1:1	10:12	1	0.18
PmIFG005[b]	2n×1n[c]	1:1:1:1	8:9:13:2	3	7.75
PmIFG015	22×13	1:1	19:13	1	1.13
PmIFG102	34×12	1:1:1:1	6:10:6:7	3	1.48
PmIFG112	22×12	1:1	15:13	1	0.14
PmIFG118	23×11	1:1	8:2	1	3.60
PmIFG121	23×14	1:1:1:1	8:5:6:7	3	0.77
PmIFG123	23×13	1:1:1:1	8:6:5:6	3	0.76
PmIFG320	33×34	1:1	12:9	1	0.43
PmIFG343	12×13	1:1:1:1	13:5:5:8	3	5.52

[a] Prefix PtIFG denotes loci detected with loblolly pine cDNA probes (Devey et al. 1994)
[b] Prefix PmIFG denotes loci detected with Douglas-fir genomic-DNA probes
[c] N, no visible band
* Statistically significant at P=0.05

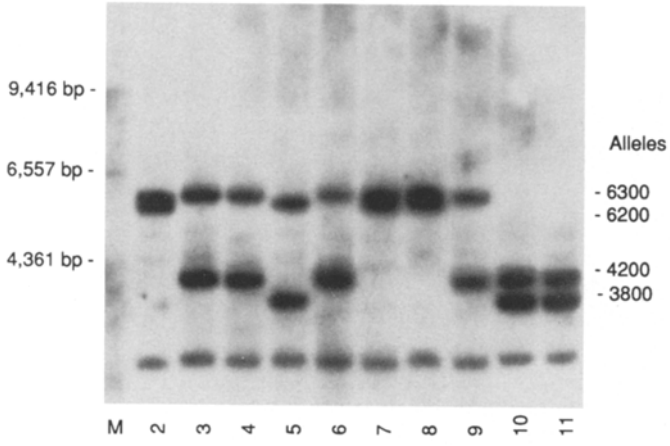

Fig. 4 Southern-blot analysis of ten Douglas-fir progeny DNAs hybridized with Douglas-fir genomic-DNA probe pPmIFG121. *Lane one*, lambda/*Hin*dIII marker (degraded). Alleles are identified by visual comparison to molecular-weight standards

Table 3 Number of alleles detected among a sample of 16 Douglas-fir seed-orchard clones at 16 RFLP loci

Locus	Number of alleles
PtIFG1949	4
PtIFG2006a	–[a]
PtIFG2006b	4
PtIFG2025	3
PtIFG2271	–
PtIFG2356	–
PtIFG2553	2
PmIFG102	–
PmIFG121	5
PmIFG112	3
PmIFG343	3
PmIFG320	6
PmIFG015	3
PmIFG005	–
PmIFG118	7
PmIFG123	4
Avg. no. alleles	4.0

[a] Not possible to score alleles at these loci in the sample of seed-orchard clones because accurate genetic interpretations could not be made

hybridized gave high-copy or repetitive band patterns.Seventy-one percent of the loblolly pine cDNA probes cross-hybridized and, of these, 66% revealed polymorphism in the parents (Fig. 3 and Table 1).

Progeny hybridizations

A total of 16 segregating loci were revealed by hybridizations with 15 probes and analyzed for goodness-of-fit to expected Mendelian ratios (Fig. 4 and Table 2). Probe pPtIFG2006 detected two segregating loci; all other probes detected only one segregating locus. One locus, pPtIFG2025, did not fit expected ratios at the 5% probability level.

Population study

The same 15 probes that were used for segregation analysis were also hybridized to blots containing DNAs of 16 Douglas-fir seed-orchard clones (Fig. 5). New alleles, not previously identified in the mapping pedigree, were sometimes difficult to assign to a known locus. Probes detect-

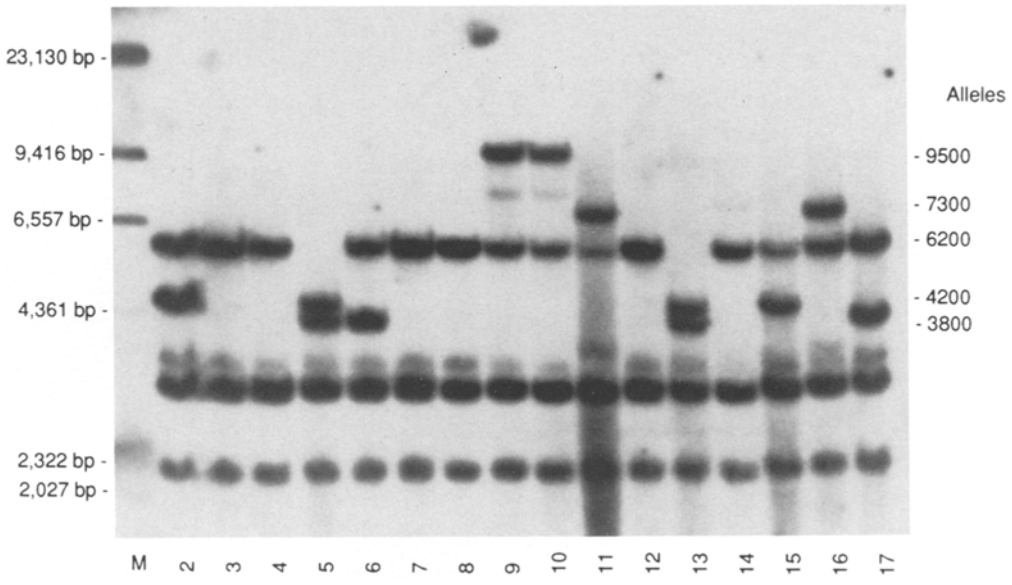

Fig. 5 Southern-blot analysis of 16 Douglas-fir DNAs hybridized with Douglas-fir genomic-DNA probe pPmIFG121. *Lane 1*, lambda/*Hin*dIII marker (*M*). Alleles are identified by visual comparison to molecular-weight standards

Table 4 Number of RAPD loci detected in female parent tree 412-2 and male parent tree 013-1 for three informative mating types and two non-informative mating types

Mating-type configuration	Female 412-2	Male 013-1	No. loci identified
Paternally informative backcross (PIBC)	aa	Aa	9
Maternally informative backcross (MIBC)	Aa	aa	14
Intercross (IC)	Aa	Aa	51
Non-informative backcross (NIBC)	AA	Aa	10
Non-informative backcross (NIBC)	Aa	AA	11
Total			95

ing only one or two well-defined and separated loci were easier to interpret than probes that revealed complicated band patterns. Accurate genetic interpretations could be made at 11 of the 16 loci. The average number of alleles detected in the sample of 16 seed-orchard clones was 4.0 (Table 3).

RAPDs

Parental screening

We applied 142 primers to DNA from six megagametophytes from each parent; 124 primers produced RAPD bands and 18 (13%) failed to yield amplification products. Interpretable polymorphisms were detected in amplifications with 40 of the 124 primers (33%), and a total of 95 loci (2.4 loci/primer) were scored for zygosity in both parents. Twenty-three loci showed parental alleles in the informative backcross (Aa×aa or aa×Aa) configuration and 51 loci were found to be in the intercross configuration (Aa×Aa)(Table 4). Twenty-one loci were found to be in the non-informative backcross configuration.

Segregation in F_2 progeny

Twenty-nine loci (19 in the informative backcross configuration and ten in the intercross configuration) were assayed in the F_2 progeny (Fig. 6). All but one locus segregated according to expected ratios (Table 5).

Haploid segregation analysis

Twenty-three primers revealed 70 heterozygous loci in the male parent, 013–1. Initially, only 23 megagametophyte DNAs were assayed with all 23 primers (Fig. 7). Subsequently, DNAs from an additional 48 megagametophytes were extracted and added to the sample. A portion of the 23 primers applied to the sample of 23 megagametophyte DNAs failed to amplify in a repeatable pattern when applied to the additional 48 megagametophyte DNAs. However, other primers amplified in consistent and repeatable band patterns. Therefore, sample size varied with respect to the primer employed. In some cases, segregating bands appeared in amplifications in the second set of megagametophytes that were not detected in the initial screening.

Table 5 Single-locus segregation analysis of 29 RAPD loci in F_2 progeny

Locus (MW)[a]	Mating type	Observed ratio	Chi-square
OPB_18 (675)	IC	34:14	0.44
OPB_18 (800)	PIBC	30:18	3.00
OPB_18 (950)	IC	32:16	1.78
OPB_18 (1050)	IC	32:16	1.78
OPB_18 (1125)	PIBC	29:19	2.08
OPC_01 (775)	PIBC	26:18	1.45
OPC_01 (800)	PIBC	21:23	0.09
OPC_01 (1200)	PIBC	23:21	0.09
OPC_01 (1500)	PIBC	23:21	0.09
OPC_06 (700)	IC	29:13	0.79
OPC_07 (700)	IC	38:9	0.86
OPC_07 (1050)	PIBC	24:13	4.70*
OPC_07 (1350)	MIBC	27:20	1.04
OPC_19 (700)	IC	35:11	0.03
OPC_19 (1250)	PIBC	23:23	0.00
OPD_12 (2000)	MIBC	23:16	1.26
OPD_15 (775)	MIBC	17:20	0.24
OPE_07 (1075)	PIBC	22:19	0.22
OPE_14 (800)	IC	38:9	0.86
OPE_14 (900)	PIBC	19:28	1.72
OPE_15 (675)	IC	34:11	0.01
OPG_05 (800)	MIBC	23:22	0.02
OPG_11 (925)	PIBC	25:19	0.41
OPG_15 (1400)	MIBC	23:20	0.21
OPH_07 (650)	IC	31:14	0.90
OPH_08 (975)	MIBC	24:21	0.20
OPH_15 (450)	MIBC	23:20	0.21
OPH_15 (1050)	IC	36:11	0.06
OPH_15 (1200)	MIBC	26:21	0.27

[a] Molecular weight
* Statistically sigificant at P=0.05

This difference resulted in a net gain of ten heterozygous loci over the 70 heterozygous loci identified in the preliminary screening of primers. Thus, a total of 80 loci were scored and single-locus segregation ratios analysed for goodness-of-fit to expected Mendelian ratios (Table 6). Eight loci did not conform to the expected 1:1 segregation ratio.

Allozymes

Fourteen enzyme systems were assayed revealing 19 loci – seven of which were polymorphic in the cross. *G6pd* was polymorphic but the parents were homozygous for alternate alleles. Six loci (*Pgi-2*, *Mdh-4*, *Lap-1*, *Ugpp-1*, *Idh* and *Me-2*) are expected to segregate in the F2 progeny.

Discussion

We have identified 225 (151 RFLP and 74 RAPD) new polymorphic loci in a three-generation outbred Douglas-fir pedigree and have demonstrated inheritance of 16 RFLP and 29 RAPD markers based on segregations in the F_2 progeny. In addition, 80 RAPD loci have been identified based on haploid segregation analysis in parent tree 013-1. These genetic markers will form the foundation of a saturated genetic map neccessary for the identification of quantitative trait loci affecting bud phenology and possibly other complex traits.

Loblolly pine cDNA probes were applied to the Douglas-fir mapping population with only a slight reduction of stringency in hybridization conditions. The application of loblolly pine RFLP probes in Douglas-fir will allow a comparison of genome organization within the *Pinaceae*. We recently completed the construction of a Douglas-fir cDNA library for additional mapping probes. The Douglas-fir genomic-DNA probes often gave high-copy, repetitive signals; thus, we will not use genomic probes in future analyses.

One RFLP locus, PtIFG2025, showed a distorted segregation ratio in the F_2 progeny. This locus is revealed by a loblolly pine cDNA probe encoding the *rbcS* gene (C. Kinlaw, personal communication). Single-locus segregation distortion in Douglas-fir has been reported for the allozyme loci *6pgd*-1 (1) (Adams et al. 1990) and *Idh* (Wheeler and Jech 1992).

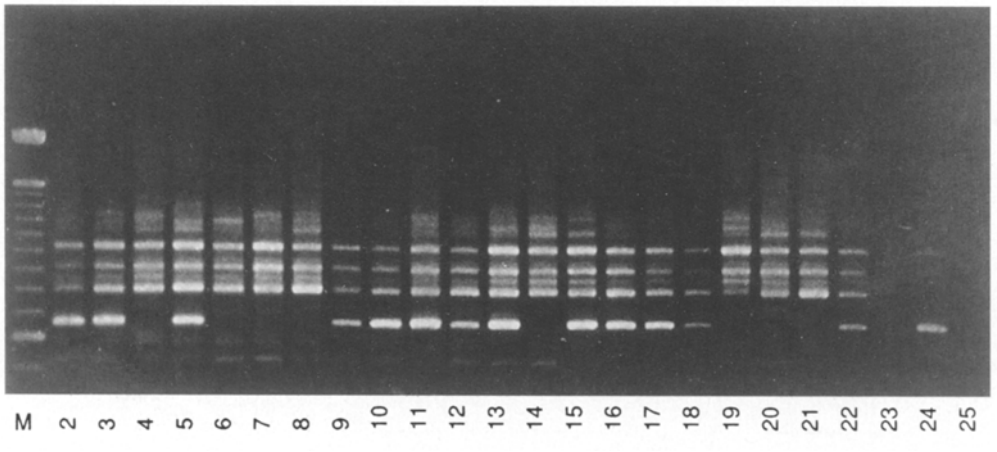

Fig. 6 Segregating RAPD loci revealed by Operon primer H7, based on a sample of 24 progeny DNAs. *Lane 1*, 100-base-pair ladder (BRL); *lanes 3* through 25, progeny DNA samples

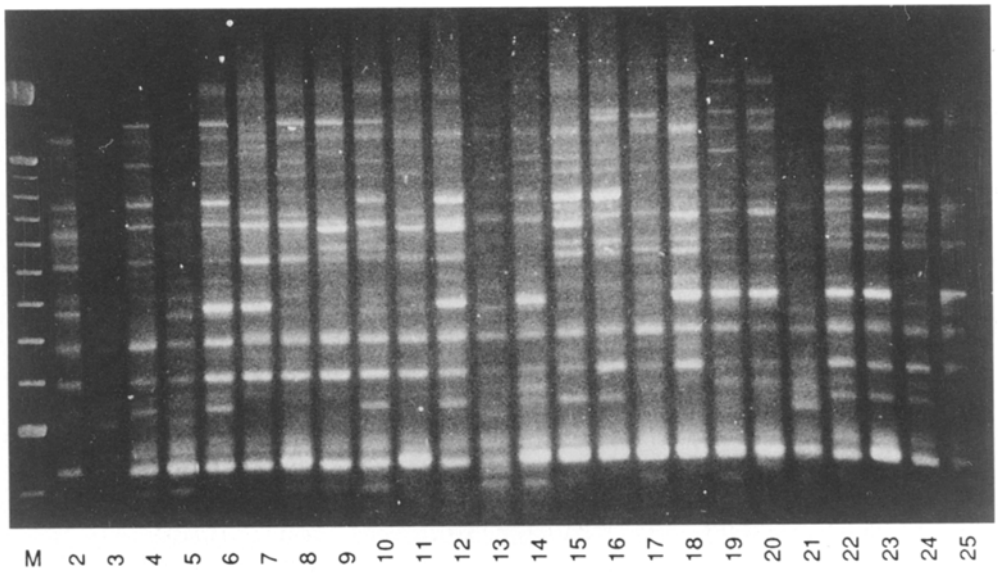

Fig. 7 Segregating RAPD loci revealed by Operon primer D11, based on a sample of 23 megagametophytes of parent tree 013-1. *Lane 1*, 100 base-pair ladder (BRL); *lane 2*, 013-1 diploid DNA from needle tissue; *lanes 3 through 25*, megagametophyte DNA

Table 6 Single-locus segregation analysis of 80 RAPD loci from parent tree 013–1 based on a sample of 23–71 megagametophytes

Locus (MW)[a]	Segregations observed	G-Statistic
OPB_01 (1100)	14:9	1.096
OPB_01 (800)	35:26	1.333
OPB_01 (550)	13:10	0.392
OPB_11 (575)	13:10	0.392
OPB_14 (475)	41:29	2.067
OPB_14 (325)	16:7	3.618
OPC_01 (1200)	40:17	9.551*
OPC_01 (1100)	26:31	0.439
OPC_01 (775)	34:23	2.136
OPC_05 (625)	24:24	0.000
OPC_06 (1425)	10:11	0.048
OPC_06 (1400)	15:6	3.985*
OPC_06 (875)	32:30	0.065
OPC_06 (600)	28:34	0.582
OPC_06 (550)	35:28	0.779
OPC_07 (1550)	10:9	0.053
OPC_07 (625)	13:6	2.641
OPC_07 (525)	11:8	0.476
OPD_05 (500)	14:7	2.379
OPD_05 (475)	38:17	8.225*
OPD_05 (375)	21:34	3.102
OPD_07 (875)	10:11	0.048
OPD_07 (825)	14:7	2.379
OPD_07 (625)	14:7	2.379
OPD_07 (475)	11:11	0.000
OPD_11 (1800)	10:12	0.182
OPD_11 (1225)	9:13	0.731
OPD_11 (1025)	10:12	0.182
OPD_11 (1000)	14:8	1.657
OPD_11 (900)	10:12	0.182
OPD_11 (625)	10:12	0.182
OPD_15 (1400)	10:10	0.000
OPD_15 (1250)	14:6	3.291
OPD_15 (1200)	8:12	0.805
OPD_15 (1075)	10:10	0.000
OPD_15 (900)	12:8	0.805
OPD_15 (800)	12:8	0.805
OPD_15 (700)	9:11	0.200
OPD_15 (600)	9:11	0.200
OPE_07 (1125)	9:10	0.053
OPE_07 (1075)	10:9	0.053
OPE_07 (450)	21:20	0.024

Table 6 (continued)

Locus (MW)[a]	Segregations observed	G-Statistic
OPE_09 (1400)	9:10	0.053
OPE_09 (800)	9:10	0.053
OPE_09 (700)	11:8	0.476
OPE_18 (800)	8:14	1.657
OPE_18 (650)	8:14	1.657
OPE_18 (450)	13:9	0.731
OPE_19 (1200)	17:27	2.293
OPE_19 (575)	27:17	2.293
OPE_19 (500)	26:18	1.463
OPF_07 (950)	38:15	10.321*
OPF_07 (600)	21:32	2.300
OPG_05 (950)	9:14	1.096
OPG_05 (575)	40:10	19.274*
OPG_05 (475)	11:12	0.043
OPG_05 (425)	10:13	0.392
OPG_05 (350)	29:27	0.071
OPG_09 (1300)	12:11	0.043
OPG_09 (1125)	13:10	0.392
OPG_09 (900)	10:13	0.392
OPG_09 (775)	10:13	0.392
OPG_09 (650)	10:13	0.392
OPG_09 (625)	9:14	1.096
OPG_11 (1275)	23:39	4.176*
OPG_11 (950)	34:28	0.582
OPG_11 (675)	10:12	0.182
OPG_15 (800)	13:9	0.731
OPG_15 (600)	12:10	0.182
OPH_07 (850)	15:7	2.977
OPH_07 (825)	16:6	4.717*
OPH_07 (700)	33:24	1.427
OPH_08 (800)	14:9	1.096
OPH_08 (550)	13:10	0.392
OPH_08 (450)	20:17	0.244
OPH_11 (960)	11:12	0.043
OPH_11 (950)	37:29	0.972
OPH_11 (725)	38:29	1.213
OPH_11 (600)	31:36	0.373
OPH_11 (475)	24:43	5.463*

[a] Molecular weight
* Statistically significant at P=0.05

We plan to integrate RAPD markers identified in the F_2 population with those identified from the haploid segregation analysis of the male parent, 013-1. Some of these RAPD markers appear to be synonymous in both mapping populations; however, further study is needed for verification of this observation. This will allow for the placement of all RFLP and RAPD markers onto a single consensus map.

We have made a preliminary assessment of the potential for using RFLP probes for studying genetic diversity in Douglas-fir. It is unknown whether or not RFLP alleles can be accurately assigned to individual loci on autoradiograms when using samples other than defined genetic crosses. In our sample of 16 Douglas-fir seed orchard-clones, we found that allelism could be assigned for most RFLP loci. RFLPs may also detect more variation than isozyme markers. RFLPs detected 4.0 alleles/locus while previous allozyme data (23 loci) on the same clones detected 3.1 alleles/locus. We eventually plan to identify RFLP markers for genes associated with phenotypic traits which vary across environmental gradients in response to selection. Markers of this type should provide greater insight into adaptive genetic variation in populations of Douglas-fir and other forest trees.

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