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A sex-averaged genetic linkage map in coastal Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco var '*menziesii*') based on RFLP and RAPD markers

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Abstract We have constructed a sex-averaged genetic linkage map in coastal Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco var '*menziesii*') using a three-generation outcrossed pedigree and molecular markers. Our research objectives are to learn about genome organization and to identify markers associated with adaptive traits. The map reported here is comprised of 141 markers organized into 17 linkage groups and covers 1,062 centiMorgans (cM). Of the markers positioned on the map, 94 were derived from a Douglas-fir complimentary-DNA (cDNA) library that was constructed from new-growth needle tissue. Other markers include 11 Douglas-fir genomic-DNAs, 20 loblolly pine (*Pinus taeda* L.) cDNAs, 15 random amplified polymorphic DNAs (RAPDs) and a PCR-amplified phytochrome probe. A high degree of variation was detected in each of the two parents of our mapping population, and many of the restriction fragment length polymorphism (RFLP) and RAPD phenotypes were complex. Marker data were analyzed for linkage using mapping software JOINMAP version 2.0.

Key words RFLP · RAPD · Genetic map · Null loci · Gene family

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Introduction

Coastal Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco var '*menziesii*') is one of the most highly valued commercial conifers in the Pacific Northwestern regions of the United States and Canada. Traits of economic interest, such as growth rate, form, wood quality, reproduction, phenology, and adaptiveness have been studied extensively by forest geneticists and tree breeders (White 1987; Nakamura and Wheeler 1992; Li and Adams 1993; Vargus-Hernandez and Adams 1994; Roth and Newton 1996). Douglas-fir populations are highly adapted to diverse environmental habitats across their range, with ecotypes occupying microsites and environmental gradients and clines (Campbell and Sorensen 1978; Campbell 1986; Rehfeldt 1979, 1989). Selection of genotypes expressing specific adaptive qualities can require several to many years in the field. Detailed genetic linkage maps with markers correlated to adaptive traits would support early selection of desired ecotypes.

Early inheritance and linkage studies in Douglas-fir have been performed using isozymes (El-Kassaby et al. 1982; Neale et al. 1984; Adams et al. 1990). In these studies, the number of markers was limited by the number of polymorphic enzymes available for analysis. However, the current and expansive resource of molecular markers has made genetic mapping in conifers more efficient and applicable (Neale and Williams 1991). Linkage maps have been constructed in numerous conifer species using random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) markers (Tulsieram et al. 1992; Devey et al. 1994, 1996; Groover et al. 1994; Nelson et al. 1994). In Douglas-fir, the inheritance of RAPDs and RFLPs was demonstrated by Carlson et al. (1991) and Jermstad et al. (1994), respectively. RAPD-based linkage maps for Douglas-fir have been constructed by Agrema and Carlson (1995) and (Krutovskii et al. 1998).

One objective for constructing a genetic linkage map in Douglas-fir is to learn of its genome organization and how it compares with other conifers of the *Pinaceae*. Douglas-fir is the only member of the *Pinaceae*, including other *Pseudotsuga* species, that has 13 pairs of chromosomes ($2x = 2n = 26$) instead of the typical 12 pairs (Barner and Christiansen 1962). A second objective is to identify genomic regions that contain genes affecting bud phenology and other adaptive traits. Marker-aided breeding might be applied for early selection of these traits or other economically important traits such as growth and wood quality.

Several difficulties are encountered in making genetic maps in conifers. Sexual maturity occurs between 8 and 15 years in conifers, making the construction of pedigrees time consuming. Conifers are predominantly outcrossed and suffer from inbreeding depression in full-sib matings or self-pollination. Therefore, producing inbred lines is not desirable if traits are to be measured in the mapping population. And lastly, conifer genomes are larger than those of most agricultural species, making it more difficult to detect low-copy sequences using RFLP techniques. The genome size of Douglas-fir has been estimated at 25–34 pg per haploid nucleus (Ingle et al. 1975; Carlson et al. 1991). The high degree of gene duplications makes RFLP phenotypes difficult to interpret. There are two factors that facilitate genetic mapping in conifers. In general, conifers possess a high level of heterozygosity which allows easy detection of polymorphic loci and, secondly, their longevity enables mapping populations to be easily immortalized.

We have constructed a sex-averaged genetic linkage map in a three-generation outcrossed pedigree of coastal Douglas-fir using RFLPs and RAPDs. The map was constructed using JOINMAP version 2.0 (Stam and Van Ooijen 1995) and consists of 141 markers distributed among 17 linkage groups and covers a genetic distance of 1,062 centiMorgans (cM).

Materials and methods

Plant material

The mapping population is a three-generation outbred pedigree consisting of four grandparents, two F_1 parents, and 240 progeny that segregate for the date of budflush (Fig. 1). Forty-eight progeny were grown in a greenhouse to produce needle tissue for DNA isolation and construction of a preliminary linkage map. Rooted cuttings were obtained from 192 progeny for use in genetic tests for quantitative trait loci (QTL) mapping. Needle tissue from parents, grandparents, 48 progeny (grown in the greenhouse), and 192 outplanted clones was harvested and ground in liquid nitrogen to a coarse powder and stored at $-80°C$ for DNA extraction.

RFLP and RAPD markers

DNA extractions were performed as described in Devey et al. (1991) with the following modifications. Prior to restriction digest, a working solution of 5 m*M* $CaCl_2$ was added to DNA to precipitate pectins and unwanted polysaccharides, followed by an iso-propanol/ammonium acetate precipitation [1/4 volume 10 *M* NH_4Ac, 2/3 volumes iso-propanol]. Digested DNA fragments were fractionated on agarose gels, and transferred to ZetaProbe nylon membrane (BioRad) with 0.3 *N* NaOH, 0.3 *M* NaCl using the downward transfer method described in Koetsier et al. (1993).

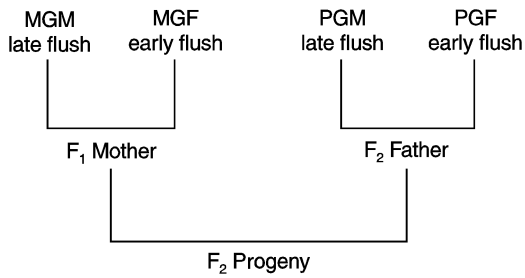

Fig. 1 Douglas-fir three-generation outbred pedigree constructed for genetic mapping and estimation of QTLs affecting phenology and other adaptive traits. Grandparents were selected for early and late date of budflush on the basis of the results of progeny tests. *MGM* Maternal grandmother, *MGF* maternal grandfather, *PGM* paternal grandmother, *PGF* paternal grandfather

Four classes of RFLP probes were used in our mapping analysis: (1) Douglas-fir genomic-DNAs (Jermstad et al. 1994), (2) Douglas-fir cDNAs (Jermstad et al. 1998), (3) loblolly pine cDNAs (Devey et al. 1991), and (4) cloned polymerase chain reaction (PCR) products from Douglas-fir. The Douglas-fir cDNA library was constructed from RNA that was extracted from new-growth needle tissue using the procedure described in Chang et al. (1993). Messenger RNA was separated from total RNA using the mRNA Purification Dynabead Kit (Dynal) and quantified by spectrophotometry. Complimentary DNA was synthesized and uni-directionally cloned into the Uni-ZAP XR *Lambda* vector using the ZAP-cDNA Synthesis Kit (Stratagene). The phagemid Bluescript SK (–) was excised from the phage vector using the in vivo excision protocol provided by Stratagene. DH5 alpha MCR library efficient competent cells (Gibco-BRL) were transformed with recombinant phagemid and plated on ampicillin-agar (50 mg/ml). Inserts were amplified in *E. coli* and isolated as described in Jermstad et al. (1994). Restriction digests with *Bam*HI and *Eco*RI were performed on recombinant phagemid to isolate and measure the size of inserts. The inserts ranged from 300 base pairs to 3,400 base pairs in length. cDNAs that were utilized in linkage analysis were sequenced and searched for similarities to genes of known function (Jermstad et al. 1998).

Radio-labeling, Southern pre-hybridization, hybridization, and post-hybridization washes were performed as described in Devey et al. (1991). The second and final washes of blots hybridized with loblolly pine probes were conducted at a lower stringency ($0.5\times$ SSPE, 0.1% SDS) than for Douglas-fir probes.

Parents and grandparents were screened for polymorphisms with 178 genomic-DNA probes, 401 random Douglas-fir cDNA probes, 171 loblolly pine cDNA probes, and one cloned phytochrome PCR product (stsIFG_1104). Loblolly pine cDNAs had been previously mapped in loblolly pine (Groover et al. 1994; Devey et al. 1994). All loci revealing polymorphisms were then assayed in 48 progeny for the construction of a preliminary map.

RAPDs were assayed in the mapping population and are described in detail in Jermstad et al. (1994). For detection of polymorphism, 142 10-mer primers (Operon Technologies) were assayed in six megagametophytes from each of the two parents. Loci in the pseudo-testcross configuration (Grattapaglia et al. 1995) were identified and then assayed in the mapping population of 48 progeny.

Nomenclature for RFLP and RAPD markers is consistent with nomenclature found in the TreeGenes database (http://s27w007.

pswfs.gov/Treegenes/index.html). However, because of space constraints, the marker names have been abbreviated in Table 1 and Fig. 7.

Scoring and single-locus segregation

Segregation type codes were designated "ab × aa" (maternally informative); "aa × ab" (paternally informative); "ab × ac" and "ab × cd" (fully informative); and "ab × ab" (intercross) according to JOINMAP 2.0 (Stam and Van Ooijen 1995) nomenclature. RFLP bands were given allelic assignments when genetic interpretation could be inferred from parental genotypes. When allelism could not be inferred from segregating bands, the bands were scored as either present or absent. Pseudo-testcross RAPD loci were scored present or absent. Single-band RFLP loci and RAPD loci of the intercross segregation type (a_ × a_) were not considered for linkage analysis because of ambiguity caused by dominance.

All loci were scored independently by two individuals and then tested for conformity to Mendelian segregation ratios. Loci showing segregation distortion ($P < 0.043$) and all single-band loci segregating 3 : 1 in the progeny were omitted from further analysis. Prior to linkage analysis single-locus segregation was re-analyzed and confirmed with JOINMAP's module, JMSLA (Stam and Van Ooijen 1995).

Linkage analysis

Initially, only the mapping sample of 48 progeny was assayed for all polymorphic loci and analyzed for Mendelian segregation and linkage using JOINMAP version 2.0 (Stam and Van Ooijen 1995). JOINMAP version 2.0, after establishing groups of linked markers, uses a least squares approach to simultaneously order multiple markers by the pairwise distances between markers of a linkage group (LG). A chi-square test is then performed on the regression residuals to determine the fit of the map order within each linkage group.

Markers were assigned to tentative linkage groups by using JOINMAP's JMGRP module to test LOD thresholds 3.5–7.0 at 0.5 increments. Linkage groups were ultimately assigned on the basis of a LOD threshold of 4.0. Markers within the groups were then analyzed for pairwise linkages using JMREC. For this module, REC and LOD thresholds of 0.499 and 0.01, respectively, were used according to the JOINMAP's recommendations. The linkage groups were then ordered with JMMAP using the following parameters: a 0.1 JMMAP LOD threshold, a 0.49 REC threshold, 3 jump threshold, 7 triplet threshold, 3 ripple value, and Kosambi's mapping function.

This initial map was then used to identify informative and evenly distributed markers for estimating QTLs affecting phenology traits (results not reported here). This subset of evenly distributed markers (110) was then assayed in 192 clonal progeny, the segregation data added to the linkage analysis, and a final map was constructed using the same parameters described above.

Male and female maps

In addition to constructing a sex-averaged map, we constructed sex-specific maps using a previous version of JOINMAP (v. 1.4). Our objective was to compare marker density, marker distribution, and genetic length of the paternal and maternal maps. Fully informative (FI) and intercross (IC) segregation-type loci were recoded into backcross segregation-type loci and included in both the male and female dataset. A LOD threshold of 4.0 was used for grouping markers, and a LOD threshold of 0.1 was used for ordering markers within linkage groups.

Results

Polymorphism and genotyping

Molecular marker analysis revealed a large amount of polymorphism in our mapping population. However, only a subset of the total number of polymorphic RFLP and RAPD bands was scored and analyzed for linkage, whereas many were discarded because they were complex and uninterpretable or gave nebulous or weak signals. Of the loci entered into analysis, RFLP probes revealed an average of 1.6 loci/probe, and RAPD analysis revealed 2.0 loci/primer. There was a small degree of redundancy within the Douglas-fir cDNA library, as well as between the Douglas-fir cDNA library and the loblolly pine cDNA library (Table 1).

Of 178 genomic-DNA probes, 141 (79%) were successfully cross-hybridized to Douglas-fir genomic DNA. However, only 32 of these probes detected polymorphism (Fig. 2). Nineteen loci were scored and analyzed for linkage, and 11 were positioned on the map (Table 2). Of the 401 Douglas-fir cDNAs screened, 208 (52%) successfully cross-hybridized, and 196 detected polymorphism. One hundred thirty-nine polymorphic loci were identified and entered into the analysis, and 94 of these loci were positioned on the map. Of the 171 loblolly pine cDNAs screened, 122 (71%) successfully cross-hybridized, and 66 of these revealed polymorphism. Thirty polymorphic loci were identified and entered into the linkage analysis, and 20 were positioned on the map. A relatively small proportion of the RAPD primers (9%) detected loci in the pseudo-testcross mating configuration that were both robust and repeatable. Consequently, only 27 RAPD loci were included in the linkage analysis. Of these 27 loci analyzed, 15 were placed on the map.

The level of heterozygosity in the male and female parents of our pedigree was similar, as demonstrated by the near-equal number of maternally informative and paternally informative loci that were entered into analysis (Table 2). A small number of loci of the intercross segregation type were identified in our mapping

Table 1 cDNAs that were assayed in the Douglas-fir mapping population that gave similar RFLP phenotypes

Douglas-fir cDNAs	Loblolly pine cDNAs
Pm1068, Pm1241, Pm1148	Pt1635, Pt2025
Pm1057, Pm1203	Pt2006
Pm1329	Pt1949, Pt1934
Pm1009, Pm1565	
Pm1075, Pm1180, Pm1596	Pt2538
Pm1238, Pm1266	
Pm1306, Pm1542	
Pm1250, Pm1590, Pm1094, Pm1169	

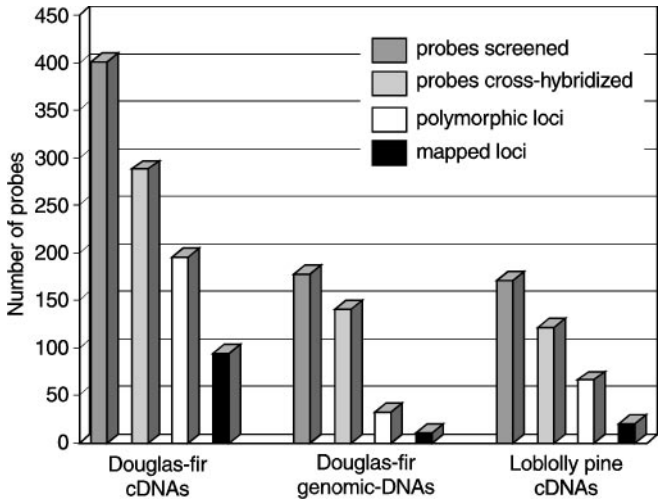

Fig. 2 The development of RFLP probes for construction of a genetic linkage map

Table 2 Segregation type and marker type of loci analyzed and positioned on the genetic linkage map

Segregation type[a]	Number of markers analyzed	Number of markers mapped
MI (ab × aa)	87	58
PI (aa × ab)	85	49
IC (ab × ab)	2	1
FI (ab × ac or ab × cd)	43	33
Total	217	141
Marker type		
Douglas-fir cDNAs	139	94
Douglas-fir genomic-DNAs	19	11
Loblolly pine cDNAs	30	20
RAPDs	27	15
STS	2	1
Total	217	141

[a] MI, Maternally informative; PI, paternally informative; IC, intercross; FI, fully informative

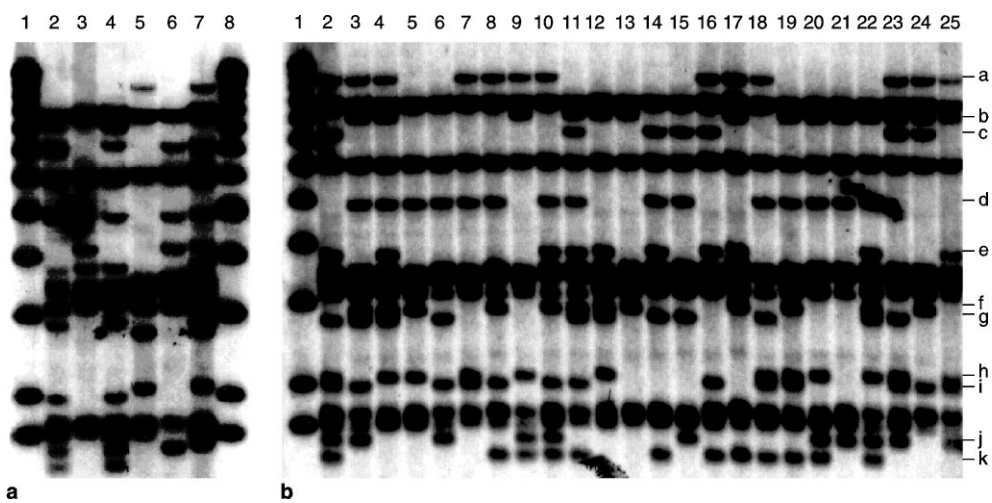

Fig. 3a, b Southern blots hybridized with Douglas-fir cDNA probe PmIFG_1052. **a** Parent/grandparent blot. *Lane 1* 1-kb ladder, *lane 2* maternal grandmother, *lane 3* maternal grandfather, *lane 4* mother, *lane 5* father, *lane 6* paternal grandmother, *lane 7* paternal grandfather, *lane 8* 1-kb ladder. **b** Progeny blot. *Lane 1* 1-kb ladder, *lanes 2–25* progeny. Only bands that could be scored with certainty were included in analysis. Loci (*a–k*) detected with this cDNA probe segregate independently and map to dispersed locations

experiment; only 2 loci were included in the analysis. This marker type may have gone unidentified due to the misinterpretation of double RFLP bands in the parents and grandparents as two separate monomorphic loci. Often, double bands that were detected in both parents and which appeared to represent the intercross segregation type (because of grandparental homozygosity at alternate alleles) instead segregated independently in the progeny as two single-banded loci.

Of the RFLP loci analyzed, 52% (99/190) were scored as present or absent because of the inability to identify allelic configurations. Figure 3 shows an example of a complex RFLP pattern, where each band appears to be segregating independently of each other. Linkage analysis of these loci resulted in each mapping to a different location. When 2 or more bands were found to be co-segregating, a multi-allelic interpretation was attempted. If the new interpretation resulted in a single locus mapping to the same position as the single-band loci, then the new interpretation was accepted. For example, each of the 4 bands denoted in Fig. 4 were initially scored and mapped as single loci. The discovery of these bands mapping to the same position prompted re-examination; these bands were then scored as four co-segregating alleles at a single locus and tested for Mendelian segregation. Linkage analysis, based on this interpretation, resulted in a map position the same as each of the single bands. In some cases, RFLP band patterns were low copy, revealing either an interpretable multi-allelic locus (Fig. 5) or single bands segregating independently (Fig. 6). These solitary bands (Fischer et al. 1995) were interpreted as loci with null alleles and were scored (+/−) or (−/−). Dominant nulls (segregation type a_ × a_) segregated 3:1 and were not considered for linkage analysis (Fig. 6).

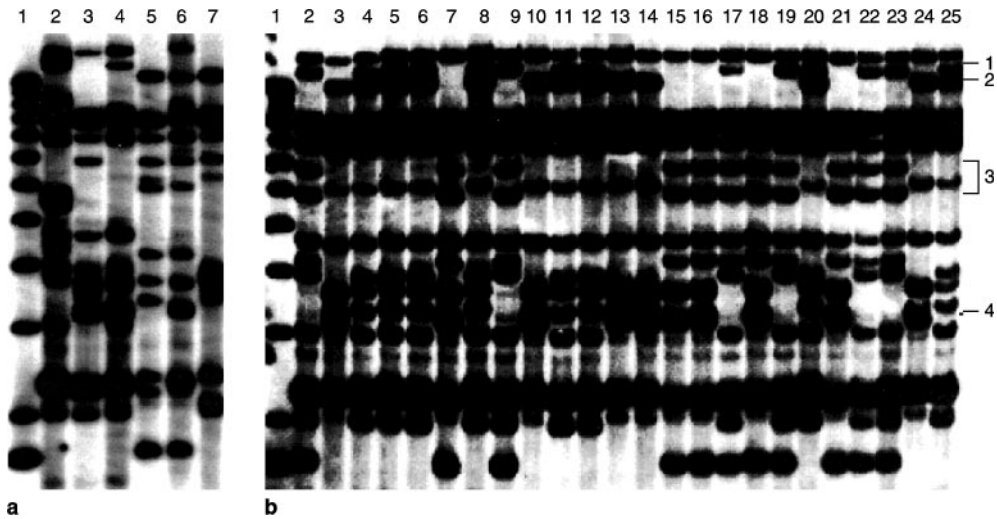

Fig. 4a, b Douglas-fir cDNA PmIFG_0339 depicting a complex RFLP banding pattern. **a** Parent/grandparent Southern autoradiogram. *Lane 1* 1-kb ladder, *lane 2* maternal grandmother, *lane 3* maternal grandfather, *lane 4* mother, *lane 5* father, *lane 6* paternal grandmother, *lane 7* paternal grandfather. **b** Progeny blot. *Lane 1* 1-kb ladder, *lanes 2–25* progeny. Bands labeled *1–4* segregate as four alleles in the progeny in which four unique genotypes were detected. Alleles 1, 2, 3, and 4 were scored for linkage analysis

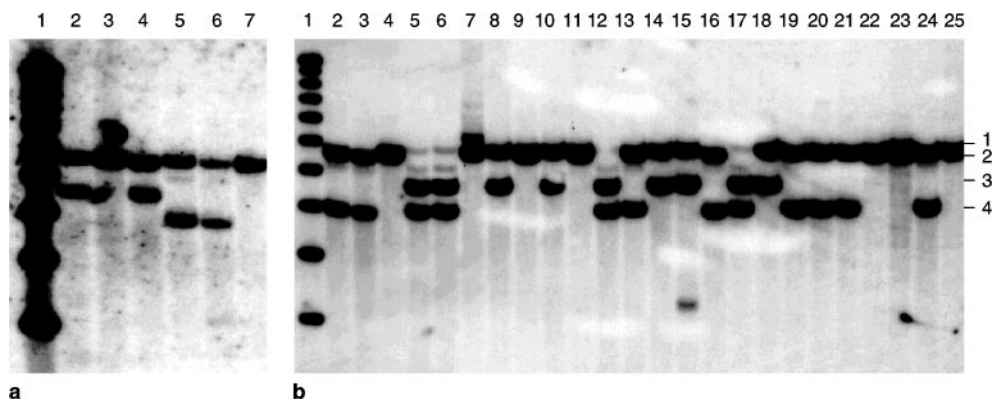

Fig. 5 Southern blots hybridized with RFLP probe PmIFG_1490. **a** Parent/grandparent blot. *Lane 1* 1-kb ladder, *lane 2* maternal grandmother, *lane 3* maternal grandfather, *lane 4* mother, *lane 5* father, *lane 6* paternal grandmother, *lane 7* paternal grandfather. **b** Progeny blot. *Lane 1* 1-kb ladder, lanes *2–24* progeny. The transmission of parental genotypes (23×14) resulted in segregation of four genotypes in the progeny (12, 34, 13, 24)

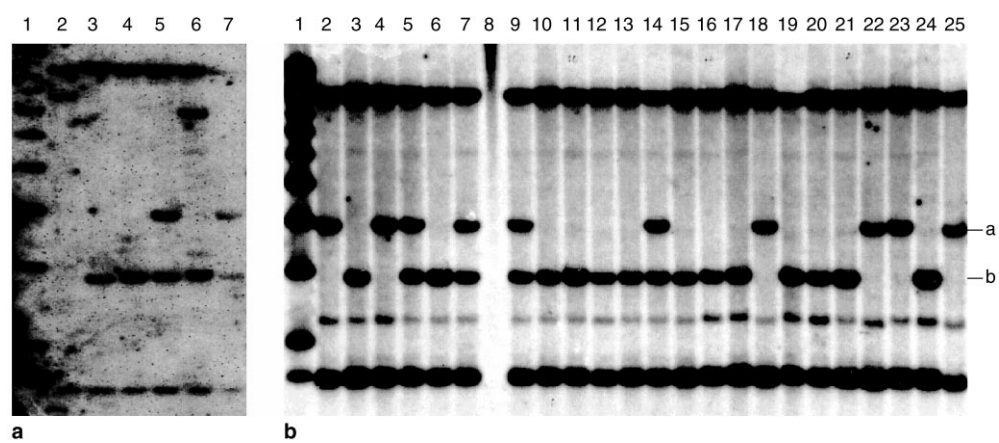

Fig. 6a, b Southern autoradiograms of Douglas-fir cDNA PmIFG_ 1331 showing a simple band pattern involving null loci. **a** Parent/grandparent autoradiogram showing polymorphism in parents. *Lane 1* 1-kb ladder, *lane 2* maternal grandmother, *lane 3* maternal grandfather, *lane 4* mother, *lane 5* father, *lane 6* paternal grandmother, *lane 7* paternal grandfather. **b** Progeny blot. *Lane 1* 1-kb ladder, lanes *2–25* progeny. Band *a* segregates 1 : 1 and was positioned on the map (LG 9). Band *b* segregates 3 : 1 and was omitted from analysis

Single-locus segregation analysis

Chi-square tests identified 8 loci which did not conform to Mendelian expectations ($P < 0.043$). These 8 loci were omitted from the dataset. A final goodness-of-fit test using JOINMAP JMSLA confirmed that all loci analyzed for linkage had probabilities higher than 0.043. Six loci had probabilities in the range of 0.043–0.050 and were included in the linkage analysis. Three of these failed to link to any other markers, and the other 3 mapped to LG 7 (PtIFG_2356_c), LG 1 (PmIFG_1011_a), and LG 13 (PmIFG_1513_b) (Fig. 7)

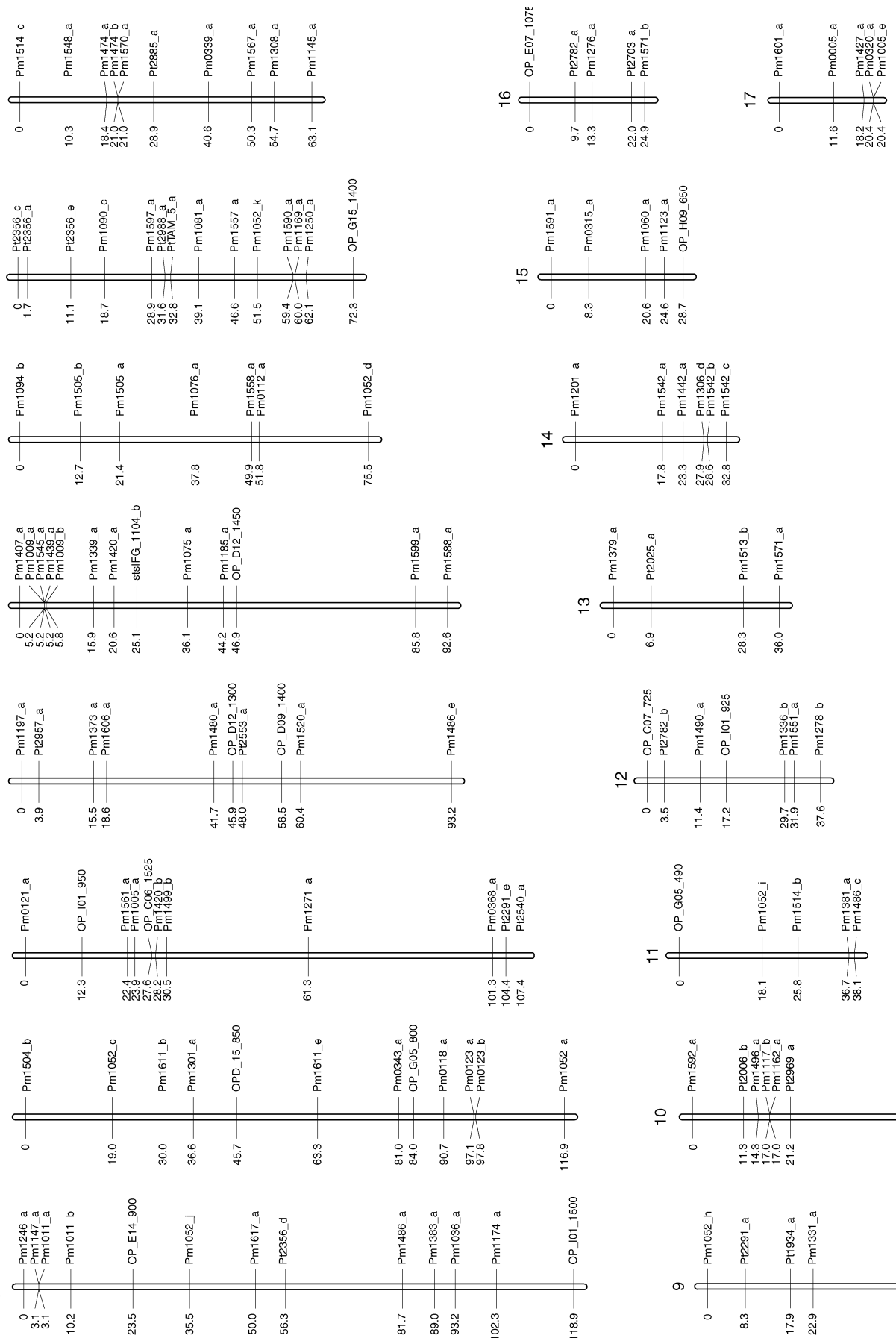

Fig. 7 Sex-averaged genetic linkage map in coastal Douglas-fir consisting of 141 RFLP and RAPD markers

Removal of these markers did not alter the order of markers in LGs; therefore these markers were not omitted from the linkage map.

Linkage analysis

A total of 217 markers were analyzed for linkage and resulted in a genetic map comprised of 141 markers organized into 17 linkage groups with a total genetic distance of 1,062 centiMorgans (cM) (Fig. 7). The average distance between markers was 7.5 cM. Thirty-two markers formed 2-marker linkage groups covering a genetic distance of 150 cM. Thirty markers failed to link to any other markers.

Our first linkage analysis was conducted when our dataset consisted of 167 markers. We considered that collecting more marker data would bring the unlinked markers into linkage groups. Hence, data was collected for an additional 50 RFLP markers and included in the linkage analysis. As these additional markers were added to linkage analyses, we expected a portion of the unlinked markers and the 2-marker LGs to become linked to larger groups or to bridge existing linkage groups. In two cases, newly added markers bridged 2 previously formed linkage groups. But, overall, newly added markers had little effect on the number of markers remaining unlinked. A similar result was reported in lettuce in which the analysis of 159 additional markers did not greatly reduce the number of linkage groups or unlinked markers (Kessili et al. 1994). Although reducing the grouping LOD to 2.5 or 3.0 decreased the number of unlinked markers, it had minor effects on the coalescence of LGs and made LGs unstable when ordered at higher JMMAP LOD thresholds. The JMGRP LOD threshold that was applied in the final linkage analysis was empirically determined by examining JMGRP results testing LOD thresholds 3.5 through 7.0 in increments of 0.5. We found that progressively increasing the JMGRP LOD slightly increased the number of LGs but progressively decreased the number of markers within groups. A JMGRP LOD threshold of 4.0 resulted in the optimum number of markers in linkage groups in which linkage order and distances were maintained when the JMMAP LOD was methodically increased. JOINMAP 2.0 recommends using a JMMAP LOD in the range of 0.01 to 0.5 for ordering markers and a REC value between 0.45 and 0.49 to create pairwise distances between markers. A step-wise increase in the JMMAP LOD threshold from 0.01 to 0.2 in the ordering phase of analysis made little difference in map order and distances, confirming integrity of marker data and group results. Analysis was conducted with a JMMAP LOD threshold of 0.1. REC threshold values were not empirically tested.

Three markers, PmIFG_0102_a, PtIFG_2969_b, and PmIFG_1506_a, comprised a group but could not be ordered. Two markers, PmIFG_1278_a from LG 12 and PtIFG_2006_a from LG 17, were removed because they formed exclusively tight linkage in the "first pair" such that other markers of the group could not be ordered. Nine markers were "jumped" by JOINMAP in the first and second round of ordering because the goodness-of-fit threshold value was exceeded when these markers were positioned on the map. In such cases, the second round map was used and these troublesome markers left out of the reported map.

Male and female maps

The paternal map was 698 cM in length and consisted of 87 markers, 35 of which originated as fully informative or intercross segregation-type loci. The average distance between markers was 8.0 cM. The maternal map was 882.4 cM in length and consisted of 99 markers, 37 which originated as fully informative or intercross segregation-type loci. The average distance between markers was 8.9 cM. Three linkage groups were uni-parentally informative: LG 11 is comprised solely of maternally informative markers, and LGs 9 and 17 are comprised solely of paternally informative markers. Because these LGs contain no markers that segregate in both the male parent and female parent, a sex-averaged map could not be constructed for these LGs.

Discussion

We have constructed a sex-averaged genetic map for Douglas-fir that is comprised of 141 markers distributed among 17 linkage groups and covers a genetic distance of 1,062 cM. The nuclear genome of Douglas-fir is organized into 13 pairs of chromosomes ($n = 13$). The actual genetic length of the Douglas-fir genome is unknown but is thought to be similar to that of pines (2,000–2,600 cM) (Echt and Nelson 1997). Single-tree RAPD-based maps have been constructed in Douglas-fir with genetic distances ranging from 2,030 cM to 2,468 cM (Agrema and Carlson 1995; Krutovskii et al. 1998). We have no explanation as to why our sex-averaged map covers 1,062 cM while conifer maps made with other types of markers are twice the genetic length. In general, shorter genetic distances are characteristic of RFLP maps in pines (Groover et al. 1994; Devey et al. 1994, 1996). Limitations to the number of markers used in linkage analysis and decreased polymorphism due to the use of a single mapping population might account for the shorter genetic distances reported for RFLP maps. However, Sewell et al. (1998) reports a RFLP map for loblolly pine that is comprised of 359 markers and is based on two separate mapping pedigrees. The genetic distance is 1,300 cM. The

reasons for shorter genetic distances of RFLP maps remain unclear.

Null loci were commonly detected in our Southern analyses and have been reported in other mapping experiments (Devey et al. 1996; Lui et al. 1996). Only nulls segregating 1 : 1 were included in our mapping analysis. Although there is speculation about how null alleles arise (Chang et al. 1988; Fischer et al. 1995), the explanation for the number of null loci we detected is not clear. Chang et al. (1988) suggested that undetected alleles are of high molecular weight and go undetected in Southern analysis. It is also feasible that some alleles have undergone large deletions and are undetected because of their low molecular weight. Our Southern blots contain fragmented DNA in the range of 500 bp to 18 kb where the majority of restricted-DNA fragments are revealed with interpretable resolution. How many of these null alleles are of low molecular weight and excluded from Southern blotting is unknown, but they are unlikely to represent the large percentage of null alleles we detected. It is also feasible that transposable elements have created large rearrangements in coding regions, causing aberrations and highly diverged allelic sequences that fail to cross-hybridize to a labeled probe (Fischer et al. 1995). SanMiguel et al. (1996) reported that maize has a long history of multiple-nested insertions of transposable elements adjacent and within coding regions. Transposable elements have been shown to exist and to have been active in the history of some conifers (Kinlaw and Neale 1997) and could possibly be responsible for alleles that fail to cross-hybridize in our Douglas-fir population. Some null loci, such as those revealed by a Douglas-fir cDNA, PmIFG_1052 (Fig. 3), were positioned at dispersed locations on the map (LGs 1, 2, 6, 7, 9 and 10), while others were positioned in proximity to one another, such as PmIFG_1542 on LG 14 (Fig. 7).

Multi-gene families are characteristic of eukaryotic genomes (Fotaki and Iatrou 1993; Krumlauf 1994; Cooke et al. 1997). Various models have been proposed for gene duplications to dispersed as well as clustered map locations (Krumlauf 1994; Fischer et al. 1995; Witsenboer et al. 1995; Dixon et al. 1996; Kinlaw and Neale 1997). In our Douglas-fir linkage map, RFLP probes detecting multiple polymorphic loci were positioned in clusters as well as being dispersed. For example, PtIFG_2356 revealed 3 segregating loci that mapped close to each other on linkage group 7, while 3 loci revealed by PmIFG_1486 mapped to LGs 1, 4 and 11. In each case, it has not been determined which of the mapped loci are transcriptionally active. A number of cDNAs produced identical or similar multi-locus RFLP phenotypes on autoradiograms. It is unknown whether identical RFLP phenotypes produced by multiple Douglas-fir cDNAs are identical in sequence, or whether the individual cDNAs represent unique members of a multi-gene family.

The first application of the genetic map reported here is to identify quantitative trait loci controlling bud phenology and related adaptive traits. We have focused primarily on cDNA markers which map to coding regions of the genome. cDNAs are a good source of informative, multi-allelic markers that are useful for QTL mapping. They can also, by DNA sequencing, reveal the identity of specific genes residing in the regions of quantitative trait loci. To further support these objectives, we are constructing a shoot meristem-specific cDNA library for additional mapping and sequencing. Also, we are currently attempting to place candidate genes, known to be involved in the regulation of other adaptive traits such as cold and drought tolerance, on the Douglas-fir map by PCR amplification of conserved gene sequences.

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