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A genetic linkage map for *Pinus radiata* based on RFLP, RAPD, and microsatellite markers

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Abstract A genetic linkage map for radiata pine (*Pinus radiata* D. Don) has been constructed using segregation data from a three-generation outbred pedigree. A total of 208 loci were analyzed including 165 restriction fragment length polymorphism (RFLP), 41 random amplified polymorphic DNA (RAPD) and 2 microsatellite markers. The markers were assembled into 22 linkage groups of 2 or more loci and covered a total distance of 1382 cM. Thirteen loci were unlinked to any other marker. Of the RFLP loci that were mapped, 93 were detected by loblolly pine (*P. taeda* L.) cDNA probes that had been previously mapped or evaluated in that species. The remaining 72 RFLP loci were detected by radiata pine probes from a *Pst*I genomic DNA library. Two hundred and eighty RAPD primers were evaluated, and 41 loci which were segregating in a 1:1 ratio were mapped. Two microsatellite markers were also placed on the map. This map and the markers derived from it will have wide applicability to genetic studies in *P. radiata* and other pine species.

Key words *Pinus radiata* · Genetic linkage map · RFLP · RAPD

Introduction

Radiata pine (*Pinus radiata* D. Don, also known as Monterey pine) is the most important plantation species in Australia and New Zealand, with a total area planted of 0.9 and 1.2 million hectares, respectively. It is also widely planted in Chile, South Africa and other countries of the Southern Hemisphere. Radiata pine is native to three small areas along the coast of California and two coastal islands; in North America the species is not of any significant commercial value.

Long generation times have hindered traditional breeding and selection of pine trees. Molecular genetic approaches to tree improvement have also been hindered to some extent by large genome sizes (Wakamiya et al. 1993) and large amounts of dispersed repetitive DNA. Radiata pine is a diploid organism (2n = 24); genetic variation is high, it being an outbred species, although not as high as in some other pine species (Moran et al. 1987; Conkle 1992).

The value of restriction fragment length polymorphism (RFLP) markers to obtain linkage maps and then to use the maps for analysis of quantitative traits has been amply demonstrated in both agronomic crops (Stuber et al. 1992; Paterson et al. 1991; Yu et al. 1991; Keim et al. 1990) and forest trees (Groover et al. 1994; Bradshaw and Stettler 1995). A further advantage of RFLPs is that they can be used across species to establish linkage relationships and to construct synteny maps (Ahn and Tanksley 1993; Tanksley et al. 1992; Whitkus et al. 1992). In the study presented here, about half of the RFLP probes used to map radiata pine had originally been mapped in loblolly pine (*P. taeda*) (Devey et al. 1994). The loblolly probes have also been shown to work well in other conifer speces (Ahuja et al. 1994).

Random amplified polymorphic DNA (RAPD) markers (Williams et al. 1990) have received much attention among conifer geneticists because of their simplicity and ease of use, and because of the haploid genetic system that occurs in pine megagmetophytes (Tulsieram et al. 1992). A number of RAPD maps in conifers have been constructed on the basis of segregation of haploid megagametophytes (Neale and Sederoff 1991; Nelson et al. 1993, 1994). Although RAPDs are dominant markers, they can also be used to map diploid material using the "pseudo-testcross" approach (Grattapaglia and Sederoff 1994). With this method, markers heterozygous present in one parent and homozygous absent in the

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other segregate 1:1 in F_2 progeny, as in a tescross, and can be mapped.

Microsatellite markers have been developed as part of the radiata pine mapping project (Smith and Devey 1994). Because microsatellites are highly polymorphic in pines, as in other species, existing breeding trials, which generally consist of large numbers of families with a limited number of progeny per family, can be more fully utilized for analysis of quantitative traits. In contrast to pine RFLPs, microsatellites only reveal a single locus per primer pair, which makes genetic interpretation much easier.

The study described herein uses RFLP, RAPD and microsatellite markers to construct a genetic linkage map for radiata pine.

Materials and methods

Plant materials

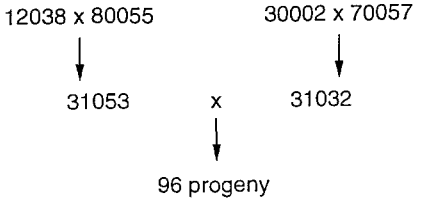
Segregation data from a three-generation pedigree (Fig. 1) including four grandparents, two parents, and 96 progeny was used to construct the map. Needle tissue and seeds of this materials were kindly provided by Dr. L. Pederick of the Department of Conservation and Natural Resources, Victoria. Needle tissue from the grandparents and parents were obtained from the Victorian Plantations Corporation seed orchard at Creswick, Victoria. Needle samples for the progeny were obtained from two sources: a progeny test including 48 progeny from this cross, which is being grown in Myrtleford, Victoria; and seed from the same cross that was grown in the glasshouse at CSIRO Forestry and Forest Products in Canberra. Forty-eight seedlings from an available 356 were included from the glasshouse-grown material, giving a total of 96 progeny in the mapping population. The glasshouse-grown seedlings were also clonally propagated and planted out in replicated trials in Victoria and South Australia for subsequent studies involving quantitative trait loci.

RFLP procedures

Radiata pine genomic DNA was isolated as previously described for loblolly pine (Devey et al. 1991), except that 10% polyvinyl pyrrolidone (MW 40000) was substituted for 10% polyethylene glycol (MW 8000) in the extraction buffer. DNA samples were digested with either *Hin*dIII or *Eco*RI and then electrophoresed in 0.8% agarose gels in IX TPE (TRIS-phosphate-EDTA) and Transferred to Hybond N+ (Amersham). Hybridizations and probe preparations were as described in Devey et al. (1991). If a probe revealed more than one band but the two parents were monomorphic, it would also be evaluated on a small blot consisting of digested DNA from several progeny as well as the parents, i.e. to determine whether both parents were heterozygous for the particular locus. Selected probes revealing polymorphic loci were then hybridized to DNA samples from grandparents, parents and 96 progeny, as well as the two parents from the loblolly mapping pedigree, digested with the appropriate restriction enzyme. The loblolly parents were included as a reference to help determine which loci were homologous in the two species.

RFLP probes for this study were derived from loblolly and radiata pine. The loblolly pine probes were from a cDNA library and had been previously mapped or evaluated in that species (Devey et al. 1994). The radiata pine probes were from a *Pst*I genomic DNA library. Total genomic DNA from radiata pine was digested to completion with *Pst*I. The DNA fragments were size-selected by electrophoresis on low-melting temperature agarose (Nusieve, FMC), and fragments 0.4–2 kb were cut out, extracted with phenol and phenol/chloroform and ethanol-precipitated. The fragments were redissolved in water, ligated into pUC18 and transformed into bacterial strain $DH5_{\alpha}$. Bacterial strains containing recombinant plasmids were selected on the basis of ampicillin resistance and the 5-bromo-4-chloro-3-indolyl-β-D-galactoside (Xgal) and isopropylthio-β-D-galactoside (IPTG) screening procedure (Sambrook et al. 1989).

Fig. 1 Three-generation radiata pine pedigree used for genetic mapping



RAPD procedures

DNA extractions from needle tissue for RAPDs were the same as for the RFLP analysis described above. Extractions for megagametophyte DNA were as described by Dellaporta et al. (1983).

Templates for polymerase chain reactions (PCR) consisted of 10 ng of DNA from either an individual megagametophyte or needle sample. The reactions were run in 10 m*M* TRIS (pH 8.3 at 25 °C), 50 m*M* KCL, 2.0 m*M* $MgCl_2$, 0.1 m*M* of each dNTP, 0.33 μM primer and 0.9 units of Ampli-*Taq* DNA polymerase (Perkin-Elmer Cetus) in a total volume of 15 µl. RAPD primers were obtained from Operon Technologies (Alameda, Calif.). A total of 40 cycles of PCR were done on a Perkin-Elimer 9600 machine: 3 × (2 min 94°, 2 min 35°, 2 min 72°) and 37 × (1 min 92 °C, 1 min 35 °C, 2 min 72 °C). PCR amplification products were electrophoresed on 1.5% agarose gels in 0.5 × TBE and visualized by ethidium bromide fluorescence.

Six megagametophytes from each parent were used to determine whether a RAPD polymorphism was in the appropriate configuration, e.g. segregating in one parent and absent in the other. For those loci which were heterozygous in one parent and homozygous absent in the other, the products were then amplified from diploid DNA obtained from the 96 individuals in the mapping pedigree.

Microsatellite procedures

The procedures for isolation and characterization of microsatellite loci in radiata pine have been described by Smith and Devey (1994). Segregation data for 2 of these loci were obtained for the 96 progeny in the mapping pedigree.

Linkage analysis

Multipoint linkage analysis was performed using JoinMap version 1.4 (Stam 1993). This program allows two or more mapping populations or as in the present case, maps from maternal and paternal parents, to be combined into one map (Groover et al. 1994). Two sets of data were constructed for those markers segregating on the maternal and paternal side of the pedigree. Loci for which both parents were heterozygous for the same alleles (e.g. *A1A2* × *A1A2*) or those loci segregating for three or four alleles were included in both data sets after recoding to obtain only maternal or paternal segregations; for example progeny from a cross with alleles *A1A2* × *A2A3* were coded as if segregating from a *A1A2* × *A2A2* cross for the maternal data set and a *A1A1* × *A2A3* cross for the paternal data set. Markers segregating on both sides of the pedigree were used to join maternal and paternal maps together. The map was constructed using a LOD score of 3.0 except for linkage groups 1 and 3, which were analyzed at a LOD of 4.0; a "map LOD" score of 0.1 was used for all linkage groups (see JoinMap manual). CentiMorgan (cM) values were calculated using the Haldane mapping function.

CRI-MAP (Green et al. 1988) was used to verify results obtained with JoinMap. CRI-MAP uses all of the available information from

grandparents, parents and progeny, including data from markers segregating for three or four alleles. Each linkage group must be constructed individually, first by inspection of two-point analysis of segregation data and then by establishing order among linked loci. Possible genotyping errors were checked by looking for double recombinants using the Map Manager program (Manly and Cudmore 1994). The map was drawn using the Draw Map program (van Ooijen 1994).

Results

The RFLP probes used in this study were derived from either loblolly or radiata pine (Table 1). Most of the loblolly pine probes had detected loci that had been previously mapped in that species (Devey et al. 1994); the others, although not detecting polymorphic loci in the loblolly mapping pedigree, were thought to be good candidates for mapping in other crosses. Probes from loblolly pine hybridized to radiata pine genomic DNA digests worked nearly or equally as well as they did in loblolly pine under the same stringency conditions for hybridization and washing. From 147 probes, 91 detected discrete and easily visualized bands. No attempt was made to vary the hybridization or washing conditions for probes that did not give satisfactory results; some of these detected multiple bands in a complex pattern, and in others the signal intensity was too weak to score. Ultimately, 93 loci detected by 57 loblolly pine probes were mapped in radiata pine. Of the 65 probes which detected mapped loci in loblolly pine, 39 also detected mapable loci in radiata using only *Hin*dIII or *Eco*RI restriction enzymes.

More than 1000 probes from a *Pst*I-digested genomic DNA library of radiata pine were also evaluated in the mapping pedigree (Table 1). However, only about 1 probe in 10 was polymorphic and of sufficient quality to allow mapping of the RFLP loci detected. Most were discarded due to high copy number, complexity of the hybridization pattern or inadequate signal intensity. Further, only one-third (59/174) of the good probes detected polymorphic loci with *Hin*dIII or *Eco*RI in this pedigree. The others, while not detecting polymorphic loci between the two parents of the mapping pedigree, may be useful probes for other pedigrees or may reveal polymorphic loci with other restriction enzymes.

A high proportion of RFLP loci segregated in a 1:1 ratio (Table 1) as a result of one of two factors. First, many of these loci were in a backcross configuration, e.g. as from a cross with alleles $A1A2 \times A1A1$, which may be a result of the lower levels of genetic variation that occur in radiata pine relative to most other pines (Moran et al. 1987). Second, RFLP probes in radiata and other pines often reveal dozens of restriction fragments, and it was not always possible to determine which fragments were allelic. Consequently, it was sometimes necessary to score loci as presence versus absence of detectable fragments. If 2 fragments scored in this way were in fact allelic, the linkage analysis would show them to be linked and they could then be combined as a single codominant locus.

Two hundered and eighty RAPD primers, Operon number OPB-1 to OPO-20, were evaluated using 6 megagametophytes from each parent. From these 41 were chosen which were heterozygous present in one parent and homozygous absent in the other, and which could be mapped in this pedigree. Twenty-eight segregated on the maternal side of the pedigree and 13 segregated on the paternal side. There were a number of inconsistencies in amplification products observed in haploid and diploid DNA samples. Some RAPD loci which should have been segregating based on megagametophyte assays either were not present or were monomorphic in the F_2 progeny.

Segregation data were obtained from PCR amplification of parent and progeny DNAs using the primer pairs for mPr4.6 and mPr9.3 (Smith and Devey 1994). Both primer pairs identify a single locus that segregates in each parent, and each locus has three alleles in this pedigree.

Linkage analysis

A genetic linkage map was constructed based on the segregation of 165 RFLP, 41 RAPD, and 2 microsatellite markers using the JoinMap linkage program (Stam 1993) (Fig. 2). Twenty-two linkage groups were established covering a total map distance of 1382 cM, with an average distance between pairs of loci of 7 cM. Forty-five loci segregated on both sides of the map, and 33 of these were fully informative. The 12 largest linkage

Table 1 Summary of RFLP probes evaluated and loci assayed in radiata pine cross 31053 × 31032

Source of probes	Number of probes evaluated	"Good" probes[a]	Number of probes detecting polymorphic loci	Number of loci scored	Number of loci with expected segregation ratios		
					1:1	1:2:1	1:1:1:1
cDNA[b]	147	91	57	93	75	5	13
gDNA	1025	174	59	72	47	7	18
Total	1172	265	116	165	122	12	31

[a] Excludes high-copy probes or those which did not produce an adequate hybridization signal, but includes probes that did not detect polymorphic loci in the cross but may be useful in other crosses or with other restriction enzymes

[b] The cDNA probes and 2 gDNA probes were previously mapped or evaluated in loblolly pine (Devey et al. 1994)

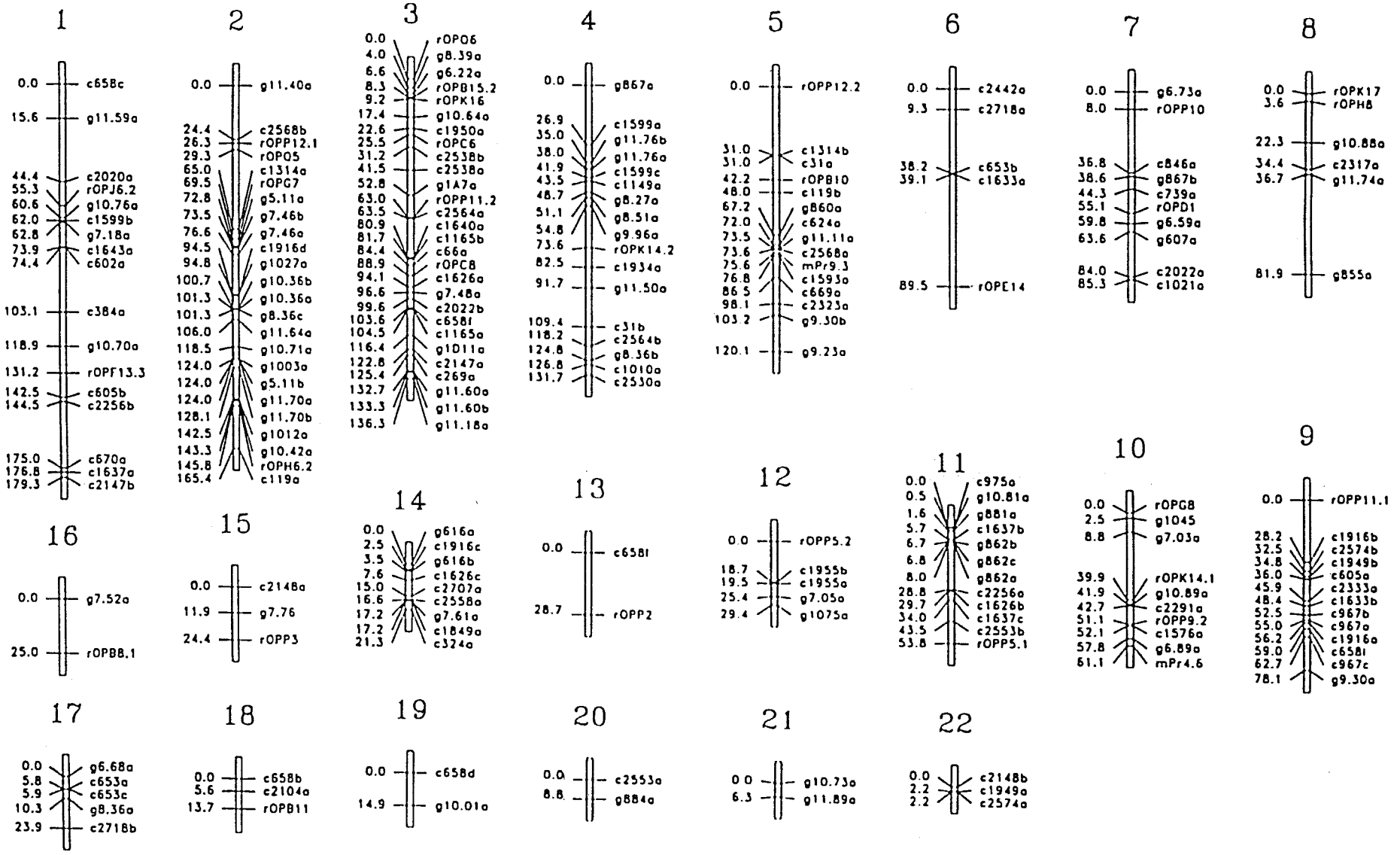

1
0.0 c658c
15.6 g11.59a
44.4 c2020a
55.3 rOPJ6.2
60.6 g10.76a
62.0 c1599b
62.8 g7.18a
73.9 c1643a
74.4 c602a
103.1 c384a
118.9 g10.70a
131.2 rOPF13.3
142.5 c605b
144.5 c2256b
175.0 c670a
176.8 c1637a
179.3 c2147b
2
0.0 g11.40a
24.4 c2568b
26.3 rOPP12.1
29.3 rOPQ5
65.0 c1314a
69.5 rOPG7
72.8 g5.11a
73.5 g7.46b
76.6 g7.46a
94.5 c1916d
94.8 g1027a
100.7 g10.36b
101.3 g10.36a
101.3 g8.36c
106.0 g11.64a
118.5 g10.71a
124.0 g1003a
124.0 g5.11b
124.0 g11.70a
128.1 g11.70b
142.5 g1012a
143.3 g10.42a
145.8 rOPH6.2
165.4 c119a
3
0.0 rOPO6
4.0 g8.39a
6.6 g6.22a
8.3 rOPB15.2
9.2 rOPK16
17.4 g10.64a
22.6 c1950a
25.5 rOPC6
31.2 c2538b
41.5 c2538a
52.8 g1A7a
63.0 rOPP11.2
63.5 c2564a
80.9 c1640a
81.7 c1165b
84.4 c66a
88.9 rOPC8
94.1 c1626a
96.6 g7.48a
99.6 c2022b
103.6 c658l
104.5 c1165a
116.4 g1D11a
122.8 c2147a
125.4 c269a
132.7 g11.60a
133.3 g11.60b
136.3 g11.18a
4
0.0 g867a
26.9 c1599a
35.0 g11.76b
38.0 g11.76a
41.9 c1599c
43.5 c1149a
48.7 g8.27a
51.1 g8.51a
54.8 g9.96a
73.6 rOPK14.2
82.5 c1934a
91.7 g11.50a
109.4 c31b
118.2 c2564b
124.8 g8.36b
126.8 c1010a
131.7 c2530a
5
0.0 rOPP12.2
31.0 c1314b
31.0 c31a
42.2 rOPB10
48.0 c119b
67.2 g860a
72.0 c624a
73.5 g11.11a
73.6 c2568a
75.6 mPr9.3
76.8 c1593a
86.5 c669a
98.1 c2323a
103.2 g9.30b
120.1 g9.23a
6
0.0 c2442a
9.3 c2718a
38.2 c653b
39.1 c1633a
89.5 rOPE14
7
0.0 g6.73a
8.0 rOPP10
36.8 c846a
38.6 g867b
44.3 c739a
55.1 rOPD1
59.8 g6.59a
63.6 g607a
84.0 c2022a
85.3 c1021a
8
0.0 rOPK17
3.6 rOPH8
22.3 g10.88a
34.4 c2317a
36.7 g11.74a
81.9 g855a
9
0.0 rOPP11.1
28.2 c1916b
32.5 c2574b
34.8 c1949b
36.0 c605a
45.9 c2333a
48.4 c1633b
52.5 c967b
55.0 c967a
56.2 c1916a
59.0 c658l
62.7 c967c
78.1 g9.30a
10
0.0 rOPG8
2.5 g1045
8.8 g7.03a
39.9 rOPK14.1
41.9 g10.89a
42.7 c2291a
51.1 rOPP9.2
52.1 c1576a
57.8 g6.89a
61.1 mPr4.6
11
0.0 c975a
0.5 g10.81a
1.6 g881a
5.7 c1637b
6.7 g862b
6.8 g862c
8.0 g862a
28.8 c2256a
29.7 c1626b
34.0 c1637c
43.5 c2553b
53.8 rOPP5.1
12
0.0 rOPP5.2
18.7 c1955b
19.5 c1955a
25.4 g7.05a
29.4 g1075a
13
0.0 c658l
28.7 rOPP2
14
0.0 g616a
2.5 c1916c
3.5 g616b
7.6 c1626c
15.0 c2707a
16.6 c2558a
17.2 g7.61a
17.2 c1849a
21.3 c324a
15
0.0 c2148a
11.9 g7.76
24.4 rOPP3
16
0.0 g7.52a
25.0 rOPB8.1
17
0.0 g6.68a
5.8 c653a
5.9 c653c
10.3 g8.36a
23.9 c2718b
18
0.0 c658b
5.6 c2104a
13.7 rOPB11
19
0.0 c658d
14.9 g10.01a
20
0.0 c2553a
8.8 g884a
21
0.0 g10.73a
6.3 g11.89a
22
0.0 c2148b
2.2 c1949a
2.2 c2574a

Fig. 2 Genetic linkage map for radiata pine. The loci are listed on the *right* and cummulative map distances (in cM) are shown on the *left*. Loci beginning with *c* are detected by loblolly pine cDNA probes; *g1A7a* and *g1D11a* are detected by loblolly pine genomic DNA probes, all other loci beginning with *g* are detected by radiata pine genomic DNA probes. Loci begnning with *r* are revealed by Operon (Alameda Calif.) RAPD primers. The two loci beginning with *m* are radiata pine microsatellite loci. To correspond to the format in the TreeGenes database (http://s27w007.pswfs.gov/) all loblolly pine locus names should be prefixed by PtIFG and all radiata pine locus names should be prefixed by PrCSI_

groups consist of from 5 to 17 loci and from 29.4 to 179.3 cM; the remaining 10 linkage groups consist of from 2 to 9 loci and 2.2 to 28.7 cM. Two RFLP and 11 RAPD loci were not linked to any other locus at a LOD of 3.0. One of the linkage groups appeared to be unusually long when analyzed at a LOD of 3.0; however, when analyzed at a LOD of 4.0 it was broken into two groups (Groups 1 and 3). These groups were joined by one codominant locus, *g10.76a*, segregating on both the maternal and paternal sides of the pedigree, which was linked to *c1165a* and which segregated only on the maternal side of the pedigree. Locus *c1165a* was not linked to any other locus in linkage group 1. The following 13 loci were unlinked: *c1089a*, *g10.42b*, *rOPB13.1*, *rOPB13.2*, *rOPB15.1*, *rOPH6.1*, *rOPJ6.1*, *rOPJ7*, *rOPK15*, *rOPK18.1*, *rOPK18.2*, *rOPO20* and *rOPP9.1*.

The program CRI-MAP was used to verify results obtained with JoinMap. CRI-MAP was often unable to place loci in which the phase was unknown, even if the phase unknown likelihood tolerance (PUK-LINK-TOL) was set to 0 (see CRI-MAP manual). When phase-unknown loci were omitted from the analysis, the program could analyze and order an indefinite number of loci. The best order for phase-known loci between CRI-MAP and JoinMap were identical in all but 2 linkage groups, and in both of these cases it was not possible to determine an exact order. In 1 of these groups, Group 8, CRI-MAP reversed the order of *c2317a* and *g11.74a* relative to JoinMap; however these loci were only 2.3 cM apart. In Group 10, only 1 locus segregated on both sides of the pedigree, and CRI-MAP inverted the order of those loci segregating on the maternal side relative to those segregating on the paternal side.

Thirty-seven probes revealed more than 2 segregating loci that could be scored and mapped. Ten of these probes produced multiple loci that mapped to the same linkage region (within 10 cM). For 2 of these, the loci appear to be distinct, but for the others it is not clear whether the same locus is being scored or whether there are multigene families within these regions. There were also a number of cases where groups of related loci were present on different linkage groups. For example, the pair *c2256a/c1637c* on linkage group 11 is similar to a related pair, *c2256b/c1637a*, on linkage group 1. Similarly, the pair *c1314b/c2568a* on group 5 mirrors a pair, *c2568b/c1314a*, on group 2. There may be some indication of duplicated regions across linkage groups, but more extensive data is needed to confirm this.

For linked loci that were fully informative, recombination on the female and male sides of the pedigree could be compared. There was a slight trend for recombination to be higher on the male side than on the female side, over 17 pairs of linked loci from 5 linkage groups, but it was not significant. Only at 1 locus pair *(g1D11a/g7.48a)* on group 3 was the difference in recombination estimates between the sexes significant, with recombination higher on the male side.

Discussion

A genetic linkage map for radiata pine has been constructed based on the segregation of RFLP, RAPD and a small number of microsatellite markers. The total distance covered is 1382 cM with an average of 7 cM between loci, which should provide adequate coverage of the genome to begin quantitative trait analysis. RAPD maps for pines have ranged from 782 to 1687 cM (Neale and Sederoff 1991; Nelson et al. 1993, 1994; Plomion et al. 1995). The RFLP map for loblolly pine reported by Groover et al. (1994) was 521 cM on the maternal side and 499 cM on the paternal side of the pedigree. There is some question as to what the total map distance is for pines. Plomian et al. (1995) estimated it to be 1500 cm; however, it could be as high as 3000 cM (Neale and Williams 1991; Nelson et al. 1994).

Moran et al. (1983) showed that for 1 pair of linked isozyme loci in radiata pine, recombination was 43% higher on the male side than on the female side. A recent study (Groover et al. 1995) in loblolly pine found that the mean estimate of recombination was 26% higher in the paternal parents, but a large part of this difference could be accounted for by differences in 2 linkage groups. In contrast, the study herein examined 17 locus pairs and found a 7% greater recombination on the male side. However, we can not conclude from this that there is greater recombination in male gamete formation across the entire genome of pines.

Thirty-nine RFLP probes revealed mapped loci in both loblolly and radiata pine; however, no attempt has been made at this time to align the maps for the two species. Although the parents for the loblolly pine mapping pedigree were run on the same gel with the radiata parents and progeny, it was still difficult in many cases to determine which loci were homologous. This is because the probes often detected many fragments, multiple loci and alleles of different sizes; moreover, with some probes a different restriction enzyme was used in radiata than was used in loblolly pine to detect the polymorphism. Where probes detected only a single locus with 2 fragments per individual, homology was obvious; however, there were not very many of these probes. Radiata pine RFLP probes and microsatellites will be used for mapping in loblolly pine with the aim of constructing a synteny map between the two species.

Forty-one RAPD markers segregating in a 1:1 ratio were analyzed in this pedigree. A number of other loci appeared to be in the appropriate backcross configuration based on megagametophyte segregation; however, in diploid needle DNA samples, the bands were either not present or were monomorphic. RAPD bands segregating in a 1:1 ratio were also observed in needle but not megagametophyte DNA samples. These differences are presumably due to competition effects of RAPD primer binding sites, which are two-fold greater in diploid than in haploid DNA samples. Emphasis on RAPD markers was less in this map construction because our objective was to construct a base map consisting of markers that could be easily transported across pedigrees and species. There is some question as to whether RAPD markers are transportable across pedigrees (Heun and Helentjaris 1993). In a study involving 10 RAPD markers closely linked to a gene for resistance to white pine blister rust in sugar pine (*P. lambertiana*) (Devey et al. 1995) half of the loci were easily visualized across five different families from ethidium bromide-stained agarose gels, while the other 5 loci needed more sensitive methods of detection (e.g. Southern blotting). So although it should be possible to use RAPD markers across pine pedigrees, in general this would be more difficult than with RFLP or microsatellite markers. The use of RAPD markers to compare loci in different species of pines would probably not be feasible.

Development of a genetic linkage map is the first step toward the detection of factors which control the expression of economically important traits. Much of the effort in constructing such a map is directed toward identifying useful, polymorphic markers, and once identified these markers can be used in numerous other pedigrees and related taxa. The mapping pedigree used here was selected from the Australian commercial forestry breeding program. Projects have been initiated to use the map and markers derived from it to identify factors controlling economically important traits in this and other pedigrees.

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