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An integrated genetic linkage map for eucalypts using RFLP, RAPD and isozyme markers

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Abstract An integrated genetic linkage map for *E. nitens* was constructed in an outbred three-generation pedigree. Analysis of 210 RFLP, 125 RAPD and 4 isozyme loci resulted in 330 markers linked in 12 linkage groups covering 1462 cM ($n = 11$ in eucalypts). The 12th linkage group is comprised of only 5 markers and will probably coalesce with another linkage group when further linked loci are located. Co-dominant RFLP loci segregating in both parents were used to integrate linkages identified in the male and female parents. Differences in recombination frequencies in the two parents were observed for a number of pairs of loci, and duplication of sequences was identified both within and between linkage groups. The markers were distributed randomly across the genome except for the RFLPs in linkage group 10 and for some loci showing segregation distortion, which were clustered into three regions of the map. The use of a large number of co-dominant RFLP loci in this map enables it to be used in other pedigrees of *E. nitens* and forms a basis for the detection and location of QTL in *E. nitens* and other eucalypt species.

Key words Genetic map · Linkage · Eucalypts · RFLP · RAPD

Introduction

Forest tree species are generally characterized by outbred mating systems as well as by long generation times, and many of the traits that are of commercial importance in the forest industry are expressed late in the development of the trees. These characteristics have limited the development of classical morphological markers, which is turn has hampered progress in tree breeding programs compared to that in crop species. The advent of isozyme techniques provided some genetic markers that could be used to address questions concerning genetic resources, mating systems and population structure. Linkage analysis of isozyme loci has been carried out in a number of forest tree species (*Larix laricina*, Cheliak and Pitel 1985; *Eucalyptus regnans*, Moran and Bell 1983; *Pinus* spp, Conkle 1981; *Pinus rigida*, Guries et al. 1978; *P. taeda*, Adams and Joly 1980; *P. strobus*, Eckert et al. 1981; *P. radiata*, Moran et al. 1983; *Pseudotsuga menziesii*, El-Kassaby et al. 1982). Although isozyme systems are highly variable in a few eucalypt species (Moran and Bell 1983), the numbers of loci have not been sufficient for application in breeding strategies using marker-aided selection.

Restriction fragment length polymorphism (RFLP) techniques provide a potentially infinite number of variable markers for the development of genetic linkage maps (Tanksley 1983; Helentjaris et al. 1985) and have been used in crop improvement programs in agricultural species (Tanksley et al. 1989; Stubber 1992). In tree species RFLPs have been used to construct genetic linkage maps in *Pinus taeda* (Devey et al. 1994; Groover et al. 1994), *Populus tremuloides* (Liu and Furnier 1993) and *Cryptomeria japonica* (Mukai et al. 1995), and have been used to provide markers in a map of apple (Hemmat et al. 1994). More recently, the development of random amplified polymorphic DNA (RAPD) techniques have also provided a large number of markers (Williams et al. 1990; Welsh and McClelland 1990), however, their dominant nature makes them less informative than co-dominant RFLP markers in diploid outbred organisms. The availability of haploid megagametophyte tissue in conifers and the use of RAPD loci has enabled the rapid construction of genetic linkage maps for individual trees in *Pinus elliottii* (Nelson et al. 1993) and *Picea glauca* (Tulsieram et al. 1992). In angiosperm trees, where haploid tissue is not available, a pseudo-testcross approach using outbred pedigrees has been used to construct maps of single individuals of *E. grandis* and *E. urophylla* using RAPD markers (Grat-

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tapaglia and Sederoff 1994). This approach has also been used in apple to generate maps of each parent of a cross prior to integration of the maps using co-dominant markers (Hemmat et al. 1994).

Eucalypts are important tree species that are grown both for timber production and for pulp and paper manufacture. An integrated linkage map will provide a framework for the use of molecular techniques for improved breeding stategies in the commercial eucalypts and for addressing questions of population and conservation genetics in other eucalypt species. RFLPs have been shown to be highly variable in *Eucalyptus nitens* (Byrne et al. 1994). The high level of synteny observed in linkage relationships between a number of species (Shields 1993) suggests that a map developed in one eucalypt species will be comparable with maps in other species in the genus. Hence, RFLP probes are a good source of markers for the development of an integrated linkage map that will be useful in a wide range of breeding material across eucalypt species.

A genetic linkage map using an F_2 outbred pedigree arising from interpopulational crosses has been constructed. The use of interpopulation crosses and highly variable RFLP markers increases the probability of using the map in other pedigrees of *E. nitens* and other eucalypt species. RAPD loci that occur in a pseudo-testcross configuration in this pedigree have also been mapped, along with several isozyme loci. A pedigree of *E. nitens* was chosen for the construction of the map for several reasons. Firstly, it is an important species, particularly in Australia, South Africa and Chile; secondly, interpopulational crosses were available from which a three-generation pedigree could be developed; and thirdly, isozyme (Moran 1992) and chloroplast (cp) DNA markers (Byrne and Moran 1994) suggest that the species maintains a high level of diversity, possibly due to the disjunct distribution of the populations. Thus, a pedigree involving an interpopulational cross in this species should show high levels of variability for molecular markers and may also increase the chance of segregation in quantitative traits of interest to the forest industry.

Materials and methods

Plant material

The mapping work was carried out on a three-generation outbred pedigree of *E. nitens*. Details of the origin and sampling of the pedigree is given in Byrne et al. (1994). Analysis was conducted on four first-generation (grandparents), two second-generation (parents) and 118 third-generation (progeny) individuals of the pedigree.

RFLP procedures

Construction of libraries and methods for RFLP analysis were as in Byrne et al. (1994). The parents of the pedigree were screened with genomic and cDNA probes to identify polymorphic and interpretable loci. The progeny were screened with 97 genomic and 72 cDNA probes. Most RFLP patterns were simple, and genetic interpretation of loci was made on the basis of inheritance of alleles in all three generations. Some RFLP patterns were complex, and interpretation in terms of alleles at loci was not possible. In these cases the presence or absence of fragments was scored and treated as markers segregating from one parent only.

RAPD procedures

RAPD analysis was carried out using a modification of the procedure of Williams et al. (1990) and Yu and Pauls (1992). Reaction mixtures of 20 ul contained 10 m*M* Tris-HCl pH 8.3, 50 m*M* KCl, 1.8 m*M* MgCl, 0.2 m*M* dATP, 0.2 m*M* dCTP, 0.2 m*M* dGTP, 0.2 m*M* dTTP, 0.33 u*M* primer, 0.9 unit *Taq* polymerase and 5ng DNA template. Amplification was carried out in a Perkin Elmer–Cetus 9600 thermal cycler programmed for 1 cycle of 1 min denaturation at 94 °C, 30 s of annealing at 36 °C and 1 min extention at 72 °C, then 34 cycles with the denaturation step reduced to 5 s. Amplification products were run out in 1.5% agarose gels in 0.5 × TBE buffer (4.5 m*M* Tris-HCl pH 8.0, 8.9 m*M* boric acid, 0.2 m*M* EDTA) at 150 V for 2.5 h, visualized by staining in 0.5 ug/ml ethidium bromide and viewed over UV light. The parents and grandparents of the pedigree were screened with 520 random 10-base primers (Operon Technologies, kits A–Z). Analysis in the progeny was carried out with 83 primers that detected bands in a testcross configuration (i.e. one parent heterozygous for the presence of a band and the other parent absent). Segregation of the band in the progeny was confirmed by analyzing the parents and 10 progeny, then analysis of the primer in 94 more progeny was carried out. Therefore, analysis of RAPD primers was undertaken for 104 progeny in total.

Isozyme procedures

The isozymes were detected in leaf tissue. The extraction buffer, electrophoresis conditions and staining recipes were as described in Moran and Bell (1983) and Conkle et al. (1982). The parents and grandparents of the pedigree were assessed for variation in 16 enzyme systems: malate dehydrogenase (MDH, EC 1.1.1.37), isocitrate dehydrogenase (IDH, EC 1.1.1.42), malic enzyme (ME, EC 1.1.1.82), phosphogluconate dehydrogenase (PGD, EC 1.1.1.44), glucosephosphate isomerase (GPI, EC 5.3.1.9), uridine diphosphogluconate pyrophosphatase (UPG, EC 2.7.7.9), phosphoglucomutase (PGM, EC 5.4.2.2), shikimic acid dehydrogenase (SDH, EC 1.1.1.25), esterase (EST, EC 3.1.1.1), leucine aminopeptidase (LAP, EC 3.4.11.1), menadione reductase (MR, EC 1.6.99.2), aconitase (AC, EC 4.2.1.3), peroxidase (PER, EC 3.4.13.11), fumarase (FUM, EC 4.2.1.2), triosephosphate isomerase (TPI, EC 5.3.1.1) and diaphorase (DIA, EC 1.8.1.4). Segregation in the 118 progeny was assessed for 3 enzyme systems: malate dehydrogenase, shikimic acid dehydrogenase and phosphogluconate dehydrogenase.

Linkage analysis

All loci were scored at least twice by two people separately to minimize scoring and interpretation errors. Segregation of the loci was tested for goodness-of-fit to expected Mendelian segregation ratios using a χ^2 test. Analysis of linkage between loci was carried out using Joinmap (Stam 1993). The loci were separated into three types: (1) those showing segregation from one parent (i.e. one parent was heterozygous and the other was homozygous); (2) those loci showing segregation for more than two alleles and both parents were heterozygous; and (3) those loci showing segregation for two alleles and both parents were heterozygous. The analysis requires two datasets comprising loci representing segregation from each parent. The first type of loci were split into those segregating from the female parent and those segregating from the male parent. For the second type of loci the segregation from each parent was separated and included with the first type of loci. For third type of loci, the scores for individuals where the segregation from each parent could be identified (i.e. the homozygotes) were included in the sets of loci showing segregation from each parent. Each dataset was analyzed

independantly for two-point linkage. Linkages between pairs of type-three loci are present in both datasets, therefore they were deleted from the two-point linkage data from one set. The two sets of two-point linkages were then combined and run in one analysis producing an integrated map from the two groups of loci. The loci that segregated from both parents and had more than two alleles were fully informative and were used to integrate the male and female linkages.

Linkage groups were identified using a two-point analysis with a minimum LOD score of 5.0. The most likely order of loci within groups was determined using multipoint analysis with a minimum LOD score of 5.0 and a mapLod score of 0.5. The Kosambi (1944) mapping function was used to determine distance between markers. Presence of double crossover events in consecutive loci were identified using MapManager (Manly and Cudmore 1994) and all were checked for genotyping errors. Two-point linkage analysis was also carried out using Crimap (Green 1988) to verify the linkage groups identified by Joinmap. Output files from Joinmap were used as input to Drawmap (van Ooijen 1994) to draw the map. The distribution of markers on the linkage groups was analyzed for clustering using a nearest neighbor analysis (Clark and Evans 1954) modified for one dimension.

Results

Marker analysis

Analysis of the fragment patterns produced with the 169 probes gave information at 210 loci, 119 from genomic probes and 91 from cDNA probes. Segregation ratios that significantly departed from the expected at the 5% level were observed in 13 loci (6%). RFLP loci where segregation information is obtained from both parents (i.e. both parents were heterozygous) are the most informative loci for the construction of an integrated map. In *E. nitens* approximately 40% of the RFLP loci detect three or more alleles (Byrne et al. 1994). In the mapping pedigree 67 loci detected more than three alleles and showed segregation from both parents, and 15 showed segregation from both parents but detected only two alleles. Of the remaining 128 loci 75 exhibited segregation from the maternal parent and 54 from the paternal parent.

Since RAPD analysis generally produces dominant markers their use in mapping in diploid outbreeding species is most efficient when the markers are in a testcross configuration. This configuration will occur approximately 20% of the time in an outbred pedigree. Out of the 520 primers screened, 83 gave amplification products in the testcross configuration, representing thereby 125 loci. Segregation ratios that significantly departed from the expected at the 5% level were observed in 10 loci (8%). This is similar to that observed for RFLP loci. Being dominant markers RAPD loci segregate from only one parent, and 61 loci segregated from the maternal parent while 63 showed segregation from the paternal parent. One co-dominant RAPD locus was identified where both parents were heterozygous for the presence of two amplification products. The presence of the products in the progeny showed segregation in a 1:2:1 ratio for the three classes of genotypes as was expected under Mendelian principles.

Variation in the mapping pedigree was observed for 3 of the 16 isozyme systems. The 3 systems gave zones of activity representing 5 loci of which 4, *Mdh*-1, *Mdh*-2, *Sdh* and *Pgd*-1, were polymorphic in the mapping pedigree. All loci segregated according to expected Mendelian ratios. Two showed segregation from the maternal side and 2 showed segregation from the paternal side of the pedigree.

Linkage analysis

Two-point linkage analysis of the 339 loci resulted in 12 linkage groups containing 330 loci (Fig. 1). Nine loci (3 RFLP and 6 RAPD loci) were not assigned to any linkage group. Multipoint linkage analysis of each group produced linkage groups ranging from 21 cM to 156 cM with the average size being 122 cM. The number of markers per group ranged from 5 to 48 with the average being 27.5. The total length of the map was 1462 cM with an average distance between loci of 4.4 cM. The distance between adjacent markers was less than 20 cM except for six pairs of markers, and the largest distance between these markers was 40 cM. Eucalypts have a haploid chromosome number of 11, therefore this analysis identified 1 more linkage group than was expected. The smallest group, group 12, contains 5 loci all segregating from the male parent. This group may well represent a group of loci that will map to the end of another group when appropriate loci are identified to provide the linkage. Loci have been assigned to linkage groups with a high degree of confidence since analysis with both Joinmap and Crimap programs identified the same linkage groups. The order of the loci for most groups is quite robust, indicating consistency in the data. Variation of the linkage parameter (mapLOD) from 0.05 to 1.0 or removal of loci from the analysis results only in an occasional flipping in the order of close adjacent markers, and some reordering of a few markers occurs in 3 groups.

Analysis of the distribution of markers was carried out using a nearest neighbor test. The distribution of markers across linkage groups showed no significant deviation from a random distribution at the 0.05 level (Table 1). Similarly, analysis of RFLPs and RAPDs alone showed no significant deviation from a random distribution except for the RFLPs on group 10, which showed clustering (Table 1). Loci segregating from the female or male parents or both were spread across the linkage groups, except for group 12, which is comprised only of loci segregating from the male parent. The average distance between loci segregating from both parents is 15.5 cM. Multiple RFLP loci were detected by 41 probes, and the loci from 21 of these mapped to the same linkage groups. The distance between these loci ranged from 0 to 40 cM with the average distance being 6.5 cM. Those loci that mapped to within 1 cM represented segregation that could not be genetically interpreted as 1 locus. The loci from the other 20 probes

Table 1 Analysis of clustering of markers on linkage groups using test for serial correlation

Linkage group	Size (cM)	Number of loci	All loci	RFLPs	RAPDs
1	140.2	48	0.97	1.14	1.01
2	147.0	26	0.76	0.81	0.34
3	155.8	23	0.85	0.77	1.39
4	156.8	47	1.12	1.16	0.75
5	138.4	21	0.66	0.97	0.65
6	123.6	37	1.14	0.96	1.16
7	131.9	19	1.07	1.57	0.96
8	155.5	38	0.94	1.03	0.69
9	116.6	33	1.00	1.14	0.98
10	96.4	17	0.49	0.22*	1.53
11	78.5	16	0.98	1.22	1.19
12	21.2	5	0.50		

* Significance at the 0.05 level

mapped to different linkage groups. No pattern of conservation of duplicated sequences between linkage groups was observed except for a duplication involving two pairs of loci on the same linkage group separated by 2 cM, which appears to be a tandem duplication at this resolution of the map. None of the RAPD loci that were amplified by the same primer mapped to the same position in a linkage group.

A small proportion of loci showed distortion in their segregation ratios. An analysis carried out excluding these loci resulted in only minor changes in the order of a few loci in some linkage groups. Therefore, these loci were included in the map. For the RFLP loci that showed segregation from both parents the distortion in the ratios came from either one parent or the other. Only 1 of these loci showed distorted ratios from both parents. One other locus showed distorted segregation from both parents, but the parents were heterozygous for two alleles and therefore the source of the distortion could not be determined. The majority of these loci were clustered into three regions of the map. Loci with distortion from the female parent mapped to a region of linkage group 1. Loci showing distortion from the male parent mapped to a region on linkage group 2 and a region of linkage group five. Six other loci were scattered across the linkage groups with no clustering.

Large differences in recombination frequencies between the parents were observed for some pairs of loci that segregated from both parents. Approximately 10% of the pairs of loci that segregated from both parents showed differences between the parents of more than 10 recombination units. These pairs of loci were not grouped together in any particular linkage group, and there was no consistent pattern of increased recombination frequency from either parent. The average of the two recombination frequencies is used as the distance between the markers in the integrated map.

Fig. 1 Genetic linkage map of *E. nitens*. Marker identification is given to the *right* of the bars; cumulative distance (in cM) of markers along groups is indicated to the *left* of the bars

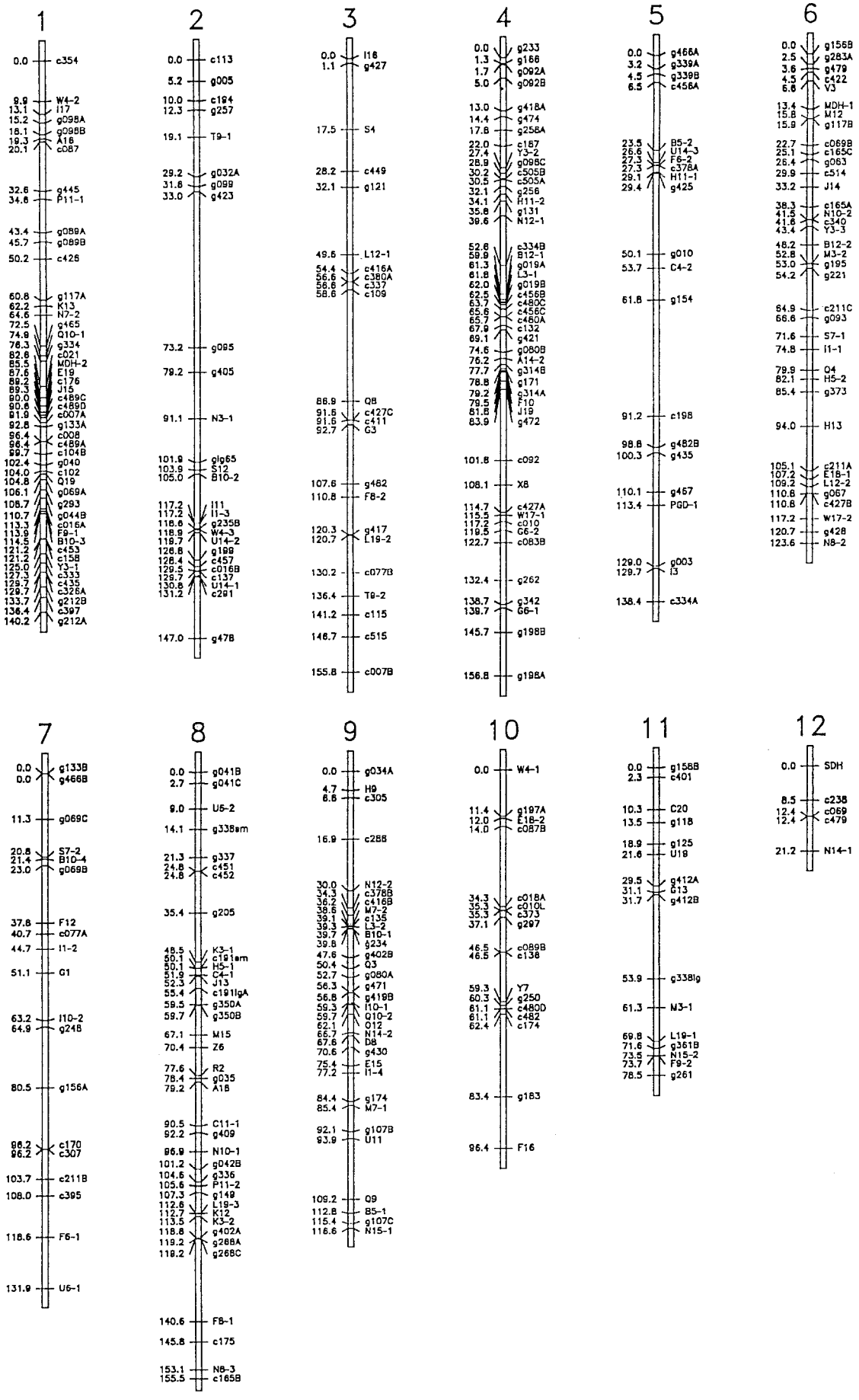
Discussion

The construction of an integrated map in an outbred pedigree relies on the use of fully informative co-dominant markers. In this map 22% of all the markers are fully informative, and these markers are well-spaced across the linkage groups. The number of these markers and their distribution across the linkage groups enables the integration of linkages identified by the other loci on either the female or male side of the cross. The assignment of loci to linkage groups is very reliable. The order of the loci in the linkage groups is quite robust in this pedigree although this is not a critical requirement for this map since it is intended to be a baseline map for this and other eucalypt species. Once genes for specific traits have been located to particular linkage regions, these regions will be studied more intensively in a number of pedigrees, and the unambiguous order of loci will be more important.

The 12th linkage group comprises loci segregating from the male parent only. It is probable that this group of loci represent a region of a chromosome also represented by another linkage group. Identification of fully informative loci in this region will be required to determine the linkage relationships of group 12 with another group. The 3 RFLP loci that are not linked segregate from the female parent. These loci may well also come from this region of the genome, and the loci required to determine their linkage relationships have not been identified. A bulk segregant analysis (Michelmore et al. 1991) could be used to screen both RFLP and RAPD loci for linkage to this group in order to target the identification of loci linked to this region and coalesce this group with a larger linkage group. This approach was successfully used to join a small linkage group containing a downy mildew resistance gene to a larger linkage group in lettuce (Kesseli et al. 1994) and to identify a marker linked to cereal cyst nematode resistance in wheat (Eastwood et al. 1994)

Genetic linkage maps have been constructed for two other eucalypt species, *E. grandis* and *E. urophylla* (Grattapaglia and Sederoff 1994). These maps were constructed from linkage information from single individuals of each species using RAPD loci segregating in a testcross configuration. The linkage distance covered in our map is similar to the distance covered in the maps of *E. grandis* (1551 cM) and *E. urophylla* (1101 cM).

One co-dominant RAPD locus was identified and confirmed with the linkage analysis. Codominant RAPD loci have also been identified in slash pine (Nelson et al. 1993) and *E. grandis* and *E. urophylla* (Grattapaglia and Sederoff 1994), all with a frequency of less


1
0.0 c354
8.9 W4-2
13.1 I17
15.2 g098A
18.1 g098B
19.3 A16
20.1 c087
32.6 g445
34.6 P11-1
43.4 g089A
45.7 g089B
50.2 c426
60.8 g117A
62.2 K13
64.6 N7-2
72.5 g465
74.8 Q10-1
76.3 g334
82.8 c021
85.5 MDH-2
87.6 E19
88.2 c176
89.3 J15
90.0 c489C
90.6 c489D
91.9 c007A
92.8 g133A
96.4 c008
98.4 c489A
99.7 c104B
102.4 g040
104.0 c102
104.8 Q19
106.1 g069A
108.7 g293
110.7 g044B
113.3 c016A
113.9 F9-1
114.5 B10-3
121.2 c453
121.2 c158
125.0 Y3-1
127.3 c333
128.7 c435
129.7 c326A
133.7 g212B
136.4 c397
140.2 g212A
2
0.0 c113
5.2 g005
10.0 c194
12.3 g257
19.1 T9-1
29.2 g032A
31.8 g099
33.0 g423
73.2 g085
79.2 g405
91.1 N3-1
101.9 clg65
103.9 S12
105.0 B10-2
117.2 I11
117.2 I1-3
118.6 g235B
118.9 W4-3
119.7 U14-2
126.8 g188
128.4 c457
129.5 c016B
129.7 c137
130.8 U14-1
131.2 c291
147.0 g47B
3
0.0 I18
1.1 g427
17.5 S4
28.2 c449
32.1 g121
49.6 L12-1
54.4 c416A
56.6 c380A
56.6 c337
58.6 c109
86.9 Q8
91.6 c427C
91.6 c411
92.7 G3
107.6 g462
110.8 F8-2
120.3 g417
120.7 L19-2
130.2 c077B
136.4 T8-2
141.2 c115
146.7 c515
155.8 c007B
4
0.0 g233
1.3 g166
1.7 g092A
5.0 g092B
13.0 g418A
14.4 g474
17.8 g258A
22.0 c187
27.4 Y3-2
28.9 g098C
30.2 c505B
30.5 c505A
32.1 g256
34.1 H11-2
35.8 g131
39.6 N12-1
52.6 c334B
59.9 B12-1
61.3 g019A
61.8 L3-1
62.0 g019B
62.5 c456B
63.7 c480C
65.6 c456C
65.7 c460A
67.9 c132
69.1 g421
74.6 g080B
76.2 A14-2
77.7 g314B
78.8 g171
79.2 g314A
79.5 F10
81.8 J19
83.9 g472
101.8 c092
108.1 X8
114.7 c427A
115.5 W17-1
117.2 c010
119.5 G6-2
122.7 c083B
132.4 g262
138.7 g342
139.7 G6-1
145.7 g198B
156.8 g198A
5
0.0 g466A
3.2 g339A
4.5 g339B
6.5 c456A
23.5 B5-2
26.6 U14-3
27.3 F6-2
27.3 c378A
29.1 H11-1
29.4 g425
50.1 g010
53.7 C4-2
61.8 g154
91.2 c198
98.8 g482B
100.3 g435
110.1 g467
113.4 PGD-1
129.0 g003
129.7 I3
138.4 c334A
6
0.0 g156B
2.5 g283A
3.6 g479
4.5 c422
6.6 V3
13.4 MDH-1
15.8 M12
15.9 g117B
22.7 c069B
25.1 c165C
26.4 g063
29.9 c514
33.2 J14
38.3 c165A
41.5 N10-2
41.6 c340
43.4 Y3-3
48.2 B12-2
52.8 M3-2
53.0 g195
54.2 g221
64.9 c211C
66.6 g093
71.6 S7-1
74.8 I1-1
79.9 O4
82.1 H5-2
85.4 g373
94.0 H13
105.1 c211A
107.2 E18-1
109.2 L12-2
110.8 g067
110.8 c427B
117.2 W17-2
120.7 g428
123.6 N8-2
7
0.0 g133B
0.0 g466B
11.3 g069C
20.8 S7-2
21.4 B10-4
23.0 g069B
37.8 F12
40.7 c077A
44.7 I1-2
51.1 G1
63.2 I10-2
64.9 g248
80.5 g156A
86.2 c170
86.2 c307
103.7 c211B
108.0 c395
118.6 F6-1
131.9 U6-1
8
0.0 g041B
2.7 g041C
9.0 U6-2
14.1 g338sm
21.3 g337
24.8 c451
24.8 c452
35.4 g205
48.5 K3-1
50.1 c191sm
50.1 H5-1
51.9 C4-1
52.3 J13
55.4 c191lgA
59.5 g350A
59.7 g350B
67.1 M15
70.4 Z6
77.6 R2
78.4 g035
79.2 A18
90.5 C11-1
92.2 g409
96.9 N10-1
101.2 g042B
104.6 g336
105.6 P11-2
107.3 g149
112.6 L19-3
112.7 K12
113.5 K3-2
118.8 g402A
119.2 g268A
119.2 g268C
140.6 F8-1
145.8 c175
153.1 N8-3
155.5 c165B
9
0.0 g034A
4.7 H9
6.6 c305
16.9 c288
30.0 N12-2
34.3 c378B
36.2 c416B
38.6 M7-2
39.1 c135
39.3 L3-2
39.7 B10-1
39.8 g234
47.6 g402B
50.4 Q3
52.7 g080A
56.3 g471
56.8 g419B
59.3 I10-1
59.7 Q10-2
62.1 O12
66.7 N14-2
67.6 D8
70.6 g430
75.4 E15
77.2 I1-4
84.4 g174
85.4 M7-1
92.1 g107B
93.9 U11
109.2 Q9
112.8 B5-1
115.4 g107C
116.6 N15-1
10
0.0 W4-1
11.4 g197A
12.0 E18-2
14.0 c087B
34.3 c018A
35.3 c010L
35.3 c373
37.1 g297
46.5 c089B
46.5 c138
59.3 Y7
60.3 g250
61.1 c480D
61.1 c482
62.4 c174
83.4 g163
96.4 F16
11
0.0 g158B
2.3 c401
10.3 C20
13.5 g118
18.9 g125
21.6 U18
29.5 g412A
31.1 G13
31.7 g412B
53.9 g338lg
61.3 M3-1
69.8 L19-1
71.6 g361B
73.5 N15-2
73.7 F9-2
78.5 g261
12
0.0 SDH
8.5 c238
12.4 c069
12.4 c479
21.2 N14-1

than 5%. Multiple loci identified with the same primers were not linked and thus represented independent loci in the genome. The independence of these loci is an important aspect of the use of RAPD loci identified by the same primers in the identification of individuals or diversity studies. Multiple RFLP loci identified by the same probe must represent sequences that share homology, however the polymorphism identified by the different loci does not necessarily result from the same mutation events. If the size of the genome is assumed to be 530 Mbp/1C (Grattapaglia and Bradshaw 1994) and the estimate of the genetic size to be about 1500 cM as determined here, then 1 cM represents approximately 350 kb. The average size of fragments identified with RFLP probes for the enzymes *Bgl*II, *Dra*I and *Eco*RI is 5.5 kb (Byrne et al. 1994). Thus, DNA fragments at 2 loci identified by the same probe may map to the same position but still be up to 350 kb away from each other and represent different mutation events. Half of the duplicated sequences identified by polymorphic fragment patterns were duplicated loci on the same linkage groups and half were duplicated on different linkage groups. No evidence for the duplication of large sections of linkage groups was observed in *E. nitens*. Similar random duplication of loci without conservation was observed in lettuce (Kesseli et al. 1994), whilst the extensive conservation of duplicated loci between linkage groups was observed for maize (Helentjaris et al. 1986) and *Brassica rapa* (Song et al. 1991). Chromosome duplication has been hypothesized in the evolution of both maize and *Brassica rapa* (Helentjaris et al. 1986; Song et al. 1991).

The differences in recombination frequency between the two parents of the mapping pedigree for a number of pairs of markers could be due to several factors. There may be some regions of the genome where recombination can vary significantly in different genetic backgrounds, differences in recombination rates when an individual acts as a male or female in a cross or random effects between individuals. Recombination between markers in a number of individuals and in the same individual acting as a male and a female parent in a reciprocal cross is being investigated.

No significant clustering of markers on linkage groups was identified, except for the RFLP loci in group 10. Some clustering of markers has been reported for other species (Kesseli et al. 1994; Grattapaglia and Sederoff 1994; Tanksley et al. 1992; Vallejos et al. 1992). The clustering of loci showing distortion in their segregation was observed, and a similar clustering of distorted loci has been observed in other species (Kesseli et al. 1994; Vallejos et al. 1992; Gebhardt et al. 1991). Such a clustering of loci may indicate a biological basis for the observed distortion. Analysis of the segregation patterns of these loci in these regions in other crosses will give further indications of specific causes for the distortion, such as the presence of lethal genes. Large gaps between markers have been reported in a detailed map of lettuce (Kesseli et al. 1994), and several gaps greater than 20 cM are also present in our map. These gaps may represent regions of increased recombination or may represent highly conserved regions where polymorphic markers are fewer and therefore harder to identify. They could also be regions of repetitive DNA where fewer markers will be identified since selection against these markers has occurred in the construction and screening of the RFLP libraries. Targeting of these regions using a bulk segregant approach may identify more markers in these regions of the linkage groups.

Markers linked with genes controlling economically important traits may be used for marker-aided selection in breeding programs. The linkage data developed in this map will be used directly for the detection and location of genes controlling quantitative trait loci in *E. nitens*. The seedling progeny of the mapping pedigree were assessed for several traits in the glasshouse. Analysis of the quantitative data and the linkage information developed in this map is currently in progress to detect quantitative trait loci controlling these traits. The progeny of the mapping pedigree have been planted in a field trial and will be assessed for other quantitative traits in the future. These markers may also be used to tag chromosome segments containing genes of interest and monitor their introgression into other elite genetic material in breeding programs. A large number of highly variable markers will also be useful for the identification of breeding lines and determination of pollen flow in seed orchards.

This map can be used as a basis for the use of RFLP markers both in *E. nitens* and other species within the genus since these markers can be used in other eucalypt species and appear to detect the same loci (Byrne and Moran, unpublished data). A high level of synteny has been identified in the genomes of some other species (e.g. sugar cane , sorghum and maize, Grivet et al. 1994; rice and maize, Ahn and Tanksley 1993; pea and lentil, Weeden et al. 1992; maize and sorghum, Whitkus et al. 1992; potato and tomato, Gebhardt et al. 1991). It would be expected that linkage relationships in other eucalypt species may well be comparable with those established in *E. nitens*, particularly for the main commercial species in the subgenus Symphyomyrtus to which *E. nitens* belongs (Pryor and Johnson 1971). Determination of the linkage relationships of these RFLP loci in other eucalypt species will allow investigations of genome organization and similarity in the genus. Markers for which linkage information is available will allow appropriate selection of independant markers from across the genome for studies of diversity, population structure and phylogeny in many eucalypt species.

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