Theor Appl Genet (1994) 89: 397–402 © Springer-Verlag 1994

M. Byrne · G. F. Moran · J. C. Murrell · W. N. Tibbits

Detection and inheritance of RFLPs in *Eucalyptus nitens*

Received: 27 January 1994 / Accepted: 2 February 1994

Abstract The level of polymorphism using genomic and cDNA probes with a number of restriction enzymes and the inheritance of the RFLP loci was investigated in *E. nitens*. The polymorphism detected with 366 genomic and cDNA probes and three to six restriction enzymes was analysed in three-generation outbred pedigrees. No difference in the level of polymorphism detected with genomic versus cDNA probes was observed. There was a difference in the efficiency of detection of polymorphism with six different restriction enzymes, with three of the enzymes (*Bgl*II, *Dra*I and *Eco*RI) showing substantially more polymorphism than the others. There was no significant correlation between the size of the DNA fragments generated by the enzymes and the detection of polymorphism. Several cases of restriction-site mutations resulting in a polymorphism were observed. The inheritance of 69 loci was analysed in two pedigrees resulting from interpopulational crosses. The majority of the loci segregated according to expected ratios with distortion observed in only 3% of loci. Probes from the cDNA library detected a greater proportion of loci with more than two alleles than did probes from the genomic library. The high polymorphism, large number of alleles, and ease of interpretation of RFLPs in *E. nitens* means that they will be useful in a range of applications such as genetic linkage maps and paternity analysis.

Key words RFLP · Eucalypts · Inheritance

Introduction

Eucalypts are an important source of timber and fibre for pulp and paper manufacture. As for other forest trees, long generation times mean that gains in traditional breeding programs are slow to achieve. Marker-aided selection has the potential to increase the efficiency of selection in breeding programs, but requires the availability of a large number of markers that are reasonably polymorphic. The number of markers available with isozyme loci is limited, although some of these loci are highly polymorphic (Moran and Bell 1983). Some analysis of linkage has been carried out for isozyme loci in *Eucalyptus regnans*. The loci Lap-2 and Pgi-2 are known to be linked whilst combinations of seven other loci have been shown to segregate independently (Moran and Bell 1983).

The use of restriction fragment length polymorphisms (RFLPs) has provided an almost unlimited set of genetic markers in many species. The level of polymorphism detected with RFLPs within species can be highly variable, e.g., high levels of variation were detected in maize (Helentjaris et al. 1985) and alfalfa (Brummer et al. 1991) but only low levels in tomato (Helentjaris et al. 1985), lentil (Havey and Muehlbauer 1989) and soybean (Apuya et al. 1988). Analysis of RFLPs in a number of species have shown that the source of probes and the choice of restriction enzymes affect the level of polymorphism detected. Some studies have found that probes originating from cDNA libraries detect substantially more polymorphism than probes from genomic libraries (Landry et al. 1987b; Havey and Muehlbauer 1989; Miller and Tanksley 1990). Several studies have also found a correlation between the size of the fragments generated by a restriction enzyme and the polymorphism detected (Landry et al. 1987b; McCouch et al. 1988; Wang and Tanksley 1989; Miller and Tanksley 1990). Since the construction of genetic linkage maps is time consuming and expensive the optimization of detection of polymorphism using RFLPs is an important consideration.

The level of polymorphism observed with genomic and cDNA probes in combination with a number of restriction enzymes, and the inheritance of the RFLP loci detected, have been investigated in *E. nitens* prior to the construction of a genetic linkage map in this species. *E. nitens* is a commercially important species particularly in Australia,

Communicated by P. M. A. Tigerstedt

M. Byrne (✉) · G. F. Moran · J.C. Murrell
Division of Forestry, CSIRO, P.O. Box 4008,
Queen Victoria Terrace, Canberra, ACT 2600, Australia

W. N. Tibbits
North Forest Products, P.O. Box 63, Ridgley, Tasmania 7321, Australia

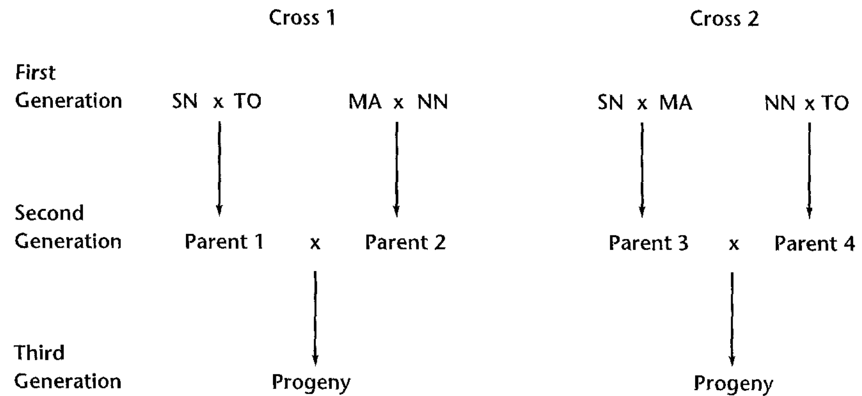

Fig. 1 The two pedigrees used to analyse RFLPs in *E. nitens*. The first generation trees come from four different populations: *SN*, southern New South Wales; *TO*, Torongo; *MA*, Macalister; *NN*, northern New South Wales

South Africa and Chile. This species has less total genetic diversity for isozyme loci than other species with similar distributions, but does show high levels of genetic differentiation between regions (Moran 1992). The genetic linkage map developed in *E. nitens* may have applications in other eucalypt species and will provide a basis for a comparison of genome organization between species of this large genus.

Materials and methods

Pedigree materials

Two three-generation outbred pedigrees have been developed for linkage mapping in *E. nitens* (Fig. 1). Both these pedigrees involve the same four first-generation trees which originated from different natural populations of the species (see Byrne and Moran 1994 for species distribution). The second generation of the pedigrees was developed by North Forest Products and the third generation was developed by the CSIRO Division of Forestry. The first- and second-generation individuals were sampled in the field. The third-generation individuals were collected as seed, then cold/moist stratified for 3 weeks and germinated in sand under mist. Seedlings were transplanted to 5-inch pots in non-soil medium and grown in the glasshouse at 24 °C day temperature and 16 °C night temperature. Seedlings were sampled for DNA extraction at 3 months of age. All first- and second-generation plants were evaluated for RFLP variation. Plants from the third generation of the pedigrees were evaluated for allelic segregation at RFLP loci.

Source of probes

Probes used to identify RFLPs originated from either a genomic library or a cDNA library. The genomic library was prepared from the DNA of *E. nitens* seedlings. DNA extraction was as described in Byrne et al. (1993). The DNA was digested with *Pst*I, treated with Gene Clean (Bresatec) then ligated into *Pst*I-digested pUC19. Plasmids were transformed into bacterial strain JPA101 by electroporation. Clones containing recombinant plasmids were selected using IPTG/Xgal screening and maintained as glycerol stocks at –80 °C. The cDNA library was prepared in λ Zap II (Stratagene) by S. Southerton, CSIRO Division of Plant Industry, from RNA isolated from pre-anthesis buds of *E. globulus*.

The genomic and cDNA clones were given identifiers, e.g., pEng001 or pEglc001, where p denotes plasmid, En or Egl indicates whether the clone originated from *E. nitens* or *E. globulus* material, g or c denotes whether the clone is of genomic or cDNA origin and 001 is the identification number.

DNA procedures

Genomic DNA was extracted from adult leaves of the first- and second-generation trees and from seedling leaves of the third generation as described in Byrne et al. (1993). Restriction of DNA and hybridization conditions were as previously described (Byrne et al. 1993), except that filters of third-generation individuals were capillary blotted in 0.4 M NaOH overnight rather than vacuum blotted, and post-hybridization washes were in 2×SSC, 0.1% SDS. Probes were prepared for use in hybridizations either by excision of DNA inserts with *Pst*I or PCR amplification of inserts and, following gel electrophoresis in low-melting-point agarose, the insert DNA was cut out, the agarose melted in the presence of 20 mM Tris/1 mM EDTA, organically extracted with phenol then phenol/chloroform and the DNA precipitated with ethanol. Probes were labelled with ^{32}P-dCTP to high specific activity using the random priming method (Feinberg and Vogelstein 1983).

Initially six restriction enzymes (*Bgl*II, *Bam*HI, *Dra*I, *Eco*RI, *Eco*RV or *Hin*dIII) were used to evaluate polymorphism in the first and second generations of all pedigrees, but after analysis with 43 probes only three restriction enzymes were subsequently used (*Bgl*II, *Dra*I, *Eco*RI). Polymorphism in the third generation was evaluated with only one restriction enzyme per probe.

Results

Initial screening of probes

Probes from the genomic library were pre-screened for high-copy sequences by hybridization of slot blots with total genomic DNA. Inserts with high-copy sequences were identified in 86 out of 401 (21%) plasmids. The level of variation detected in DNA digested with the initial six enzymes was evaluated. Table 1 details the number of probes which detected polymorphism and the size of the fragments generated for each enzyme. An analysis of the relationship between the degree of polymorphism detectable with each enzyme and the size of the fragments generated showed no significant correlation (r^2=0.13). Polymorphism was detected with the enzymes *Bgl*II, *Dra*I, and *Eco*RI two–three times more often than with *Bam*HI, *Eco*RV and *Hin*dIII and therefore subsequent probe evaluation was carried out using *Bgl*II, *Dra*I, and *Eco*RI only.

One-hundred-and-ninety-seven genomic DNA probes and 169 cDNA probes were screened for RFLP variation in the first and second generations. Of these, 22 genomic

DNA and 15 cDNA probes showed a high copy or very complex banding patterns, and 42 genomic DNA and 44 cDNA probes were monomorphic in all first generation individuals. Not all probes that identified polymorphisms were easily interpreted. RFLP patterns that could be hypothetically interpreted in terms of alleles and loci in the first and second generations were detected with 95 genomic and 75 cDNA probes. Table 2 summarizes the polymorphism observed with the probes.

Apparent maternal inheritance of bands was observed with one genomic DNA and one cDNA probe. Detection of the same RFLP pattern was observed with six pairs of cDNA probes but not with any genomic probes.

Segregation of RFLP loci in the third generation

Segregation of RFLP loci was assessed in two pedigrees resulting from the original first- and second-generation individuals (Fig. 1). Selection of probe-enzyme combinations was made on the basis of RFLP pattern in the first and second generations. Between 117 and 132 third-generation individuals of Cross one were analysed for RFLP variation with 29 genomic and 33 cDNA probes. Between 33 and 39 individuals from Cross two were also analysed for RFLP variation with 19 of the genomic probes and 21 of the cDNA probes that were analysed for Cross one. Not all of the probes analysed in Cross one could be analysed in Cross two since some loci that segregated in Cross one did not segregate in Cross two, e.g., if they were monomorphic or the second-generation individuals in Cross two were alternate homozygotes for some loci and therefore their progeny were all heterozygotes.

Segregation of two alleles at the locus pEng248 is shown in Fig. 2. The progeny segregate in a 1:2:1 ratio as expected for the Mendelian inheritance of two alleles when the parents are both heterozygous. The segregation patterns in the third generation for 30 of the 69 loci detected are detailed in Table 3. The goodness of fit to expected segregation ratios based on the Mendelian inheritance of alleles was calculated for all loci. Some loci showed an almost perfect fit to expected segregation ratios whilst others showed some departure from a perfect fit. Two loci showed segregation patterns that were significantly different from expected at the 5% significance level in Cross one. One of these loci was analysed in Cross two and did not show any distorted segregation. One locus that fitted expected segregation ratios in Cross one showed a deviation from expected in Cross two.

The 29 genomic probes analysed detected 34 loci with an average of 2.5 alleles per locus. The 33 cDNA probes detected 35 loci with 2.7 alleles per locus on average. The genomic probes detected loci with up to six alleles present in the first-generation individuals whilst the cDNA probes detected loci with up to five alleles. The distribution of alleles at all loci is shown in Table 4. Although the genomic probes detected more alleles in total, the majority of loci showed only two alleles. In contrast, the cDNA probes detected fewer alleles in total but showed a higher propor-

Table 1 The average size of fragments (in kilobases) generated by six restriction enzymes and the number of probes that detected polymorphic RFLP patterns for each enzyme

Enzyme	No. of polymorphic probes (%)	Av. fragment size (kb)
*Bam*HI	7 (16)	7.10
*Bgl*II	19 (44)	5.72
*Dra*I	25 (58)	5.22
*Eco*RI	21 (50)	5.42
*Eco*RV	9 (21)	6.00
*Hin*dIII	9 (21)	4.48

Table 2 Summary of RFLP variation observed between first- and second-generation individuals using genomic and cDNA sequences as probes

Probe type	No. probes	No. (%) high copy	No. (%) polymorphic	No. (%) interpretable
Genomic DNA	197	22 (11)	133 (68)	95 (48)
cDNA	169	15 (9)	110 (65)	75 (44)
Total	366	37 (10)	243 (66)	170 (46)

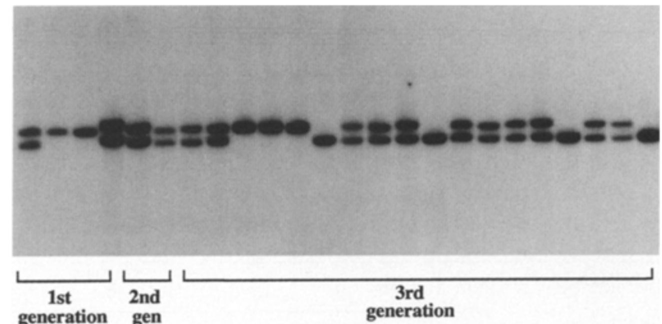

Fig. 2 The RFLP pattern for probe pEng248 hybridized to *Bgl*II-digested DNA of Cross one. *Lanes 1–4* are first-generation individuals, *lanes 5 and 6* are the second-generation individuals, and *lanes 7–25* are third-generation individuals

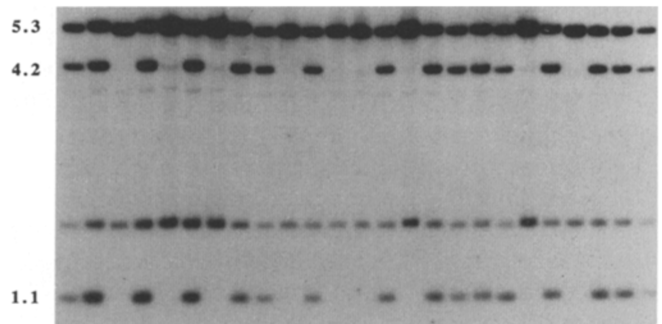

Fig. 3 The RFLP pattern for probe pEglc008 hybridized to *Bgl*II-digested DNA of Cross one showing a polymorphism due to a restriction-site mutation. Sizes of fragments (in kilobases) are given at the left

Table 3 Segregation and chi-square goodness of fit of 30 RFLP loci in two crosses; * indicates significant deviation from expected at the 0.05 level

Locus	Cross one				Cross two			
	Segregation	Expected	χ^2	Probability	Segregation	Expected	χ^2	Probability
pEglc007	31:59:27	1:2:1	0.28	0.7–0.9	18:21	1:1	0.23	0.5–0.7
pEglc069	58:60	1:1	0.03	0.7–0.9				
pEglc083A	30:28:31:29	1:1:1:1	0.16	>0.95	19:15	1:1	0.47	0.3–0.5
pEglc092	58:60	1:1	0.03	0.7–0.9	21:18	1:1	0.23	0.5–0.7
pEglc104	57:59	1:1	0.03	0.7–0.9				
pEglc115	57:75	1:1	2.45	0.1–0.2	16:18	1:1	0.11	0.7–0.9
pEglc136	59:55	1:1	0.14	0.7–0.9	15:24	1:1	2.07	0.1–0.2
pEglc137	20:8:41:49	1:1:1:1	36.1	<0.001*	9:11:9:5	1:1:1:1	2.23	0.5–0.7
pEglc158	31:30:28:29	1:1:1:1	0.16	>0.95	14:20	1:1	1.05	0.3–0.5
pEglc176	34:25:24:35	1:1:1:1	3.42	0.3–0.5	17:16	1:1	0.03	0.7–0.9
pEglc198	59:59	1:1	0	1.0				
pEglc238	61:57	1:1	0.13	0.7–0.9	8:31	1:1	13.56	<0.001*
pEglc333	52:66	1:1	1.66	0.1–0.2	21:18	1:1	0.23	0.7–0.9
pEglc373	56:62	1:1	0.3	0.5–0.7	9:13:9:8	1:1:1:1	1.51	0.5–0.7
pEglc395	52:66	1:1	1.66	0.1–0.2				
pEng003	56:62	1:1	0.3	0.5–0.7	19:20	1:1	0.02	0.7–0.9
pEng010	22:71:25	1:2:1	5.03	0.05–0.1				
pEng067	58:60	1:1	0.03	0.7–0.9	18:21	1:1	0.23	0.5–0.7
pEng092B	62:56	1:1	0.3	0.5–0.7	22:17	1:1	0.64	0.3–0.5
pEng149	64:67	1:1	0.06	0.7–0.9	10:8:9:6	1:1:1:1	1.06	0.7–0.9
pEng156B	66:66	1:1	0	1.0				
pEng195	50:68	1:1	2.74	0.05–0.1	19:20	1:1	0.02	0.7–0.9
pEng198A	61:57	1:1	0.13	0.7–0.9				
pEng221	25:74:33	1:2:1	2.9	0.2–0.3				
pEng250	63:69	1:1	0.27	0.5–0.7				
pEng257A	63:68	1:1	0.19	0.5–0.7				
pEng258A	28:60:29	1:2:1	0.1	0.95	11:22:6	1:2:1	1.92	0.3–0.5
pEng297	64:53	1:1	1.03	0.3–0.5				
pEng417	21:34:30:31	1:1:1:1	3.24	0.3–0.5	16:23	1:1	1.25	0.2–0.3
pEng482	24:34:30:30	1:1:1:1	1.72	0.5–0.7	5:12:13:7	1:1:1:1	4.83	0.1–0.2

Table 4 Distribution of the number of alleles in the first-generation individuals for 69 loci analysed for segregation

Locus type	No. loci (%)				
	2 alleles	3 alleles	4 alleles	5 alleles	6 alleles
Genomic DNA	24 (70)	5 (15)	3 (9)	1 (3)	1 (3)
cDNA	19 (54)	10 (29)	5 (14)	1 (3)	0
Total	43 (62)	15 22)	8 (12)	2 (3)	1 (1)

tion of loci with more than two alleles. In three of the loci two-banded alleles were present and the size of the paired fragments was equal to the size of the fragment representing the other allele (Fig. 3). The polymorphism in these loci can be attributed to an additional restriction site in the larger fragment.

Discussion

Construction of a genomic library with the methylation-sensitive enzyme *Pst*I produced a library enriched for low-copy sequences. This library contained 79% low-copy sequences which is similar to the levels of low-copy sequences identified in libraries of other species made in this way (Figdore et al. 1988; Helentjaris et al. 1988; Devey et al. 1991). *Pst*I does not cut efficiently in *E. nitens* (data not presented) and the presence of a large proportion of low-copy sequences in a *Pst*I library suggests that repeated sequences in eucalypt DNA are highly methylated. This is similar to other species (e.g., maize, Burr et al. 1988; tomato, Miller and Tanksley 1990) but is in contrast to rice where the DNA appears to be less methylated than in other species (McCouch et al. 1988). Both the genomic library and the cDNA library showed similar levels of efficiency in terms of the number of repeated-sequence clones and the number of clones that were polymorphic and interpretable as alleles and loci. Studies in lettuce (Landry et al. 1987b), lentil (Havey and Muehlbauer 1989), and tomato (Miller and Tanksley 1990) have presented evidence that cDNA libraries detect more polymorphism than genomic libraries whilst no such difference in the efficiency of different libraries has been found in other species. There was no difference in the efficiency of detection of polymorphism between probes from the cDNA and genomic libraries in *E. nitens*. Havey and Muehlbauer (1989) suggested that the increased efficiency of detection of polymorphism with cDNA libraries may be related to the relatively large genome size of lentil and lettuce. The lack of difference between the two libraries in *E. nitens* is consistent with this suggestion since eucalypts have a small genome (Grattapaglia and Bradshaw 1994). The presence of gene families

and large stretches of repeated DNA in plants with large genomes may reduce the efficiency of probes from genomic libraries in the detection of polymorphism in these species. The lack of extensive gene families in *E. nitens* was also indicated by the majority of the probes detecting only one locus. Although no difference in the efficiency of detection of polymorphism between the two libraries was evident, there was a difference in the number of alleles detected per locus. Probes from the genomic library detected loci with more alleles in the four first-generation individuals than did the cDNA library; however, a greater proportion of the loci detected by the cDNA probes had more than two alleles. Thus the cDNA probes were more efficient in detecting loci with more than two alleles. For mapping purposes there is no significant advantage in the presence of more than four alleles at a locus since this is the maximum number of alleles that can segregate in any one cross. However, probes detecting highly variable sequences are more likely to be polymorphic in other pedigrees, thus making the map more transportable between pedigrees. Probes detecting sequences with a high number of alleles will be very useful for other applications such as fingerprinting and individual identification, paternity analysis, and the determination of levels of gene flow.

Chloroplast (cp) and mitochondrial (mt) sequences have been detected in libraries in other species (Figdore et al. 1988). The genomic and cDNA libraries were not screened directly for cpDNA or mtDNA sequences; however, two probes, one from each library, showed maternal inheritance of restriction fragments indicating that these clones contained sequences originating from the cp or mt genomes. Extensive duplication of sequences have been reported in genomic libraries of lettuce (Landry et al. 1987b) and lentil (Havey and Muehlbauer 1989), and to a lesser extent in a cDNA library of loblolly pine (Devey et al. 1991). Some redundancy in the eucalypt cDNA library was also evident since 2% of the clones were identical. No evidence of redundancy was detected in the genomic library of *E. nitens*.

Many investigations using RFLPs have found that some restriction enzymes are better at detecting polymorphism than others. The level of polymorphism detected appears to be correlated with the frequency of cutting and the size of the fragments that are generated (Landry et al. 1987b; McCouch et al. 1988; Wang and Tanksley 1989; Miller and Tanksley 1990). The larger the fragments generated by an enzyme the greater the probability that polymorphism will be detected with that enzyme. This would be expected if insertions/deletions were the primary cause of the polymorphisms. In *E. nitens*, however, whilst there was a range in the average size of the fragments generated by the enzymes there was a clear separation of the enzymes into two groups in terms of the level of polymorphism, and there was no significant correlation between fragment size and the level of polymorphism. This suggests that insertions and deletions may not be the main cause of polymorphism in *E. nitens*. Variation with more than one restriction enzyme has frequently been observed in other species (Apuya et al. 1988; McCouch et al. 1988; Wang and Tanksley 1989) and also indicates that the cause of the variation is primarily insertions and deletions. Polymorphism was often detected with more than one enzyme in *E. nitens*, although the fragment pattern was not necessarily consistent with the same polymorphic event for each enzyme. Polymorphism due to restriction-site mutations will not usually be discernible, since the size of the probe is generally smaller than the size of the fragments detected; yet restriction-site mutations causing polymorphism were clearly observed in three loci in *E. nitens*. The frequency of restriction-site mutations resulting in polymorphism may be higher in *E. nitens* than in other species.

A majority of the loci segregated as expected according to Mendelian principles. The loci that showed some segregation distortion did not have any consistent pattern of distortion from one parent or the other. Segregation distortion has been observed in loci in other species (Landry et al. 1987a; Gebhardt et al. 1989; Havey and Muehlbauer 1989; Gebhardt et al. 1991; Song et al. 1991; Kianian and Quiros 1992; Liu and Furnier 1993). The proportion of loci showing distortion is related to the type of cross in which the segregation is analysed. Interspecific crosses tend to show large proportions of segregation distortion (19–59%; Landry et al. 1987a; Gebhardt et al. 1989; Havey and Muehlbauer 1989; Gebhardt et al. 1991; Kianian and Quiros 1992) whilst intraspecific crosses show a small number of loci with distorted ratios (2–8%; Song et al. 1991; Kianian and Quiros 1992, Liu and Furnier 1993). The low level of distorted ratios in *E. nitens* (3%) is acceptable for these intraspecific crosses since they are between individuals from widely-separated regions.

The number of probes detecting polymorphism was relatively high for an intraspecific cross and the number of alleles present per locus was also high. The pedigrees used for analysis of RFLPs in *E. nitens* were chosen to maximize the variation detected. The species has a large range, with populations distributed in widely-separated regions. Genetic differentiation between regions has been observed in studies using isozyme (Moran, unpublished data) and cpDNA markers (Byrne and Moran 1994). The pedigrees developed result from wide interpopulational crosses where the four first-generation individuals originated from different natural populations. The level of polymorphism detected between these four individuals is similar to the levels of polymorphism detected between cultivars of rice (McCouch et al. 1988; Wang and Tanksley 1989) and potato (Gebhardt et al. 1989) and between nine trees of *Populus tremuloides* (Liu and Furnier 1993). This suggests that there is a high level of variation for random nuclear sequences in these species. The loci detected in *E. nitens* are not only highly polymorphic but are also readily interpreted. Whilst some probes detected complex RFLP patterns resulting from several loci with overlapping allele positions, a majority detected a small number of bands which could easily be resolved into alleles representing one or two loci. This ease of interpretation greatly simplifies the scoring of RFLP loci. High polymorphism, a large number of alleles, and the ease of interpretation of loci in *E. nitens* mean that RFLPs are well suited for the construction of genetic linkage maps. The high level of polymor-

phism and the large number of alleles suggests that a large number of markers in a linkage map created in one pedigree will also be polymorphic in other pedigrees in the species, which will be important for the use of markers correlated with QTLs in selection programs. These characteristics of RFLP loci in this species also mean that they will be useful in other applications such as fingerprinting, paternity analysis, measurement of gene flow in seed orchards and in natural populations, and the estimation of genetic diversity parameters.

Acknowledgements We thank M. Moncur and J. Turner for assistance with the controlled crossing to produce the third generation.

References

Apuya NR, Frazier BL, Keim P, Roth EJ, Lark KG (1988) Restriction fragment length polymorphisms as genetic markers in soybean, *Glycine max* (L.) merrill. Theor Appl Genet 75:889–901

Brummer EC, Kochert G, Bouton JH (1991) RFLP variation in diploid and tetraploid alfalfa. Theor Appl Genet 83:89–96

Burr B, Burr FA, Thompson KH, Albertson MC, Stuber CW (1988) Gene mapping with recombinant inbreds in maize. Genetics 118:519–526

Byrne M, Moran GF (1994) Population divergence in the chloroplast genome of *Eucalyptus nitens*. Heredity 73:18–28

Byrne M, Moran GF, Tibbits WN (1993) Restriction map and maternal inheritance of chloroplast DNA in *Eucalyptus nitens*. J Hered 84:218–220

Devey ME, Jermstad KD, Tauer CG, Neale DB (1991) Inheritance of RFLP loci in a loblolly pine three-generation pedigree. Theor Appl Genet 83:238–242

Feinberg AP, Vogelstein B (1983) A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Annal Biochem 132:6–13

Figdore SS, Kennard WC, Song KM, Slocum MK, Osborn TC (1988) Assessment of the degree of restriction fragment length polymorphism in *Brassica*. Theor Appl Genet 75:833–840

Gebhardt C, Ritter E, Debener T, Schachtschabel U, Walkemeier B, Uhrig H, Salamini F (1989) RFLP analysis and linkage mapping in *Solanum tuberosum*. Theor Appl Genet 78:65–75

Gebhardt C, Ritter E, Barone A, Debener T, Walkemeier B, Schachtschabel U, Kaufmann H, Thompson RD, Bonierbale MW, Ganal MW, Tanksley SD, Salamini F (1991) RFLP maps of potato and their alignment with the homeologous tomato genome. Theor Appl Genet 83:49–57

Grattapaglia D, Bradshaw H (1994) Nuclear DNA content of commercially important *Eucalyptus* species and hybrids. Can J For Res (in press)

Havey MJ, Muehlbauer FJ (1989) Linkages between restriction fragment length, isozyme and morphological markers in lentil. Theor Appl Genet 77:395–401

Helentjaris T, King G, Slocum M, Siedenstrang C, Wegman S (1985) Restriction fragment length polymorphisms as probes for plant diversity and their development as tools for applied plant breeding. Plant Mol Biol 5:109–118

Helentjaris T, Weber D, Wright S (1988) Identification of the genomic locations of duplicate nucleotide sequences in maize by analysis of restriction fragment length polymorphisms. Genetics 118:353–363

Kianian SF, Quiros CF (1992) Generation of a *Brassica oleracea*-composite RFLP map: linkage arrangements among various populations and evolutionary implications. Theor Appl Genet 84:544–554

Landry BS, Kesseli RV, Farrara B, Michelmore RW (1987a) A genetic linkage map of lettuce (*Lactuca sativa* L.) with restriction fragment length polymorphism, isozyme, disease resistance and morphological markers. Genetics 116:331–337

Landry BS, Kesseli R, Leung H, Michelmore RW (1987b) Comparison of restriction endonucleases and sources of probes for their efficiency in detecting restriction fragment length polymorphisms in lettuce *(Lactuca sativa* L.). Theor Appl Genet 74:646–653

Liu Z, Furnier GR (1993) Inheritance and linkage of allozymes and restriction fragment length polymorphisms in trembling aspen. J Hered 84:419–424

McCouch SR, Kochert G, Yu ZH, Wang ZY, Khush GS, Coffman WR, Tanksley SD (1988) Molecular mapping of rice chromosomes. Theor Appl Genet 76:815–829

Miller JC, Tanksley SD (1990) Effect of different restriction enzymes, probe source and probe length on detecting restriction fragment length polymorphism in tomato. Theor Appl Genet 80:385–389

Moran GF (1992) Patterns of genetic diversity in Australian tree species. New Forests 6:49–66

Moran GF, Bell JC (1983) Eucalyptus. In: Tanksley SD, Orton TJ (eds) Isozymes in plant genetics and breeding. Part B. Elsevier Science Publishers, Amsterdam, pp 423–441

Song KM, Suzuki JY, Slocum MK, Williams PH, Osborn TC (1991) A linkage map of *Brassica rapa* (syn. *campestris)* based on restriction fragment length polymorphism loci. Theor Appl Genet 82:296–304

Wang ZY, Tanksley SD (1989) Restriction fragment length polymorphism in *Oryza sativa* L. Genome 32:1113–1118