Heredity **81** (1998) 284–290 *Received 12 September 1997, accepted 5 March 1998*

Assessment of intraspecific variation in half-sibs of *Quercus petraea* (Matt.) Liebl. 'plus' trees

BART DE GREEF†, LUDWIG TRIEST*†, BART DE CUYPER‡ & JOS VAN SLYCKEN§

†Vrije Universiteit Brussel, Laboratorium voor Algemene Plantkunde en Natuurbeheer, Pleinlaan 2, B-1050 Brussel, Belgium, ‡Instituut voor Bosbouw en Wildbeheer, Duboislaan 14, B-1560 Hoeilaart, Belgium and §Instituut voor Bosbouw en Wildbeheer, Gaverstraat 4, B-9500 Geraardsbergen, Belgium

Quercus petraea (Matt.) Liebl. (sessile oak) is a long-lived, widespread species with forest rotation cycles that are considerably longer than many other species in European forestry. Together with other oak species they are of high economic and ecological importance. In Flanders (Belgium), apart from the creation of suitable situations for natural regeneration, native species (including sessile oak) are being actively introduced to enhance the authenticity of particular sites. To take into account the existing genetic diversity in such practices, there was a need to determine the available genetic variation in the selected 'plus' trees from which the progeny will be used in the (re)introduction programmes. We analysed the half-sibs of nine different 'plus' trees from officially approved stands, that were selected because of their extraordinary phenotype. RAPD and AFLP techniques were used to assess the level of genetic variation. AFLP was only used for the analysis of the progeny from two different 'plus' trees. A screening with eight selected decanucleotide primers (for RAPD) and six primer combinations (for AFLP) allowed a presence/absence matrix to be constructed for the estimation of similarity for each pair of individuals. This similarity matrix was the basis for cluster and principal co-ordinate analysis and the calculation of population genetic parameters (for the RAPD data). These analyses showed that the pool of investigated progenies comprised a considerable amount of genetic variation, which makes them suitable for the above-mentioned introduction initiatives.

Keywords: AFLP, genetic variation, introduction programmes, progeny, *Quercus petraea*, RAPD.

Introduction

Oak species are common in most parts of Europe and are carrier species of economically as well as ecologically important forest ecosystems. They range from the flood plains of the lowlands to the submontane or even montane regions. The genus *Quercus* comprises more than 300 species spread over Asia, North America and Europe (Camus, 1934–54). *Quercus petraea* is a widespread species covering most of Europe from Spain to Russia and from Scotland to Turkey (Kleinschmit, 1993). Its natural range is principally a continuous distribution. Throughout its distribution, *Q. petraea* is associated with *Q. robur* resulting in mixed stands where unidirectional hybridization may occur (Bacilieri *et al*., 1996). Oaks are long-lived species with forest rotation cycles of 200 or more years, which is considerably longer than any other species in European forestry. As a consequence, they are exposed to more heterogeneous environmental conditions than most other predominant tree species and may serve as model organisms in the study of genetic variability and its implications for the survival of tree populations. Indeed, genetic variation determines the adaptive potential of species and is an essential component of the stability of ecosystems (Müller-Starck & Ziehe, 1991).

Although the genetic structure of herbaceous plant species has been widely studied (Hamrick &

*Correspondence. E-mail: ltriest@vub.ac.be

Godt, 1989), only limited information is available on woody angiosperm species (Schwarzmann & Gerhold, 1991). Such information is crucial for developing programmes to protect and preserve the genetic resources of plant species as it is now thought that certain environmental perturbations could threaten the existence of some tree species (Ledig, 1992). To make responsible decisions concerning the possible impacts of such pressures, it is necessary to build a more substantive base of knowledge regarding the population genetic structure of woody angiosperm species. Indeed, increasing silvicultural use, shortage of seeds and exposure to unfavourable conditions make the study of genetic structure and variation among and within autochthonous *Quercus* populations an important task.

Currently applied practices in Flanders (Belgium) are the creation of suitable situations for natural regeneration, as well as the active introduction of species to enhance the authenticity of particular sites, including forests (B. De Cuyper, personal communication). In the case of introduction, the original natural situation needs to be respected, and the existing genetic diversity considered. Taking into account the available genetic variation implies that the introductions need to originate from naturally occurring genetic resources. This is not only necessary to safeguard authenticity, but also to assist their survival. Therefore, there is a need to determine the existing genetic variation in the selected 'plus' trees from which the progeny will be used in (re)introduction programmes. In Flanders, these 'plus' trees were selected on the basis of their outstanding phenotypic characteristics, including form and growth properties such as the uprightness of the stem, the fineness of the branches, the implantation of the branches, the fork-shape, and characteristics such as the presence of cracks as a consequence of frost. The nine trees that were finally selected out of 26 preselected trees were the ones with the best scores for the 10 form and growth properties that were examined. During the choice of the source material, it is important to concentrate on the populations or stands that are highly diverse and thus suitable for gene conservation. The origin of the resources used should be taken into account to minimize disturbance of the naturalness of the introduction site.

An accurate knowledge of the existing genetic diversity in the natural population one wants to monitor requires efficient techniques to monitor genetic variability unambiguously. In recent years, detection of variability has become possible with the establishment of molecular techniques. Isozymes are extensively used to characterize plant genetic resources (Tanksley & Orton, 1983; Peakall *et al*., 1995). RFLPs have been used to investigate genetic diversity in cultivated plants and their wild derivatives (Clegg, 1989; Lin *et al*., 1996). RAPD analysis (Williams *et al*., 1990) overcomes many of the technical limitations of RFLP, requiring only small amounts of template DNA, and no prior DNA sequence information. This technique can provide a large number of potential polymorphic loci and has been successfully used for the determination of the level of genetic differentiation in several species (Dawson *et al*., 1993; Novak & Mack, 1993; Schierenbeck *et al*., 1997), including *Q. petraea* (Le Corre *et al*., 1997).

The AFLP multilocus fingerprinting technique (Vos *et al*., 1995) allows the screening in one single experiment of many different loci for the presence of polymorphism and has been used in several diversity assessment studies (Hill *et al*., 1996; Travis *et al*., 1996). The use of marker diversity in molecular ecology and conservation genetics was reviewed by Hadrys *et al*. (1992) and Milligan *et al*. (1994). A recent overview of the molecular technologies suitable for biodiversity evaluation was published by Karp *et al*. (1997).

In order to assess the genetic diversity present in the progeny of *Q. petraea* trees that were selected by the Institute for Forestry and Game Management (Flemish Community, Belgium), we analysed the half-sibs of nine different trees from one of the six officially approved stands that were selected because of their extraordinary phenotype. RAPD and AFLP revealed the amount and distribution of genetic diversity within and between the different 'plus' trees.

Materials and methods

Plant material

Progeny from nine different 'plus' trees (nos 00, 05, 15, 17, 18, 21, 22, 23, 24), originating from one stand (Buggenhout, Belgium) of *Q. petraea* were used for DNA extraction. Leaves were collected and subsequently frozen in liquid nitrogen, before storage at −70°C.

DNA extraction and PCR conditions

Total plant DNA for PCR was isolated from leaves, according to the following protocol modified from Murray & Thompson (1980).

Table 1 Primer combinations used in AFLP analysis

	M-CAA	M-CTG	M-CAC	M-CAG	M-CTA	M-CTT	M-CTC	M-CAT
E-ACA	+	+*	+*	+	+	+	+	+
E-ACG	+	+	+*	+	+	+	+*	+
E-AGG	+*	+	+	+	+*	+	+	+
E-ACC	+	+	+	+	+	+	+	+
E-ACT	+	+	+	+	+	+	+	+
E-AGC	+	+	+	+	+	+	+	+

E, *Eco*RI; M, *Mse*I.
*These primer combinations were used for the analysis of 10 progeny individuals of *Quercus petraea* trees 15 and 21.

One to two young oak leaves were ground in a mortar containing liquid nitrogen. Then, 5 mL of DNA extraction buffer (100 mM Tris pH 8, 1.4 M NaCl, 20 mM EDTA, 2% CTAB, 1% PVPP, 1% beta-mercaptoethanol) was added and the mixture incubated at 65°C for 20 min. After cooling down to room temperature, 5 mL of chloroform-isoamyl-alcohol (24:1, v/v) (CI) was added. The mixture was centrifuged at 3000 *g* for 10 min in a 50 mL centrifuge tube (Greiner). After recovery of the upper phase, 0.1 vol. 10% CTAB and 1 vol. CI were added. This mixture was centrifuged at 3000 *g* for 10 min and 1.2 vol precipitation buffer (100 mM Tris pH 8, 20 mM EDTA, 2% CTAB) was added to the upper phase. After an incubation of more than 20 min at room temperature, the nucleic acids were pelleted for 15 min at 7700 *g*. The pellet was dissolved in 0.5 mL 1 M NaCl. Once the pellet was dissolved, 1 μL of a 10 mg/mL RNase solution was added. This mixture was incubated at 37°C for 30 min. The DNA was precipitated by adding 50 μL 4 M LiCl and 1 mL EtOH (−70°C; 30 min), followed by a centrifugation at 9500 *g* for 10 min. The DNA pellet was washed in 70% EtOH. After a final centrifugation at 9500 *g* for 10 min, the DNA pellet was dried and subsequently dissolved in 150 μL TE. The DNA concentration was determined spectrophotometrically and monitored by subjecting samples to 1% agarose gel electrophoresis in TBE buffer (Sambrook *et al*., 1989) and by visual assessment of band intensities compared with DNA standards.

For RAPD analysis a 25 μL reaction mixture was prepared containing $\approx$25 ng template DNA, 5 pmol of a single decanucleotide (Operon Technologies, Alameda, CA, USA), 100 μM dNTPs (Pharmacia) and 1 unit Taq polymerase in the incubation buffer provided by the manufacturer (Boehringer Mannheim). The mixture was overlain by two drops of mineral oil (Sigma). Amplification was carried out in a Thermojet thermocycler (Eurogentec) programmed as follows: 1 cycle of 2 min 94°C; 40 cycles of 45 s 92°C, 1 min 36°C, 2 min 72°C; 1 cycle of 5 min 72°C. Amplification products were subjected to electrophoresis in 1% agarose gels in 1 × TBE and detected by ethidium bromide staining, viewing under UV light, and photographed.

AFLP analysis was carried out according to the procedure described in AFLP Analysis System I Instruction Manual (GIBCO-BRL Life Technologies). After restriction endonuclease digestion of the DNA with *Eco*RI and *Mse*I and ligation of the corresponding adapters, a preselective amplification of the restriction fragments was carried out using an *Eco*RI primer extended with A and a *Mse*I primer extended with C. In the following selective amplification step, *Eco*RI and *Mse*I primers extended with three selective nucleotides (see Table 1) were used. The amplification products were separated by denaturing polyacrylamide gel electrophoresis.

Data analysis

The amplification products for the different samples were compared to each other and screened for the presence/absence of specific bands. These presence/absence data were then analysed to obtain an estimate of similarity for each pair of individuals. The similarity coefficient employed was: $S = M_{ab}/N_t$ where M_{ab} was the number of all matches in the two genotypes tested, being the sum of the number of present and absent bands in common. N_t was the total number of different bands identified for the primer tested and thus was a constant number for each primer. The similarity matrix was the basis for further cluster and principal co-ordinate analysis using the NTSYS-PC program (Exeter Software).

The population genetic parameters were computed according to Lynch & Milligan (1994)

using a program (RAPD-SURVEY) developed by X. Vekemans (xvekema@ulb.ac.be).

Results and discussion

A set of 88 decanucleotides of arbitrary sequence (obtained from Operon Technologies, Alameda, CA) was tested on five progeny individuals from two different trees. Ten individual progenies of all nine trees were analysed with 31 primers which gave clear amplification products. Finally, eight primers (OPA2, OPA10, OPA17, OPO5, OPO15, OPJ12, OPT7, OPT15) were retained for analysis of all samples, because they yielded easily interpreted, reproducible polymorphic banding patterns. DNA from 193 progeny individuals originating from the nine trees was analysed with these selected primers and the presence or absence of one polymorphic fragment per primer and per individual was considered. A distance matrix and ordination were calculated per tree, using the simple matching algorithm (data not shown). Only for tree 17 was the progeny clearly separated according to the first principal co-ordinate of the ordination. An ordination based on the frequency of present and absent bands per tree revealed a separation according to the first and the second principal coordinates of the ordination with trees 17 and 22 towards the edges of the plot and the other trees in between these two extremes (data not shown). When the presence/absence data for the different primers for all progeny individuals of the different trees were analysed, globally a dense cloud of individual points was observed after ordination. Closer observation revealed a separation of the progeny individuals of trees 17 and 22 according to the first principal co-ordinate of the ordination. The progenies of all the other trees were located in a more spread and intermixed manner (Fig. 1). To obtain a less complex view on the variation present, pairwise comparisons were carried out (data not shown) which showed a clear separation of the progeny of tree 17 from all the other progenies. The progeny of tree 00 harboured a high level of variation, which means that they cannot be separated from the progeny of the other trees, except tree 17. Some progeny individuals of tree 00 were genetically identical (for the primers used) with progeny individuals of trees 21 and 22.

A series of AFLP analyses was carried out using the DNA of progenies of two different trees (15 and 21). In total, 48 different primer combinations were tested. From these 48 primer combinations (Table 1), six were selected to be used for the analysis of 10 individual progenies of trees 15 and 21 because they yielded the highest number of polymorphic bands. As we did not know the outcome of the RAPD analyses, which were carried out in parallel with the AFLP experiments, we decided to analyse progenies from two different trees growing at two different sites within the studied stand. The distance between the two trees was about 800 m.

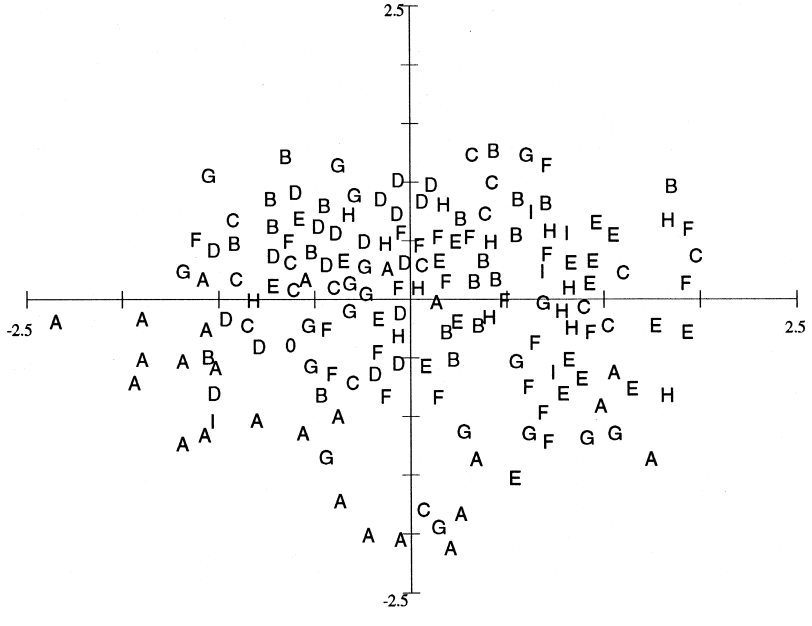

Fig. 1 Principal coordinate analysis plot of all 193 progeny individuals of the nine 'plus' *Quercus petraea* trees, analysed with RAPD. The codes used to mark the progenies of the nine trees are: 17 = A, 00 = B, 18 = C, 22 = D, 21 = E, 24 = F, 15 = G, 23 = H, 05 = I.

The aim of this preliminary study was to evaluate the ability of the technique to detect polymorphisms in individual *Q. petraea* progenies and to compare the amount of polymorphism detected with what was obtained after RAPD analysis. The analysis was technically successful and, depending on the primer combination used, a high level of polymorphism was detectable. The number of amplification products generated by AFLP per experiment was significantly higher than the number of bands obtained with RAPD, which makes the data scoring more complex. We nevertheless scored the banding patterns to check if the genetic variation observed with the two techniques was similar. We scored the presence/absence of 32 bands and these data were processed with NTSYS-PC and were presented in the same way as for the RAPD-data. This showed that the overall variation in the two families was similar, whichever method was used.

Starting from the presence/absence data for the different RAPD primers for all progeny individuals of the different trees, RAPD-SURVEY (see Materials and methods) was used to estimate several classical population genetic parameters based on the estimators described by Lynch & Milligan (1994) (Table 2; Fig. 2). We rely on the assumption that similarity of fragment size is an indicator of homology, which is approximately true for closely related populations or species (Rieseberg, 1996). Moreau *et al.* (1994) showed that amplified fragments of the same size shared the same oligonucleotide sequences and that equal-sized fragments present in *Q. petraea* and *Q. robur* shared the same sequence. Our case study of half-sibs enhances the chance that comigrating fragments are indeed homologous. The nonhomology of comigrating bands would introduce errors into the presence/absence data sets obtained after RAPD or AFLP analysis, eventually leading to inaccurate estimates of genetic relationships. As an immediate consequence it is inappropriate to deduce phylogenetic trees from the RAPD and AFLP data obtained. Population genetic parameters were not calculated from the AFLP data because of the small number of individuals.

Generally, levels of nuclear gene diversity in oak species are among the highest found in long-lived woody species. Average H_{es} values of 0.186 (for *Q. petraea* up to 0.288) appear to be significantly higher than values found for many other tree species (Hamrick *et al.*, 1992; Kremer & Petit, 1993). The fact that oak species occur very often as sympatric species might explain this high average level of nuclear gene diversity. Le Corre *et al.* (1997) estimated the genetic variation within and between 21 populations of sessile oak and found mean within-population genetic diversity and total genetic diver-

Table 2 Population-genetic parameters for *Quercus petraea*

Pop.	*n*	No. of loci	PLP	H_j	SE (H_j)
17	18	8	87.5	0.395	0.064
0	18	7	100.0	0.390	0.049
18	13	7	100.0	0.355	0.054
22	24	8	50.0	0.145	0.063
21	14	8	87.5	0.364	0.071
24	18	8	87.5	0.315	0.073
15	17	7	85.7	0.296	0.075
23	10	8	62.5	0.282	0.078
5	2	7	42.9	0.142	0.076

Pop., number of 'plus' tree; *n*, number of progeny individuals considered for calculations; PLP, percentage of polymorphic loci; H_j, mean observed gene diversity; SE (H_j), standard error of H_j.

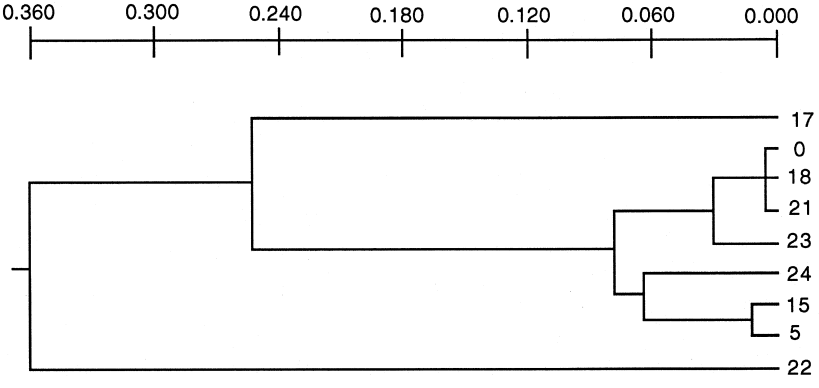

Fig. 2 Dendrogram based on genetic distances calculated by RAPD-SURVEY, representing the nine different *Quercus petraea* trees.

sity values of 0.233 and 0.239, respectively. The mean values determined for RAPD markers were significantly lower than those obtained for allozymes (H_S = 0.381, H_T = 0.392). The mean values that we obtained in our analysis of 193 progenies of nine different trees were 0.406 for H_T and 0.298 (SE = 0.032) for H_S, which are higher than the evaluations made in the other studies, but the trend that most of the variation is found within (F_{ST} = 0.272), rather than between 'populations' (in our study: half-sib families), is confirmed. The progeny of the nine different trees originated from one stand, and a more detailed sampling from a larger area might lower the mean values to the level found in other studies. It should be emphasized, though, that the aim of this study was to reveal the available genetic variation in the progeny of these particular nine trees because the stands from which they derive will be used for (re)introduction, forest modification and forest expansion programmes.

In summary, it can be stated that the pool of investigated progenies comprised a considerable amount of genetic variation, which is confirmed by the fact that 191 different genotypes were detected for the 193 progenies tested. This suggests that selection for viability and growth characteristics has not directly influenced the potential to maintain genetic variability.

Acknowledgements

This project was funded by the Fund for Scientific Research, Flanders (mandate of Research director and contract no. S2/5-ID.E 53 on ecogenetic diversity in plant populations), the Ministry of the Flemish Community (Dienst Bos en Groen, contract no. BG/17/95 on genetic diversity in tree species) and the Vrije Universiteit Brussel (OZR funding). We are very grateful to Prof. Xavier Vekemans for his help with the calculation of the population genetic parameters.

References

BACILIERI, R., DUCOUSSO, A. AND KREMER, A. 1996. Mating system and directional gene flow in a mixed oak stand. *Evolution*, **50**, 900–908.

CAMUS, A. 1934–54. *Les Chênes. Monographies du Genre* Quercus, 3 volumes. Editions Paul Le Chevalier, Paris.

CLEGG, M. T. 1989. Molecular diversity in plant populations. In: Brown, A. H. D., Clegg, M. T., Kahler, A. L. & Weir, B. S. (eds) *Plant Population Genetics, Breeding and Genetic Resources*, pp. 98–115. Sinauer Associates, Sunderland, MA.

DAWSON, I. K., CHALMERS, K. J., WAUGH, R. AND POWELL, W. 1993. Detection and analysis of genetic variation in *Hordeum spontaneum* populations from Israel using RAPD markers. *Mol. Ecol.*, **2**, 151–159.

HADRYS, H., BALICK, M. AND SCHIERWATER, B. 1992. Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. *Mol. Ecol.*, **1**, 55–63.

HAMRICK, J. L. AND GODT, M. J. W. 1989. Allozyme diversity in plant species. In: Brown, A. H. D., Clegg, M. T., Kahler, A. L. and Weir, B. S. (eds) *Plant Population Genetics, Breeding and Genetic Resources*, pp. 43–64. Sinauer Associates, Sunderland, MA.

HAMRICK, J. L., GODT, M. J. W. AND SHERMAN-BROYLES, S. L. 1992. Factors influencing levels of genetic diversity in woody plant species. *New Forests*, **6**, 95–124.

HILL, M., WITSENBOER, H., ZABEAU, M., VOS, P., KESSELI, R. AND MICHELMORE, R. 1996. PCR-based fingerprinting using AFLPs as a tool for studying genetic relationships in *Lactuca* spp. *Theor. Appl. Genet.*, **93**, 1202–1210.

KARP, A., EDWARDS, K. J., BRUFORD, M., FUNK, S., VOSMAN, B., MORGANTE, M. *ET AL*. 1997. Molecular technologies for biodiversity evaluation: opportunities and challenges. *Nature Biotechnol.*, **15**, 625–628.

KLEINSCHMIT, J. 1993. Intraspecific variation of growth and adaptive traits in European oak species. *Ann. Sci. For.*, **50** (Suppl. 1), 166s–185s.

KREMER, A. AND PETIT, R. J. 1993. Gene diversity in natural populations of oak species. *Ann. Sci. For.*, **50** (Suppl. 1), 186s–202s.

LE CORRE, V., DUMOLIN-LAPÈGUE, S. AND KREMER, A. 1997. Genetic variation at allozyme and RAPD loci in sessile oak *Quercus petraea* (Matt.) Liebl.: the role of history and geography. *Mol. Ecol.*, **6**, 519–529.

LEDIG, F. T. 1992. Genecology: the fitness of the organism and the fitness of the environment. In: Colombo, S. J., Hogan, G. and Wearn, V. (eds) *Proceedings of the Twelfth North American Forest Biology Workshop*, pp. 27–47. Ontario Ministry of Natural Resources, Ontario Forest Research Institute, and Forestry Canada, Ontario Region.

LIN, J.-J., KUO, J., MA, J., SAUNDERS, J. A., BEARD, H. S., MACDONALD, M. H. *ET AL*. 1996. Identification of molecular markers in soybean comparing RFLP, RAPD and AFLP DNA mapping techniques. *Plant Mol. Biol. Rep.*, **14**, 156–169.

LYNCH, M. AND MILLIGAN, B. G. 1994. Analysis of population genetic structure with RAPD markers. *Mol. Ecol.*, **3**, 91–99.

MILLIGAN, B., LEEBENS-MACK, J. AND STRAND, A. 1994. Conservation genetics: beyond the maintenance of marker diversity. *Mol. Ecol.*, **3**, 423–425.

MOREAU, F., KLEINSCHMIT, J. R. G. AND KREMER, A. 1994. Molecular differentiation between *Q. petraea* and *Q. robur* assessed by random amplified DNA fragments. *Forest Genet.*, **1**, 51–64.

MÜLLER-STARCK, G. AND ZIEHE, M. 1991. *Genetic Variation in European Populations of Forest Trees*. Sauerländer's Verlag, Frankfurt am Main.

MURRAY, M. AND THOMPSON, W. 1980. Rapid isolation of high molecular weight plant DNA. *Nucl. Acids Res.*, **8**, 4321–4325.

NOVAK, S. J. AND MACK, R. N. 1993. Genetic variation in *Bromus tectorum* (Poaceae): comparison between native and introduced populations. *Heredity*, **71**, 167–176.

PEAKALL, R., SMOUSE, P. E. AND HUFF, D. R. 1995. Evolutionary implications of allozyme and RAPD variation in diploid populations of dioecious buffalograss *Buchloë dactyloides*. *Mol. Ecol.*, **4**, 135–147.

RIESEBERG, L. H. 1996. Homology among RAPD fragments in interspecific comparisons. *Mol. Ecol.*, **5**, 99–105.

SAMBROOK, J., FRITSCH, E. F. AND MANIATIS, T. 1989. *Molecular Cloning: A Laboratory Manual*, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

SCHIERENBECK, K. A., SKUPSKI, M., LIEBERMAN, D. AND LIEBERMAN, M. 1997. Population structure and genetic diversity in four tropical plant species in Costa Rica. *Mol. Ecol.*, **6**, 137–144.

SCHWARZMANN, J. F. AND GERHOLD, H. D. 1991. Genetic structure and mating system of Northern Red Oak (*Quercus rubra* L.) in Pennsylvania. *Forest Sci.*, **37**, 1376–1389.

TANKSLEY, S. D. AND ORTON, T. J. 1983. *Isozymes in Plant Genetics and Breeding*. Elsevier, Amsterdam.

TRAVIS, S. E., MASCHINSKI, J. AND KEIM, P. 1996. An analysis of genetic variation in *Astragalus cremnophylax* var. *cremnophylax*, a critically endangered plant, using AFLP markers. *Mol. Ecol.*, **5**, 735–745.

VOS, P., HOGERS, R., BLEEKER, M., VAN REIJANS, M., DE LEE, T., HORNES, M. *ET AL*. 1995. AFLP: a new technique for DNA fingerprinting. *Nucl. Acids Res.*, **23**, 4407–4414.

WILLIAMS, J. G. K., KUBELIK, A. R., LIVAK, K. J., RAFALSKI, J. A. AND TINGEY, S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful genetic markers. *Nucl. Acids Res.*, **18**, 6531–6535.