# Improved AFLP analysis of tree species

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## Abstract:

Amplified fragment length polymorphism (AFLP) is a high-throughput, molecular-marker technique that is used increasingly in a variety of genetic analyses. Here, the conditions for carrying out AFLP analysis have been established for different tree species, including both angiosperm and gymnosperm trees, with genome sizes ranging from 0.54 to 38 pg DNA/2C. Specific parameters have been determined to provide informative and reproducible AFLP fingerprints of peach (Prunus persica L.), eucalypt, oak, poplar, and loblolly pine (Pines taeda L.). Typically, 80-130 amplified DNA fragments (i.e., loci analyzed per primer combination) were obtained. Subsequently, these AFLP conditions were evaluated for intra- and inter-specific genetic variability studies as well as for genome mapping purposes of woody species. This work demonstrates that AFLP is a powerful tool in forest tree genetics.

**Keywords:** Amplified fragment length polymorphism (AFLP) | forest tree genetics | genetic analysis

## Article:

### Introduction

The application of recently developed molecular marker techniques has greatly facilitated research on different aspects of the structure, organization, and evolution of genomes. Among others, these techniques allow the analysis of genetic diversity within and among species, the construction of linkage maps, and the identification of markers associated to simple and complex traits. Many of these molecular techniques rely on the advantages of polymerase chain reaction (PCR) detection, including speed, sensitivity, and selectivity.

Amplified fragment length polymorphism (AFLP; Zabeau and Vos 1993), one of the most powerful methods for DNA fingerprinting, is based on the selective amplification of a specific set of genomic DNA fragments. This highly reproducible technique allows the simultaneous

screening of a large number of anonymous molecular markers, randomly distributed throughout the genome (Vos et al. 1995). Most of the AFLP analyses published so far have dealt with the analysis of herbaceous plants (e.g., Becker et al. 1995; Bendahmane et al. 1997; Cho et al. 1996; Cnops et al. 1996; Paul et al. 1997; Schondelmaier et al. 1996; Travis et al. 1996; Wang et al. 1997). Among tree species, AFLP analysis of willow, poplar, eucalypt, loblolly pine (Pines taeda L.), maritime pine (Pines pinaster Ait.), pinyon pine (Pines edulis Engelm.), and Norway spruce (Picea abies (L.) Karst.) genomes have been reported (Beismann et al. 1997; Cervera et al. 1996a, 19966, 1999; Gaiotto et al. 1997; Marques et al. 1998; Paglia and Morgante 1998; Paglia et al. 1998; Travis et al. 1998; Remington et al. 1999; Costa et al. 2000). In contrast to many annual crop species, trees are largely undomesticated and mostly outbreeding organisms with high levels of genetic diversity. The genome sizes of different tree species, belonging to heterogenous taxa, vary by more than 80-fold. The aim of this paper is to evaluate AFLP parameters for genome analyses in trees by (i) presenting specific AFLP parameters to obtain informative AFLP patterns for tree species with genome sizes ranging between 0.54 and 38 pg/2C: peach (Prunus persica L.), poplar (Populus deltoides Bartr., Populus nigra L., Populus trichocarpa Torr. & Gray, Populus alba L., Populus simonii Carriere, Populus maximowiczii A. Henry), eucalypt (Eucalyptus urophylla S.T. Blake, Eucalyptus grandis Hill ex Maid.), oak (Quercus robur L., Quercus petraea (Matt.) Liebl.), and loblolly pine; (ii) evaluating these AFLP conditions for the analysis of inter- and intra-specific variability in trees and comparing the data with those obtained using random amplification of polymorphic DNAs (RAPDs) and proteins; and (iii) evaluating these AFLP conditions for genome mapping in trees.

## Materials and methods

### Plant material

Leaf tissue of the following poplar genotypes was provided by the Institute of Forestry and Game Management (IBW; Geraardsbergen, Belgium): six clones from both the full-sib families 87001 and 87002, derived from controlled crosses between Populus deltoides S9-2 x Populus nigra Ghoy and Populus deltoides S9-2 x Populus trichocarpa V24, respectively; 13 unrelated clones of Populus deltoides (S9-2, V12, VI, V2, V3, V7B, S235-3, S333-53, S174-1, S197-1, S329-31, S193-1, and S336-4), five unrelated clones of Populus trichocarpa (S3-31, V24, V509, V510, and V235), and six unrelated clones of Populus nigra (Ghoy, Yzerl, Loire, Terwolde, 73.081, and Essene). DNA samples of five clones of Populus trichocarpa (FPL, 19-73, 36-77, 101-74, and 212-161) and two unrelated clones of Populus nigra (SRZ and 72-501) were provided by the Institut National de la Recherche Agronomique (Orleans, France). Cuttings of seven poplar clones (the parents and five progenies of family 95.045, obtained from a controlled cross between Populus deltoides DO-006 x Populus deltoides D1-180A) from five unrelated clones of Populus alba (A.L04.003, A.L05.010, BO-1, PA080, and Villafranca), from five unrelated clones of Populus maximowiczii (MW03-093, MW05283, MW07-222, MW12-105, and MW14-083), and from five unrelated clones of Populus simonii (81.001.003, 81.002.003, 81.003.001, 81.004.002, and 81.006.007) were obtained from the Istituto Sperimentazione per la Pioppicoltura (ISP; Casale Monferrato, Italy). Leaves of two clones of Populus nigra (Irll and Irl2) were provided by Teagasc (Dublin, Ireland). Leaves of Populus nigra var. italica Zaragoza and Populus nigra (Vereecken, Fue6, and Luc2) were provided by Servicio de Investigaci6n

Agraria de Arag6n (SIA, Aragon, Spain) and leaves of Populus nigra were collected in Reims, France.

DNA samples were obtained from (i) a two-generation pedigree of Eucalyptus (one E. urophylla tree, accession Pl; one E. grandis tree, accession P2; and 4 Fl hybrid progeny; Verhaegen and Plomion 1996); (ii) a three-generation inbred pedigree of Prunus persica (two grandparents, accessions FJ and F, one Fl hybrid, accession JFI, and 5 F2 progeny); (iii) from 15 Q. robur and 15 Q. petraea unrelated clones (Barreneche et al. 1996; Bod6nes et al. 1997); and (iv) from needle and megagametophyte tissues of six loblolly pine trees each, derived from a self-cross of Pin r taeda 756.

DNA extraction

Total genomic DNA was extracted from young (smaller than 1 cm) leaves, needles, or megagametophytes by the cetyl-trimethylammonium bromide method for eucalypt, peach, pine, and oak (Bousquet et al. 1990; Doyle and Doyle 1990) and the protocol described by Dellaporta et al. (1983) for poplar.

AFLP protocol for tree species (AFLP analysis from small to large genomes)

AFLP analysis, as described by Vos et al. (1995), was carried out according to Cervera et al. (1996a) with modifications depending on the tree species analyzed. Genomic DNA analyzed ranged from 250 (peach, eucalypt, oak, and poplar) to 500 ng (pine). To improve EcoRI-MseI digestion and ligation both reactions were carried out in 10 mM Tris-HAc (pH 7.5), 10 mM MgAc, 50 mM KAc, 5 mM DTT, and 50 mM BSA. In previous work (Cervera et al. 1996a), AFLP fingerprints were obtained with biotinylated fragments. This protocol was compared with one without selection of biotinylated fragments. When no biotinylated fragments were selected, digested-ligated DNA fragments were diluted fivefold to be used as template for the first amplification reaction, i.e., the preamplification step, prior to the selective radioactive PCR. The preamplification consisted of a PCR reaction using primers that were complementary to the adapters EcoRI and MseI with one additional selective 3' nucleotide for peach, eucalypt, oak, and poplar and two additional nucleotides for pine (Table 1). The PCR reactions were performed in a 20-µL volume of 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCI, 0.2 mM of each dNTP, 30 ng of each primer (GENSET, Paris, France), 0.4 U Taq DNA polymerase (Boehringer, Mannheim, Germany), and 5 liL of diluted fragments. PCR amplifications were carried out either in a 9600 thermocycler (Perkin-Elmer, Norwalk, N.J., U.S.A.), or PTC100 thermocycler (MJ research, Watertown, Mass., U.S.A.), using 20 cycles for peach and 28 cycles for eucalypt, oak, poplar, and pine. Each cycle consisted of 30 s at 94°C, 1 min at 60°C, and 1 min at 72°C.

For the selective radioactive amplification (Cervera et al. 1996a), a different number of selective nucleotides was added at the 3' end depending on the genome size (Table 1): two (EcoRI primer) and three selective nucleotides (MseI primer) for peach; three selective nucleotides for both primers for eucalypt, oak, and poplar; and three (EcoRI primer) and four or five selective nucleotides (MseI primer) for pine. The radioactive PCR reaction and running of the gel was further performed as described by Cervera et al. (1996a). The sequences of the primers used in the preamplification and the selective amplification are given in Table 1.

**Table 1.** Primer combinations used in the AFLP analysis of different tree species.

| Species | *Eco*RI primers | *Mse*I primers |
|---|---|---|
| **Pre-amplification** | 5′-AGACTGCGTACCAATTC+(*N*) | 5′-GACGATGAGTCCTGAGTAA+(*N*) |
| *Prunus* spp. | *N* = A | *N* = C |
| *Eucalypt* spp. | *N* = A | *N* = C |
| *Quercus* spp. | *N* = A | *N* = C |
| *Populus* spp. | *N* = A | *N* = A or T |
| *Pinus* | *N* = AC | *N* = CC |
| **Selective amplification** | 5′-GACTGCGTACCAATTC+(*N*) | 5′-GATGAGTCCTGAGTAA+(*N*) |
| *Prunus* spp. | *N* = AG | *N* = CAT |
| *Eucalypt* spp. | *N* = AGA | *N* = CAT |
| *Quercus* spp. | *N* = ACA | *N* = CAT or CAA |
| *Populus* spp. | *N* = AGA or AGC or ATA | *N* = AAG or TGT or ACA |
| *Pinus* spp. | *N* = ACC | *N* = CCAG or CCAGC |

## Results

AFLP analysis of woody plants

As a first step to establish optimal conditions for AFLP analysis of tree species, we analyzed whether it was necessary to subtract all fragments containing EcoRI ends from the reaction mixture by using a 5'-biotinylated top strand of the EcoRI adapter, which binds streptavidin beads (Dynal). This step had initially been implemented to reduce DNA complexity to make subsequent amplification reactions easier (Vos et al. 1995). The AFLP patterns obtained using a selection step with biotinylated fragments prior to preamplification were exactly the same as those obtained without this step (Fig. 1), indicating that the presence of MseIMseI fragments does not interfere with an efficient amplification of EcoRI-MseI fragments.

AFLP parameters were established for different tree species having genome sizes ranging from 0.54 to 38 pg/2C (Table 2). To obtain an informative and reproducible AFLP amplified fragment pattern, primer selection is important in both the pre-amplification and the selective PCR amplification steps. For genomes smaller than 0.60 pg/2C (some Prunus species), a pre-amplification with EcoRI + 1 - MseI + 1 followed by a selective amplification with EcoRI + 2 MseI + 3 provided the most informative amplified fragment patterns, revealing approximately 80 loci (amplified fragments) per individual and per primer combination (Table 2, Fig.1). For genomes up to 2.00 pg/2C (eucalypt, oak, and poplar), a pre-amplification with EcoRI + 1 - MseI + 1 followed by a selective amplification with EcoRI + 3 - MseI + 3 was required to obtain fingerprints containing between 90 and 112 amplified fragments per lane (Table 2, Figs. 1 and 2). For some genomes with sizes ranging between 0.60 and 1.00 pg/2C, a pre-amplification with EcoRI + 1 - MseI + 1 followed by a selective amplification with a combination of two EcoRI + 3 primers and one MseI + 3 primer (selection 2 EcoRI + 3 - MseI + 3) may also provide valuable fingerprints (Cervera et al. 1998). For the pine genome a preamplification with EcoRI + 1 - MseI + 1 followed by a selective amplification with EcoRI + 3 - MseI + 3 provided background "smears" and no reproducible fingerprints (data not shown); the complexity in the amplified fragment pattern had to be further reduced. We found that a preamplification with EcoRI + 2 - MseI + 2 followed by a selective amplification with EcoRI + 3 - MseI + 4 or with EcoRI+ 3 - MseI + 5 (depending on the primer combination) provided informative fingerprints (100-130 amplified fragments/lane) (Table 2, Fig. 1).

**Table 2.** Genome size of different tree species assayed and selection used to obtain informative AFLP patterns.

| Species (no. of plants analyzed) | Nuclear DNA content(pg/2C) | Selective nucleotides (*Eco*RI-*Mse*I primers) | | Average amplified fragments per fingerprint[a] |
|---|---|---|---|---|
| | | Pre-amplification | Selective PCR | |
| *Prunus* spp. (7) | 0.54[b] | +1, +1 | +2, +3 | 80 |
| *Eucalyptus* spp. (6) | 1.32[c] | +1, +1 | +3, +3 | 112 |
| *Quercus* spp. (30) | 1.84[d] | +1, +1 | +3, +3 | 100[g] |
| *Populus* spp. (55) | 1.10[e] | +1, +1 | +3, +3 | 90 |
| *Pinus* (12) | 38.00[f] | +2, +2 | +3, +4 (or +5) | 115 |

[a] Mean number of amplified fragments obtained by analyzing one primer combination.
[b] Arumuganathan and Earle (1991).
[c] Grattapaglia and Bradshaw (1994).
[d] Favre and Brown (1996).
[e] Bradshaw and Stettler (1993).
[f] Wakamiya et al. (1993).
[g] Mean number of amplified fragments obtained by analyzing two primer combinations.

**Table 3.** Intra- and inter-specific variability obtained by using AFLP analysis of tree species.

| Species | No. of clones | Total no. of amplified fragments analyzed[a] | Total no. of polymorphic amplified fragments[b] | Percent polymorphic amplified fragments |
|---|---|---|---|---|
| **Intraspecific variability** | | | | |
| *Populus deltoides* | 15 | 54 | 33 | 61 |
| *Polulus nigra* | 15 | 61 | 39 | 64 |
| *Populus trichocarpa* | 10 | 55 | 32 | 58 |
| *Quercus robur*[c] | 15 | 188 | 101 | 54 |
| *Q. petraea*[c] | 15 | 194 | 109 | 56 |
| **Interspecific variability** | | | | |
| *Populus deltoides* vs. *Polulus nigra* | 15 vs. 15 | 72 | 68 | 94 |
| *Populus trichocarpa* vs. *Populus deltoides* | 10 vs. 15 | 77 | 70 | 91 |
| *Populus trichocarpa* vs. *Populus nigra* | 10 vs. 15 | 79 | 70 | 89 |
| *Q. robur* vs. *Q. petraea*[c] | 15 vs. 15 | 200 | 115 | 58 |

[a] Refers to the sum of the number of amplified fragments with a different size showing high or medium intensity.
[b] A polymorphic amplified fragment is an amplified fragment showing high or medium intensity (i.e., easily scorable) that is present in at least one of the clones analyzed and absent in at least one of the other clones.
[c] Data based on the analysis of two primer combinations.

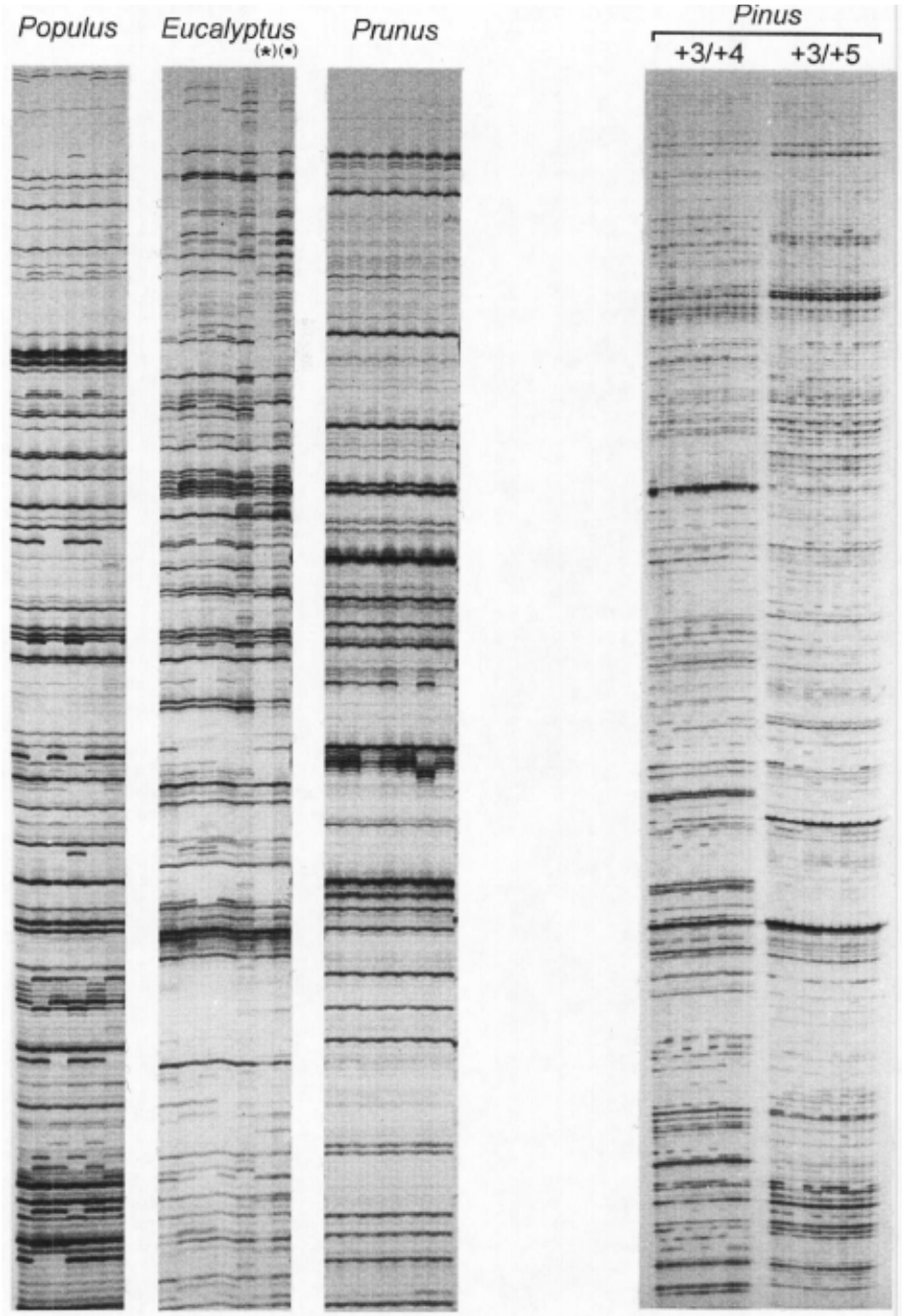


**Figure 1.** Example of AFLP fingerprints of poplar, eucalypt, peach, and pine genomes using a single primer combination. For *Populus,* six Fl hybrid trees derived from a cross between *Populus deltoides* S9-2 x *Populus nigra* Ghoy, analyzed with the primer combination *EcoRI* + AGA - *Msel* + AAG. For *Eucalyptus,* four Fl hybrid trees and their parents: *Eucalyptus urophylla* and *E. grandis,* analyzed with the primer combination *EcoRI* + AGA - *Msel* + CAT. The *E. grandis* parent **(e )** was also analyzed using streptavidin beads selection to compare the AFLP fingerprinting pattern to the one obtained without Dyna-bead selection (*). For *Prunus,* five F2 trees derived from a self-cross of *Prunus persica* JF I, obtained by crossing accession FJ and accession F (last two lanes) analyzed with the primer combination *EcoRI* + AG - *Msel* + CAT. For *Pinus,* six megagametophytes and six Fl clones obtained by self-fertilization of a *Pinus taeda* tree, analyzed with the primer combination *EcoRI* + ACC - *Msel* + CCAG and EcoRI + ACC - *Msel* + CCAGC, respectively.

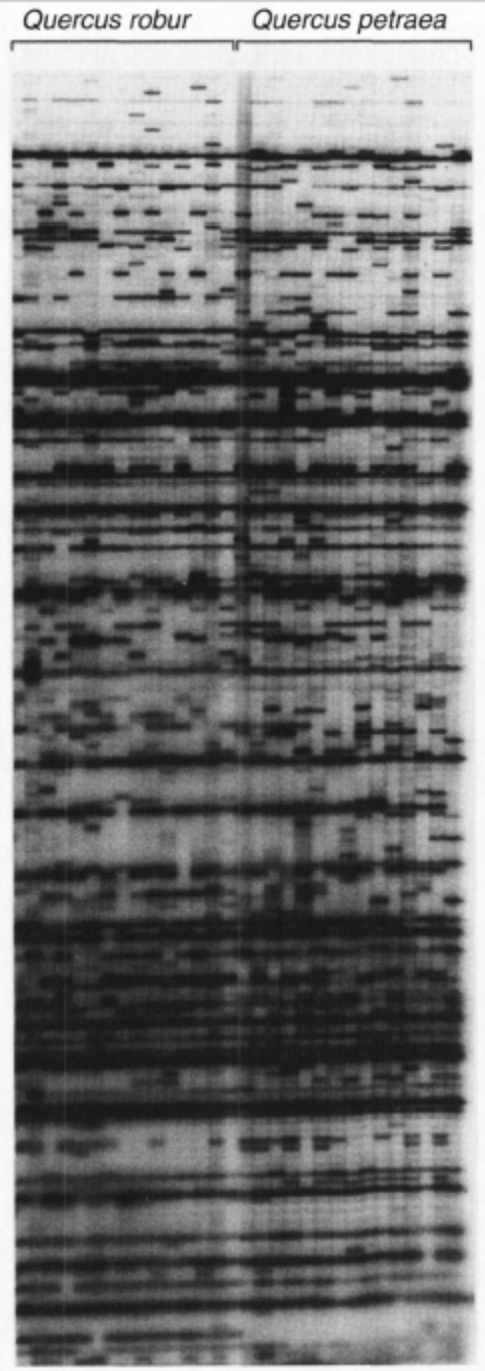


**Figure 2.** AFLP fingerprinting of oak. Fifteen clones of *Quercus robur*, and 15 clones of *Q. petraea* analyzed with *Eco*RI + ACA - *Mse*I + CAT.

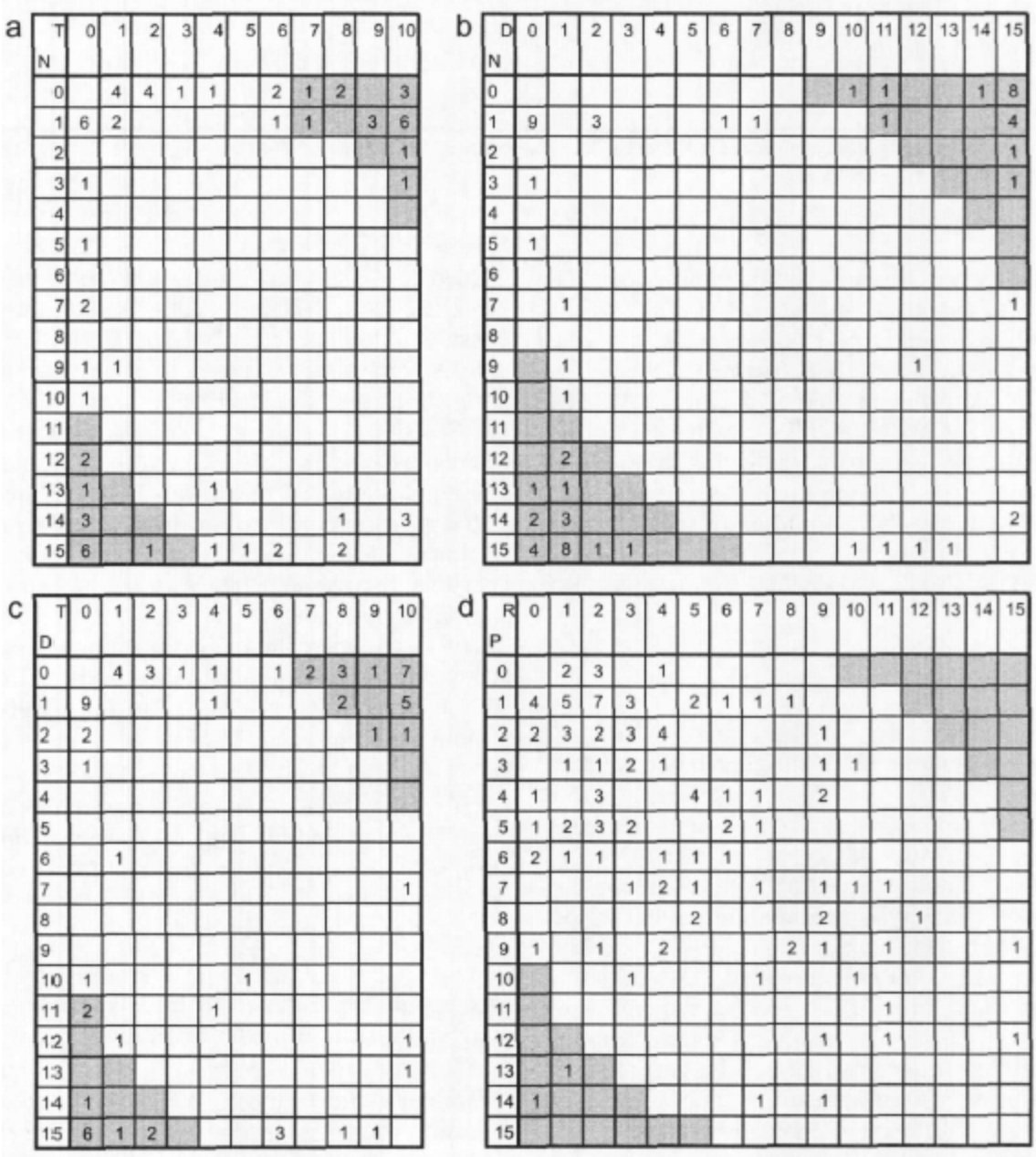


**Figure 3.** Interspecific frequency differences of polymorphic fragments between three *Populus* species and two *Quercus* species. (a) Interspecific frequency differences of 70 polymorphic fragments obtained after comparing 10 clones of *Populus trichocarpa* (T) with 15 clones of *Populus nigra* (N); the first row (solid cells) shows the number of occurrences in *Populus trichocarpa* and the first column (solid cells) shows the number of occurrences in *Populus nigra.* (b) Interspecific frequency differences of 68 polymorphic fragments obtained after comparing 15 clones of *Populus deltoides* (D) with 15 clones of *Populus nigra* (N). (c) Interspecific frequency differences of 70 polymorphic fragments obtained after comparing 10 clones of *Populus trichocarpa* (T) with 15 clones of *Populus deltoides* (D). (d) Interspecific frequency differences of 115 polymorphic fragments obtained after comparing 15 clones of *Q. petraea* (P) with 15 clones of *Q. robur* (R). Each cell contains the number of AFLP fragments with a given frequency in both species. Shaded cells correspond to AFLP fragments showing significant frequency differences between both species (overall test $p < 0.05$).

## Polymorphism of AFLP markers in parents and progenies

The construction of linkage maps has provided a wealth of information on the organization and evolution of genomes. Among others, these linkage maps allow the deterurination of the number, localization, magnitude of effect, and interaction of genetic loci that control the expression of agriculturally important traits (reviewed by Tanksley 1993). Using a pseudo-test-cross strategy (Grattapaglia and Sederoff 1994), mapping of allogamous species, such as most trees, can be done by analyzing a full-sib Fl progeny. To obtain an indication of the number of AFLP markers that can be used for mapping in Fl, the number of polymorphic AFLP markers, segregating in Fl progeny, were determined for different full-sib crosses (Table 4). An intraspecific Populus deltoides x Populus deltoides cross showed an average value of 26% polymorphic amplified fragments, whereas a self-cross of a Pinus taeda gave values ranging from 40 to 46%, when diploid or haploid (megagametophyte) tissue was analyzed, respectively (Fig. 1). When five clones of a self-cross of Prunus persica were also analyzed, 17% polymorphic amplified fragments were observed. Prunus is known to have a low level of polymorphism (Arulsekar and Parfitt 1986; Chaparro et al. 1994; Mowrey et al. 1990) compared with that of Pines or Populus. When interspecific full-sib crosses were analyzed, the percentage of polymorphic amplified fragments that could be detected ranged from 28% (for Populus deltoides x Populus nigra) to 38% (for E. urophylla x E. grandis) (Table 4, Fig. 1).

**Table 4.** Inheritance od AFLP markers in intra- and inter-specific full-sib crosses of different tree species.

| Species | No. of trees analyzed | No. of amplified fragments analyzed | No. of polymorphic amplified fragments[a] | Percent polymorphic amplified fragments |
|---|---|---|---|---|
| **Intraspecific full-sib cross** | | | | |
| *Populus deltoides* x *Populus deltoides* | 5 | 81 | 21 | 26 |
| *Prunus persica* x *Prunus persica* | 7 | 104 | 18 | 17 |
| *Pinus taeda* x *Pinus taeda*[b] | 6[c] | 115 | 46 | 40 |
| *Pinus taeda* x *Pinus taeda*[b] | 6[d] | 115 | 53 | 46 |
| **Interspecific full-sib cross** | | | | |
| *Populus deltoides* x *Populus nigra* | 6 | 97 | 27 | 28 |
| *Populus deltoides* x *Populus trichocarpa* | 6 | 102 | 32 | 31 |
| *Eucalyptus urophylla* x *E. grandis* | 5 | 127 | 48 | 38 |

[a] Given the small number of progeny trees analyzed, no difference was made between AFLP markers segregating 3:1 and 1:1 in the F1 pedigree.
[b] Selective amplification *Eco*RI + 3 - *Mse*I + 5.
[c] Diploid tissue (needles).
[d] Haploid tissue (megagagametophyte) derived from the same cross as for the previous row/

## Discussion

AFLP conditions for genome sizes ranging from 0.5 to 38 pg/2C

Different factors are critical for an optimal AFLP analysis in different species. First, the quality of the plant DNA is important to ensure a complete digestion with restriction enzymes. Both the cetyl-trimethyl-ammonium bromide method (Bousquet et al. 1990; Doyle and Doyle 1990) and the method of Dellaporta et al. (1993) provided DNA of sufficiently high quality to perform AFLP. Second, the complexity (size and composition) of the plant genome determines the number of amplified fragments that will be generated in the AFLP analysis using a fixed number of selective nucleotides. We have assayed different combinations of selective nucleotides to determine the best selection to be applied for a given genome size (Tables 1 and 2). The primer

combinations used in these assays provided between 80 and 130 amplified fragments per primer combination (one lane in the gel), ranging from 70 to 700 nucleotides. The protocol for selective PCR was standardized and ranged from EcoRI + 2 MseI + 3 (genome sizes smaller than 0.60 pg/2C); two EcoRI + 3 - MseI + 3 (genome sizes between 0.60 and 1.00 pg/2C; Cervera et al. 1998); EcoRI + 3 - MseI + 3 (genome sizes between 1.00 and 10.00 pg/2C, such as the barley genome; Qi and Lindhout 1997) always preceded by an EcoRI + 1 - MseI + 1 pre-amplification; up to EcoRI + 3 MseI + 4 or EcoRI + 3 - MseI + 5 for conifer genomes containing from 20 to 38 pg/2C. As described by Vos et al. (1995), no more than two selective nucleotides should be added to the EcoRI primers between two consecutive PCR amplifications to reduce background in the fingerprint and to ensure a high selectivity. The desired final selection for AFLP analysis of conifers was obtained by increasing the number of selective nucleotides in the pre-amplification step to EcoRI + 2 - MseI + 2. Such procedure was recently presented for plant species with very large genomes (Han et al. 1999). because of the lower annealing temperature of the MseI primers compared with that of the EcoRI primers, additional selection could be achieved by increasing the number of selective nucleotides of the MseI primers in the final amplification (four or five vs. three selective nucleotides). EcoRI primers with more than three selective nucleotides caused a loss of reproducibility in the AFLP assay, indicating tolerance of mismatches in the amplification of DNA fragments (Vos et al. 1995).

The use of EcoRI and Mse! directs the digestion to A/Trich regions in the genome. The number of fragments can also be controlled by using different combinations of restriction enzymes that cut the genome less frequently (e.g., PstI instead of EcoRI; Paglia and Morgante 1998). This option has not been explored in this work.

Assessment of inter- and intra-specific genetic variability based on AFLP markers

Our study indicated that AFLP can be very useful for population genetic analysis. When different species were analyzed at the intraspecific level, the number of polymorphic amplified fragments obtained ranged from 58 to 64% for Populus and from 54 to 56% for Quercus (Table 3). When different species of Populus and Quercus were analyzed at the interspecific level, the level of polymorphism varied between 58% for Quercus up to 94% for Populus. Our results indicate that the AFLP technology enables the detection of a high number of inter- and intra-specific polymorphic markers. This feature is being exploited in several biodiversity analyses (M.T. Cervera, unpublished results). The genetic variability assayed for Populus at the interspecific level was higher than that at the intraspecific level. However, similar variability levels were found for the two Quercus species when they were assayed at the intra- or inter-specific levels. This difference may reflect the natural area of distribution of the species analyzed. Quercus robur and Q. petraea are sympatric and interfertile, whereas the natural provenance of the three Populus species originate from different continents (Populus nigra in northern Europe, Populus deltoides in the east coast of the United States, and Populus trichocarpa on the west coast) and were separated during longer periods in comparison with Quercus species, hence increasing their genetic divergence. Also for Quercus, other results based on protein, allozyme, RAPD, and chloroplastic DNA markers indicated the existence of very tenuous molecular differences between the two species (Barreneche et al. 1996; Bodenes, et al. 1997). In general, the discovery of DNA regions discriminating two different species may contribute to our understanding on the basis of species differentiation.

Evaluation of AFLPs for tree genome mapping

All the AFLP markers detected in intra- and inter-specific full-sib progenies of different woody species were inherited from at least one of the parents. Percentages of polymorphic amplified fragments ranged from 17% for Prunus persica to 46% for Pinus taeda (Table 4). These numbers are sufficient for mapping based on a two-way pseudo-test-cross analysis (Grattapaglia and Sederoff 1994). This strategy, commonly used to approach genome mapping of highly heterozygous outbreeding species, is facilitated when a high density marker technology such as AFLP is used to obtain a high number of markers segregating 1:1 in the F1 progeny, generating the two parental genome maps (Cervera et al. 1999; Marques et al. 1998). AFLP markers segregating 3:1 allow the integration of both genome maps (Verhaegen and Plomion 1996). Saturated AFLP maps can be used to study the genetic architecture of traits of commercial interest and will also be of invaluable help for map-based cloning (Cnops et al. 1996).

There is little molecular marker data for most woody species, because they have not been important model systems. Thus, markers like AFLPs, which provide high number of fragments per primer pair, are valuable for generating a large amount of information quickly.

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