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Original article

Origin of Basque populations of radiata pine inferred from RAPD data

A Aragonés, I Barrena, S Espinel, A Herrán, E Ritter*

CIMA-Granja Modelo, Apartado 46, 01080 Vitoria-Gasteiz, Spain

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Summary – Three natural provenances of *Pinus radiata* D Don (Año Nuevo, Monterey and Cambria) and the local landrace from the Basque country in northern Spain were examined by means of RAPDs (random amplified polymorphic DNAs). A high degree of polymorphism was detected and all 27 genotypes of the assay could be distinguished by combining the RAPD patterns of only two primers. The molecular analyses indicated that the local population has closest affinities to the Año Nuevo provenance, consistent with previous observations on performance and the physiological characteristics of the different provenances.

RAPD / *Pinus radiata* / provenance / genetic distance

Résumé – Origine des populations du pin radiata du Pays Basque à partir de marqueurs RAPD. Trois provenances de *Pinus radiata* (Año Nuevo, Monterey et Cambria) ainsi que la variété locale du Pays Basque du nord de l'Espagne sont comparées à l'aide du marqueurs RAPD (*random amplified polymorphic DNAs*). Un polymorphisme très élevé est observé et les 27 génotypes utilisés dans ce travail peuvent être distingués en utilisant une combinaison de marqueurs RAPD obtenues avec deux amorces. Les analyses moléculaires indiquent que la variété locale a une affinité plus grande avec la provenance d´Año Nuevo. Cette relation est en accord avec les observations préalables sur le comportement et les caractéristiques physiologiques des différentes provenances de pin radiata.

RAPD / *Pinus radiata* / provenance / distance génétique

* Correspondence and reprints
Tel: (34) 45 28 13 00; fax: (34) 45 28 14 22; e-mail: eritter@ikt.es

INTRODUCTION

The natural distribution of the radiata pine (*Pinus radiata* D Don) is restricted to three coastal populations in California (Año Nuevo, Monterey and Cambria) and two island populations in Baja California (Guadalupe and Cedros). Owing to its rapid growth and favourable characteristics for timber production, this pine species has been widely introduced in South Africa, Chile, Australia, New Zealand and in the Basque country in northern Spain. At the moment, radiata pine represents the tree species of greatest importance in the Basque country, where two major introductions occurred, one at the beginning of this century and the other during the 1940s. However, nothing is known about the origin of these introductions. The performance of different provenances and of the local population has previously been studied in field trials (Espinel et al, 1995). Growth behaviour and the mortality observed after unfavourable climatic conditions indicated a strong relationship between the Año Nuevo provenance and the local population. Differences between *P radiata* populations have also been detected by Burdon et al (1992), including growth rate, morphological traits and susceptibility to fungal diseases. Natural populations of *P radiata* have previously been characterized by isoenzyme analysis and could be separarated by cluster analysis using allelic frequency data (Moran et al, 1988). Comparing allozyme variation in *P radiata* populations of Australia and in the five wild populations of North America, Moran and Bell (1987) found that the Monterey and Año Nuevo populations were probably the major source of the original introductions in Australia.

Molecular DNA markers have recently been developed for population genetic studies in this species. Strauss et al (1993) could differentiate between five natural *P radiata* populations and 14 populations of two other pine species by means of mitochondrial DNA RFLP using a mitochondrial *coxI* gene as probe. Chloroplast DNA variability was studied in the same populations using a chloroplast probe from *Pseudotsuga menziesii* (Hong et al, 1993). However, in this case almost no genetic variation within or between *P radiata* populations was found. On the other hand, chloroplast SSR revealed intraspecific polymorphism in *P radiata* with four of five flanking primer pairs (Cato and Richardson, 1996).

Recently, nuclear microsatellites have also been developed in this species (Smith and Devey, 1994; Fisher et al, 1996). They constitute a powerful tool for gene mapping, fingerprinting and population genetic studies. Microsatellites were abundant in the conifer genome, highly polymorphic and showed Mendelian transmission in *P radiata* (Smith and Devey, 1994). They could also be applied to linkage mapping (Devey et al, 1996).

RAPDs (random amplified polymorphic DNAs; Welsh and McClelland, 1990; Wiliams et al, 1990) have been used to analyse single genotypes of *Pseudotsuga menziessii* (Mirb) Franco and *Picea glauca* (Moench) Voss (Carlson et al, 1991), and populations of *Pinus resinosa* Ait, *Picea glauca* (Moench) Voss and *Picea mariana* (Mill) BSP (Mosseler et al, 1992). In this paper we report on the suitability of RAPD markers to ascertain the origin of the Basque *Pinus radiata* populations using a limited set of trees from the three natural mainland populations and from the local population.

MATERIALS AND METHODS

Plant material

A total of 27 trees was used for the molecular analysis. Six trees of the natural population from Año Nuevo (AN), seven trees from the Monterey population (MR) and six trees from the Cambria population (CA) were chosen. The original seed material was obtained from the CSIRO 1978 collection (Eldridge, 1983). Eight trees

were chosen from the population of the Basque country (BC). Four trees were characterized by exceptional growth (plus trees, BCp) while the other four showed normal growth characteristics (BCn) and represent the average population of this region.

Molecular analysis

DNA was extracted from 2 g of fresh needles and vegetative buds following the method of Carlson et al (1991) with some modifications. Tissues from freeze-dried material were ground in a mortar with aluminium oxide and ground in 10 mL CTAB isolation buffer [2% w/v CTAB, 1.4 M NaCl, 20 mM EDTA, 1% w/v PEG 6000, 100 mM Tris-HCl (pH 9.5)] and 0.5% v/v 2-mercaptoethanol. The mixture was incubated at 74 °C for 20 min and then cooled down to room temperature. The homogenate was extracted with 1:1 chloroform-isoamylalcohol (24:1) and centrifuged at 10 000 *g* for 10 min at 20 °C. DNA was purified following the standard phenol method according to Abelson and Simon (1987).

PCR reaction mixtures had a total volume of 25 μL. The mixture contained 0.75 units of Taq DNA polymerase (Pharmacia), 0.3 mM primer, 200 mM dNTPs and 0.5 mM magnesium chloride, the appropiate dilution of the reaction buffer prepared by the company supplying the polymerase (500 mM KCl, 15 mM $MgCl_2$ and 100 mM Tris-HCl pH 9.0), and approximately 25 ng of template DNA. Reaction mixtures were overlayed with 50 μL of mineral oil before being placed in a Linus Autocycler plus FTS-1. The PCR program had an initial cycle at 94 °C for 5 min. The 45 subsequent cycles had a denaturation step at 94 °C for 1 min, the annealing temperature was 37 °C for 1 min and the elongation step was for 2 min at 72 °C. A final elongation step at 72 °C for 10 min followed. A total of 20, 10-mer primers of arbitrary sequence (Operon Technologies, Alameda, CA) were used for PCR amplification. The amplification products were visualized on 1% agarose gels, stained with ethidium bromide, using standard methodology (Sambrook et al, 1989).

The occurrence of a specific band of amplified DNA was scored as one and absence as zero for all prominent bands within a fingerprint. Amplification of DNA was repeated once, and only those fragments amplified reproducibily were included in the analysis. NTsys-PC program (Rohlf, 1989) was used for data processing and cluster analyses. Similarity coefficients were calculated between the 27 genotypes according to Nei and Li (1979). Cluster analysis was performed based on these coefficients and using UPGMA as the clustering method, widely used for discrimination analysis (Mailer et al, 1994; Heun et al, 1994).

RESULTS

A total of 20 primers were tested in the assay. One of them (OpG-10) was found to yield monomorphic bands only. The other 19 primers revealed a total of 188 different RAPDs (177 polymorphic and 11 monomorphic fragments). Table I summarizes the observed polymorphisms between the 27 samples of *P radiata*. With our set of samples the 19 polymorphic primers revealed between 6 and 16 different RAPDs each, whereas the number of patterns oscillated between 7 for primer OpU2 and 24 for primer OpAU3. It was possible to distinguish between all 27 genotypes by combining the patterns of the two primers OpAU3 and OpAU8.

The similarities obtained between genotypes ranged from 0.476 and 0.914. A cophenetic matrix was computed from the tree matrix and compared with the original similarity matrix in order to measure the goodness of fit (Rohlf, 1972). These matrices showed a significant correlation of 93%. The results of the cluster analysis are presented in figure 1. All plus trees (BCp) and three out of four genotypes of the BCn population clustered together with all genotypes of the AN population except one outlier. Samples of provenance CA formed a distant cluster showing the least similarities to the other samples. Four trees of the Monterey provenance clustered together and joined the cluster formed by the local and the Año Nuevo population, while the other three samples of the Monterey population grouped with four samples of the Cambria population.

Table I. Characteristics and observed polymorphisms of 20 primers used with RAPD analysis in different populations of *Pinus radiata.*

Primer code[a]	*Total No of RAPDs*	*No of monomorphic fragments*	*No of patterns*
OpG10	3	3	1
OpB11	11	0	21
OpB1	7	1	8
OpE2	7	0	15
OpU2	6	0	7
OpE3	7	2	9
OpE6	8	1	14
OpE11	7	0	14
OpE4	9	1	12
OpE1	9	1	10
OpB4	11	1	15
OpB20	12	1	22
OpG6	9	1	12
OpG11	9	0	16
OpAU1	12	0	19
OpAU2	11	0	17
OpAU3	12	0	24
OpAU5	16	1	21
OpAU8	11	0	20
OpAU9	14	1	18

[a] All primers were supplied by Operon Tech.

Average similarities between single BCp and BCn genotypes and the natural provenances were computed and compared (table IIA). All BCn and three out of four BCp genotypes showed the highest average similarity with the AN population, while the genotype BCp2 had the highest average similarity with the provenance of Monterey. In the same way average similarities between populations were established, and these are presented in table IIB. With respect to interpopulation comparisons, the highest average similarities for BC were obtained with the provenance of Año Nuevo.

Similarity coefficients for interval measure data (quantitative) were computed using relative RAPD frequencies in each population. Euclidean distances were calculated for each pair of populations. The dendrogram of the corresponding cluster analysis (UPGMA) is displayed in figure 2. The closest affinity of the local population (BC) occurred again with the AN population and cluster analysis clearly separated the other two American provenances from the BC and AN population. A dendrogram obtained from cluster analysis based on average similarity data (table IIB) looked identical (results not shown).

DISCUSSION

Different DNA markers such as mitochondrial RFLP probes (Strauss et al, 1993), chloroplast SSR (Cato and Richardson, 1996) and microsatellites (Smith and Devey, 1994) could be applied successfully to dif-

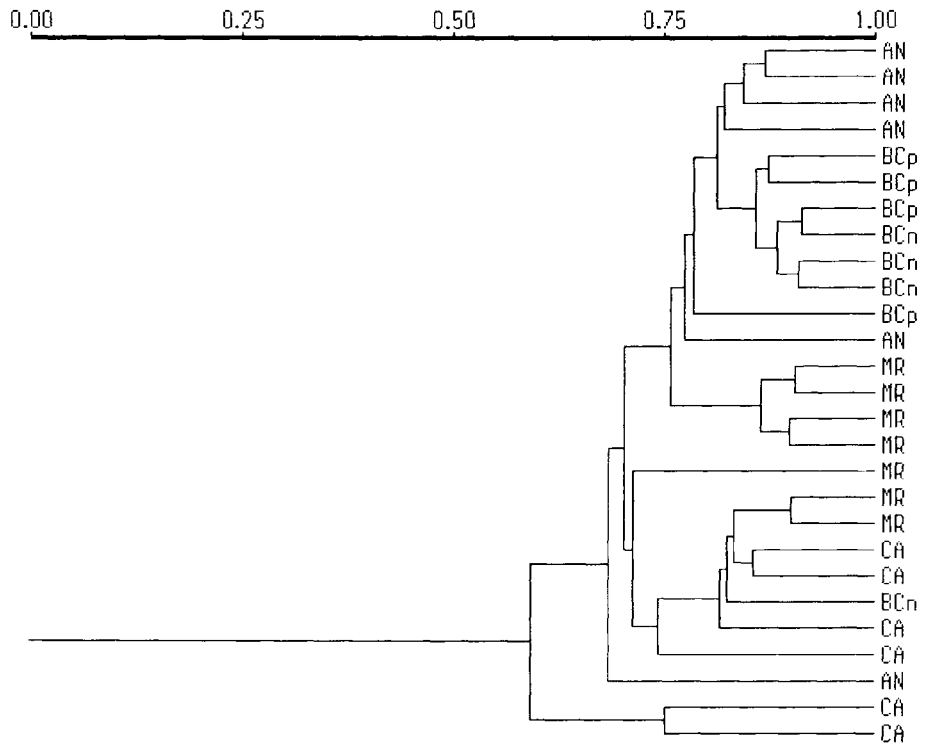

Fig 1. Dendrogram of 27 *Pinus radiata* genotypes from three provenances and from the local population of the Basque country based on similarity coefficients (Nei and Li, 1979) and the UPGMA clustering method. AN = genotypes of Año Nuevo provenance, MR = genotypes of the Monterey provenance, CA = genotypes of Cambria provenance, BCp = genotypes of the Basque population with exceptional growth characteristics, BCn = genotypes of the Basque population with normal growth.

ferentiate genotypes between and within *P radiata* populations. In the present study RAPDs also detected a high degree of polymorphisms between different samples of *P radiata* and were even useful for genotyping single individuals. With our set of individuals it was possible to distinguish all 27 genotypes by means of RAPD fingerprinting by combining the patterns of only two primers.

Moran and Bell (1987) could determine the origin of Australian breeding populations using allozyme markers. In our study the different types of genetic distance analyses based on RAPD markers indicated that the local population in the Basque country descends most probably from the provenance of Año Nuevo (AN). This hypothesis was supported by different approaches. The first indication resulted from the cluster analysis based on genetic similarities between individuals using the Nei and Li (1979) coefficient (fig 1), where seven out of eight genotypes formed a main cluster with all AN genotypes except one.

In addition, most of the BC genotypes showed the highest average similarity with the Año Nuevo population. When analysing

Table II. Genetic relationships between different provenances of *Pinus radiata* and the local population of the Basque country.

A. Average genetic similarities (Nei and Li, 1979) between genotypes of the Basque population and the natural American populations.

	AN	*MR*	*CA*
BCp1	*0.7353*[a]	0.7166	0.6228
BCp2	0.7453	*0.7571*	0.6318
BCp3	*0.7945*	0.7467	0.6720
BCp4	*0.7788*	0.7570	0.6382
BCn1	*0.7868*	0.7389	0.6653
BCn2	*0.7840*	0.7683	0.6510
BCn3	*0.8105*	0.7554	0.6675
BCn4	*0.7672*	0.6966	0.7502

B. Average genetic similarities between the different populations.

	Genetic similarity			
	AN	*MR*	*CA*	*BC*
AN	0.789			
MR	0.723	0.729		
CA	0.692	0.648	0.736	
BC	*0.775*	0.742	0.662	0.828

[a] Highest similarities of the local populations and their subgroups with the natural provenances are underlined. AN = provenance of Año Nuevo, MR= provenance of Monterey, CA = provenance of Cambria, BC= population of the Basque country. BCp/BCpi = group of genotypes/single genotypes of the Basque population with exceptional growth characteristics. BCn/BCni = group of genotypes/single genotypes of the Basque population with normal growth habitus.

average similarities between populations (table IIB), the closest relationships of the Basque populations were obtained again with the AN provenance. Another result obtained from similarity coefficients based on quantitative data by comparing RAPD frequencies between different populations confirmed this hypothesis (fig 2).

Growth behaviour and other characteristics of the different natural populations have been determined previously in different field trials (Shelbourne et al, 1979; Burdon et al, 1992; Jayawickrama and Balocchi, 1993). In a provenance test in the Basque country (Espinel et al, 1995) superior behaviour of the MR provenance and a similar behaviour between the AN and the local population was also observed. In addition, these latter two populations also showed a better adaptation in this test. Mortality of the local population and the Año Nuevo population were low (2.1 and 8.1%, respectively) while high mortality rates were found in the Monterey (29.3%) and particulary in the Cambria population (52.6%).

Technical problems and practical limitations caused by reproducibility problems and comigration of heterologous bands of similar size are well-known for RAPD analysis and were summarized by Black (1993). Nevertheless, despite the limited number of trees analysed, coherent results between field performance and molecular analysis were obtained. The reduced numbers of existing natural populations of *P radiata* in the world

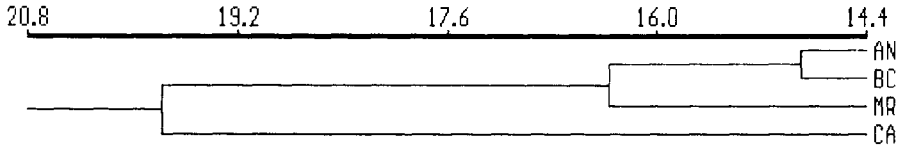

Fig 2. Dendrograms of three natural provenances and of the local population of *Pinus radiata* based on Euclidean distances derived from relative RAPD frequencies. (See table II for the legend.)

has surely facilitated the identification of the source population of Spanish radiata pine forests. However, RAPDs may represent a useful tool for the discrimination of populations at least in *P radiata*.

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