

L. Salvador · R. Alía · D. Agúndez · L. Gil

## Genetic variation and migration pathways of maritime pine (*Pinus pinaster* Ait) in the Iberian peninsula

Received: 15 February 1999 / Accepted: 30 April 1999

**Abstract** The genetic variability and migration pathways of *Pinus pinaster* after glaciation in the Iberian peninsula was studied by means of 18 loci from 12 natural populations of the species. The analysis showed the existence of three groups of populations with different levels of diversity and patterns of recolonization. The southern Iberian group displays a high level of diversity, with a stepping-stone model of variation. The presence of rare alleles in this group and their position in the phylogenetic tree suggest the existence of refugia during glaciations in this zone. The eastern Iberian group also has high levels of diversity but is clearly separate from the first group based on their genetic distances. The Atlantic group displays a low level of diversity that could be interpreted as a rapid recolonization of the entire area by the Eastern group that has not yet developed to a divergence in this area. The southern Iberian range is indicated to be the dispersal centre of the species after the last glaciation.

**Key words** *Pinus pinaster* · Genetic variability · Isozymes · Migration pathways

### Introduction

Genetic variability of Mediterranean pines is interesting both in terms of conservation of genetic resources and breeding. The Mediterranean basin is characterized by a traditional depletion of the forest and by man (Thirgood 1981) and the modification of genetic diversity of the

species by human activity (Ledig 1992). The effects, however, have not been the same throughout the entire Mediterranean range, being more important in coastal areas and fertile soils. Various ecological factors, such as forest fires, drought, soils, etc., usually play a major role in the adaptation of the species, in the isolation between stands and, to some extent, in the genetic variation of the species. The conservation of endangered native stands is, at present, a major concern in the management and breeding of Mediterranean species.

Maritime pine (*Pinus pinaster* Ait.) is an important species in the Mediterranean basin and is found throughout the western range, from France to Morocco and from Portugal to Tunisia. The role of maritime pine in the Iberian peninsula has not been clearly established from the biogeographic and botanical point of view (i.e. Barbero et al. 1998). On many occasions there has been an oversimplification of the large number of factors that affect the present distribution and genetic variation of the species.

Different aspects of *Pinus pinaster*'s genetics have been studied, mainly concerning the pattern of variation by means of terpene analysis (Baradat and Marpeau 1988), the relationship among patterns of variation using different types of markers (Petit et al. 1995) and structural and phylogenetic relationships among populations using chloroplast micro-satellites (Vendramin et al. 1998). Baradat and Marpeau (1988) defined 18 geographical races, classified into three main groups: Atlantic, Mediterranean and Maghrebi (this last including Spanish southern populations). The Iberian populations belong to 7 of these geographical races, from all three main groups, indicating the major role of this territory in the genetic variation of the species and its migration pathways. Ecological conditions in this area are extremely variable – soils ranging from calcareous to sandy and climates from Mediterranean to Atlantic – resulting in specific adaptations of the species in terms of growth pattern and survival (Alía et al. 1995).

In southern Europe, poor edaphic conditions have severely limited temperate trees, shrubs and herbs (Willis

---

Communicated by P.M.A. Tigerstedt

---

L. Salvador · L. Gil (✉)  
Unit of Anatomy, Physiology and Genetics, ETSIM,  
Ciudad Universitaria s/n. 28040, Madrid, Spain  
Fax: 34 91 543 95 57  
e-mail: lgil@montes.upm.es

R. Alía · D. Agúndez  
Department of Breeding and Biotechnology, CIFOR-INIA,  
Apdo. 8111, 28080, Madrid, Spain

**Table 1** Location of the 12 *Pinus pinaster* sampled populations

Code	Location	Country	Altitude (m)	Latitude	Longitude
MA	Estepona	Spain	500	36° 31' 05" N	5° 07' 11" W
GR	La Peza	Spain	1400	37° 16' 26" N	3° 22' 10" W
AL	Oria	Spain	1300	37° 30' 49" N	2° 20' 11" W
AB	Riopar	Spain	1200	38° 28' 05" N	2° 27' 31" W
CR	Fuencaliente	Spain	900	38° 25' 05" N	4° 11' 53" W
V	Cortes de Pallás	Spain	900	39° 10' 48" N	0° 56' 41" W
CU	Boniches	Spain	1050	39° 59' 18" N	1° 39' 03" W
CS	Benicasim	Spain	325	40° 05' 04" N	0° 00' 46" E
VA	Iscar	Spain	760	41° 20' 05" N	4° 31' 11" W
PRT	Leiria	Portugal	200	40° 00' 00" N	8° 45' 00" W
PO	Ribarteme	Spain	300	42° 07' 06" N	8° 21' 52" W
LE	Tabuyo del Monte	Spain	1050	42° 18' 13" N	6° 13' 25" W

et al. 1998). In Spain, it is usual to find a close connection between *Pinus pinaster* forests and several edaphic types (peridotites, sandy soils of inland and coastal dunes, red sandstones, etc.), and places with these conditions have served as refugia of the species. The result is that this pine appears in differently sized scattered nuclei. Nevertheless, local and regional extirpations have taken place, and pinewoods have often been replaced by a secondary maquis. In the last 40 years, reforestation programmes in the entire area (Spain and Portugal) have notably increased area inhabited by maritime pine woodlands. However, seed movement without regard to its origin was common, which is the main difficulty in making a correct assessment of the adequate populations to clarify the migration pathways of the species.

The present situation, therefore, is a complex of native populations that are of great interest with respect to genetic resources conservation and breeding and some reforestations of unknown origin. The distribution and location of the native stands in Spain, based on the first Spanish Forest Survey of 1859, have been established recently (Gil et al. 1990; Gil 1991; Alía et al. 1996). In northwestern Spain an important expansion of this species since the 19<sup>th</sup> century has occurred, mainly from original natural stands; however, in this respect no precise data are available from Portugal. Thus, the global impact of afforestation on the genetic diversity of *P. pinaster* is difficult to document.

The importance and location of different glacial refugia, the effect of isolation on marginal populations and the importance of these effects on the genetic variability of the species have not been well established. Nevertheless, Baradat and Marpeau (1989) pointed a strong differentiation linked to the parcelling of the natural range of *Pinus pinaster* after the Würm III.

In this paper, we report on the genetic variability of 12 natural populations from the Iberian peninsula as determined by 18 enzymatic loci. The geographic structures of the variability of populations with different demographic and isolation characteristics have been analysed. The migration pathways of the species are discussed taking into account the biogeography and main characteristics of the native maritime pine stands.

**Table 2** Enzyme<sup>a</sup> and buffer systems used in the analysis

Histidine pH 7.0/7.0		Ashton pH 8.3/8.3	
ACO	(E.C. 4.3.1.3.)	GOT	(E.C. 2.6.1.1.)
DIA	(E.C. 1.6.4.3.)	HK	(E.C. 2.7.1.1.)
IDH	(E.C. 1.1.1.42.)	PGM	(E.C. 2.7.5.1.)
LAP	(E.C. 3.4.11.1.)		
6PGD	(E.C. 1.1.1.44.)		
PGI	(E.C. 5.3.1.9.)		
SKDH	(E.C. 1.1.1.25.)		

<sup>a</sup> ACO, Aconitase; DIA, diaphorase; IDH, isocitrate dehydrogenase; LAP, leucine aminopeptidase; 7PGD, 6-phosphogluconate dehydrogenase; PGI, phosphoglucose isomerase; SKDH, shikimate dehydrogenase; GOT, glucamate oxaloacetate transaminase; HK, Hexokinase; PGM, phosphoglucomutase

## Materials and methods

### Material and isozyme methods

Seed samples were collected from 12 native stands of *Pinus pinaster* found over the entire range in the Iberian peninsula (Table 1). Most of the populations have been documented as having a natural origin. The Leiria population is a classic locality of the species with a historic presence, and also the population from Galicia (PO) may be assumed to be of a likely natural origin. The CR population is of very limited size, containing 200 living trees.

From each population 80 mature trees, each separated by a distance of 100 m, were sampled. The objective was to detect alleles that were present at a frequency higher than 5% in each population. One germinated female gametophyte per mother tree was analysed by means of starch gel electrophoresis when the emerging radicle was between 2 m and 5 mm long. A 0.2 M phosphate buffer, pH 7.5 (Conkle et al. 1982), was used for enzyme extraction, and two continuous buffer systems were employed in ten enzymatic systems (Table 2). Staining procedures have been described elsewhere (Brewer and Sing 1970; Conkle et al. 1982; Tanksley and Orton 1983).

The most anodal locus in an enzyme system was coded as 1. Within a locus, the most common allele was coded as 100, and the other alleles were named in the cathode direction based on the relative migration distance.

Mendelian segregation had been tested in previous investigations for all the allele combinations (Torres de Castro 1989; Salvador 1997).

**Table 3** Descriptive parameters of the populations

	Sample size <sup>a</sup>	Mean no. of alleles per locus	Polymorphic loci <sup>b</sup>		n <sub>e</sub> <sup>c</sup>	$\delta_T$ <sup>d</sup>	H <sub>e</sub> <sup>e</sup>
			95%	99%			
MA	66.1	1.72 (0.18) <sup>f</sup>	50.00	55.56	1.180	0.192	0.179 (0.051)
GR	71.3	1.94 (0.21)	38.89	61.11	1.127	0.156	0.146 (0.049)
AL	73.4	1.72 (0.18)	44.44	55.56	1.118	0.149	0.139 (0.047)
AB	67.8	1.94 (0.17)	44.44	66.67	1.152	0.173	0.157 (0.046)
CR	69.1	1.56 (0.15)	33.33	50.00	1.099	0.135	0.126 (0.046)
V	61.2	2.06 (0.24)	44.44	66.67	1.157	0.177	0.168 (0.049)
CS	69.8	1.94 (0.25)	38.89	61.11	1.134	0.161	0.150 (0.049)
CU	62.6	1.83 (0.20)	38.89	55.56	1.121	0.152	0.141 (0.044)
VA	65.4	1.78 (0.17)	27.78	61.11	1.103	0.138	0.129 (0.047)
PRT	71.2	1.72 (0.14)	44.44	66.67	1.100	0.135	0.130 (0.042)
PO	71.4	1.72 (0.19)	27.78	55.56	1.081	0.121	0.113 (0.043)
LE	68.8	1.67 (0.18)	33.33	50.00	1.099	0.135	0.126 (0.046)
Mean	68.2	1.80	38.89	58.80	1.123	0.152	0.142

<sup>a</sup> Mean number of individuals with the different loci analysed<sup>b</sup> Percentage of polymorphic loci at the 99% and 95% levels<sup>c</sup> Effective number of alleles per locus<sup>d</sup>  $\delta_T$ , Total differentiation<sup>e</sup> H<sub>e</sub>, Mean expected heterozygosity<sup>f</sup> Standard error in brackets**Table 4** Total genetic diversity (H<sub>T</sub>), within- (H<sub>S</sub>) and between- (D<sub>ST</sub>)-population components, coefficient of genetic diversity (G<sub>ST</sub>) and number of migrants (Nm) obtained by Nei's method

Locus	H <sub>T</sub>	H <sub>S</sub>	D <sub>ST</sub>	G <sub>ST</sub>	Nm <sup>a</sup>
Aco	0.088	0.084	0.004	0.049	4.80
Dia	0.258	0.224	0.033	0.129	1.68
Got1	0.023	0.022	0.001	0.048	4.94
Got2	0.000	0.000	0.000	0.000	—
Hk	0.530	0.487	0.043	0.081	2.85
Idh1	0.067	0.063	0.004	0.063	3.74
Idh2	0.099	0.093	0.006	0.061	3.83
Lap1	0.129	0.123	0.006	0.046	5.16
Lap2	0.264	0.228	0.036	0.138	1.57
Lap3	0.000	0.000	0.000	0.000	—
6pgd1	0.288	0.278	0.010	0.035	6.88
6pgd2	0.000	0.000	0.000	0.000	—
6pgd3	0.014	0.013	0.001	0.046	5.22
Pgi1	0.002	0.002	0.000	0.011	21.54
Pgi2	0.265	0.248	0.017	0.066	3.56
Pgm	0.049	0.049	0.000	0.005	47.50
Skdh1	0.661	0.612	0.049	0.073	3.15
Skdh2	0.026	0.025	0.001	0.029	8.29
Average	0.153	0.142	0.012	0.076	3.02

<sup>a</sup> Nm = ((1/G<sub>ST</sub>) - 1) / 4

#### Analysis of genetic structure of populations

Different parameters were computed for each population: number and percentage of polymorphic loci, mean number of alleles per locus, effective number of alleles (n<sub>e</sub>), expected heterozygosity (H<sub>e</sub>) and total genetic differentiation ( $\delta_T$ ). Total genetic variability (H<sub>T</sub>) was separated into within-population (H<sub>S</sub>) and between-population (D<sub>ST</sub>) components, following postulations by Nei (1973). The coefficient of genetic diversity (G<sub>ST</sub>) and number of migrants (Nm) were derived from these parameters.

Nei's unbiased genetic distance (Nei 1978) and Cavalli-Sforza and Edwards' chord distance (Cavalli-Sforza and Edwards 1967) were computed, and a phylogenetic tree was obtained by the Wagner method. The Pearson's correlation coefficient between the genetic and geographic distance has been computed. The analyses were performed using BIOSYS-1 (Swofford and Selander 1993).

## Results

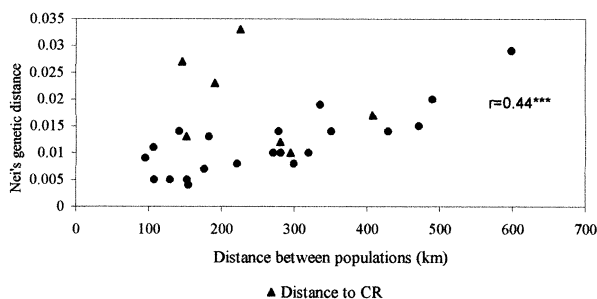
Eighteen loci were analysed from 10 enzyme systems (ACO, DIA, GOT, HK, IDH, LAP, 6PGD, PGI, PGM and SKDH), and 43 alleles were detected. Differences in

allele frequencies between populations were statistically significant for all the polymorphic loci.

The populations analysed display a high level of polymorphism (58.8 at the 99% level). The mean number of alleles is 1.80 (a value of 2.3 excluding the monomorphic loci) and the average effective number of alleles is 1.12. The mean expected heterozygosity is relatively high (0.142) and the total differentiation ( $\delta_T$ ) has a very similar value (0.152) because of the relatively high sample size. Table 3 shows the parameters of genetic variability for the populations of maritime pine studied. Large differences among populations exist with respect to the number of alleles, levels of polymorphism and heterozygosity. The highest values of these three parameters are found in the eastern and southern populations (V, AB, CS, CU and GR). A marginal population, CR, presents the lowest allelic polymorphism, followed by the northwestern populations (LE, PRT, PO and VA). The CR pine stand also presents the lowest number of alleles (28).

**Table 5** Nei's (1978) (below diagonal) and Cavalli-Sforza y Edwards' (1967) chord genetic distance (above diagonal) for the 12 *Pinus pinaster* populations analysed

	MA	GR	AL	AB	CR	V	CS	CU	VA	PRT	PO	LE
MA	*****	0.088	0.095	0.091	0.177	0.129	0.165	0.136	0.149	0.169	0.180	0.179
GR	0.007	*****	0.101	0.075	0.172	0.103	0.131	0.128	0.131	0.151	0.153	0.160
AL	0.010	0.009	*****	0.108	0.167	0.118	0.147	0.121	0.118	0.138	0.153	0.144
AB	0.010	0.004	0.011	*****	0.137	0.105	0.140	0.116	0.119	0.141	0.146	0.137
CR	0.033	0.027	0.023	0.013	*****	0.150	0.169	0.122	0.134	0.136	0.133	0.145
V	0.015	0.008	0.008	0.005	0.010	*****	0.0096	0.090	0.097	0.136	0.124	0.129
CS	0.029	0.014	0.014	0.014	0.017	0.005	*****	0.133	0.110	0.121	0.116	0.137
CU	0.020	0.019	0.010	0.013	0.012	0.005	0.014	*****	0.108	0.146	0.141	0.135
VA	0.029	0.015	0.013	0.013	0.011	0.004	0.004	0.010	*****	0.079	0.092	0.066
PRT	0.031	0.018	0.014	0.016	0.012	0.007	0.004	0.013	0.001	*****	0.081	0.106
PO	0.044	0.027	0.026	0.023	0.015	0.012	0.006	0.021	0.006	0.004	*****	0.120
LE	0.033	0.020	0.014	0.015	0.010	0.005	0.006	0.009	0.000	0.002	0.007	*****

**Fig. 1** Relationship between genetic and geographical distances for 8 populations of maritime pine in Spain ( $n=28$ ). The populations of the Atlantic group (PO, PRT, LE and VA) were excluded from this analysis

The population with the highest number of alleles is V (37), which is located in the centre of the area of distribution under study. The values decrease steadily from this place to the southern range, and rapidly to the western range of distribution. The presence of rare alleles is clearly related to geography, with the populations from eastern Spain having the most (CS and V with 4 alleles, AB and GR with 3). However, an isolated population from the Atlantic group (LE) also has a high number of rare alleles, in contrast to the other populations of the group.

Table 4 shows the resolution of the variability into among- and within-population components. The coefficient of genetic diversity depends on the loci analysed (a maximum of 0.138 for *Lap-2* and a minimum value of 0.005 of *Pgm*). The mean value ( $G_{ST}=0.077$ ) is consistent with that observed in conifers.

Table 5 shows the genetic distance according to Nei (1978) and Cavalli-Sforza and Edwards (1967). The greatest distance was found among the more distant populations, MA and PO (0.044). A linear relationship among geographic and genetic distance does not exist when all the populations are considered. However, if the Atlantic provenances are excluded, a clear relationship appears (Fig. 1) and three points clearly differ from the main line. These points correspond to the distances between the marginal population CR and the closer popula-

tions from southern Spain (AB, GR and AL), indicating an ancient isolation of this small stand and an important reduction in the population size.

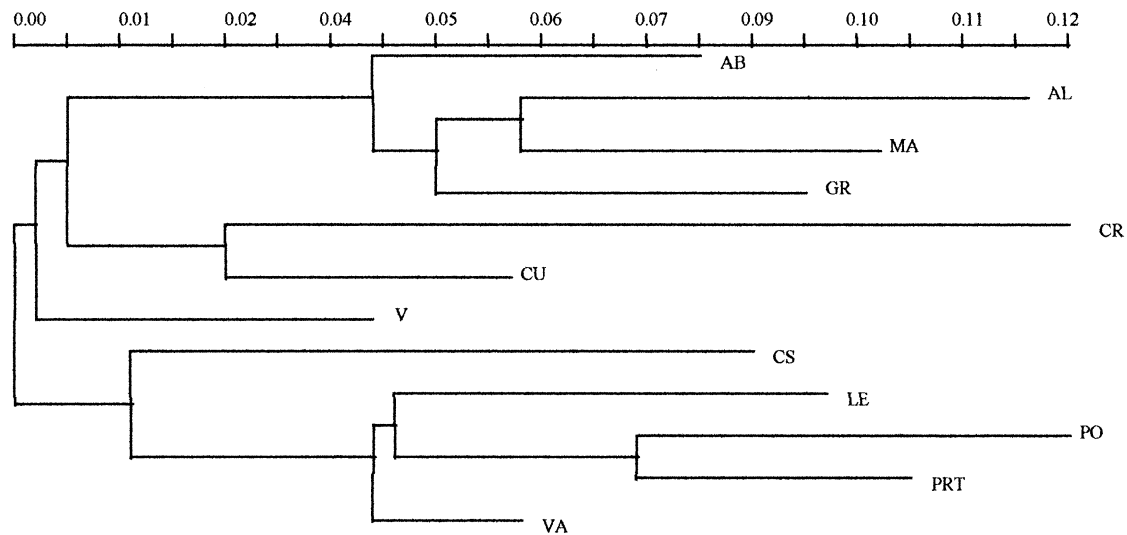
A geographic structuration is shown by the phylogenetic tree obtained by the Wagner method (Fig. 2), with a high cophenetic correlation (0.856). Three main groups could be distinguished: the first one with populations from southern Spain (AB, MA, GR y AL); the second one with populations from the Atlantic group (VA, LE, PO, PRT); and the rest of the analysed populations have an independent behaviour and were included in an Eastern group. The CS population is included in this last group based on the geographical proximity.

## Discussion and conclusion

The number of loci analysed (18) enabled us to obtain a clearer picture of the genetic variation in natural populations of *Pinus pinaster* by means of isozyme markers than that obtained in previous studies. The Iberian *Pinus pinaster* populations present a high level of polymorphism and heterozygosity ( $He=0.158$ ) compared to the genus *Pinus* ( $He=0.136$ , Hamrick et al. 1992).

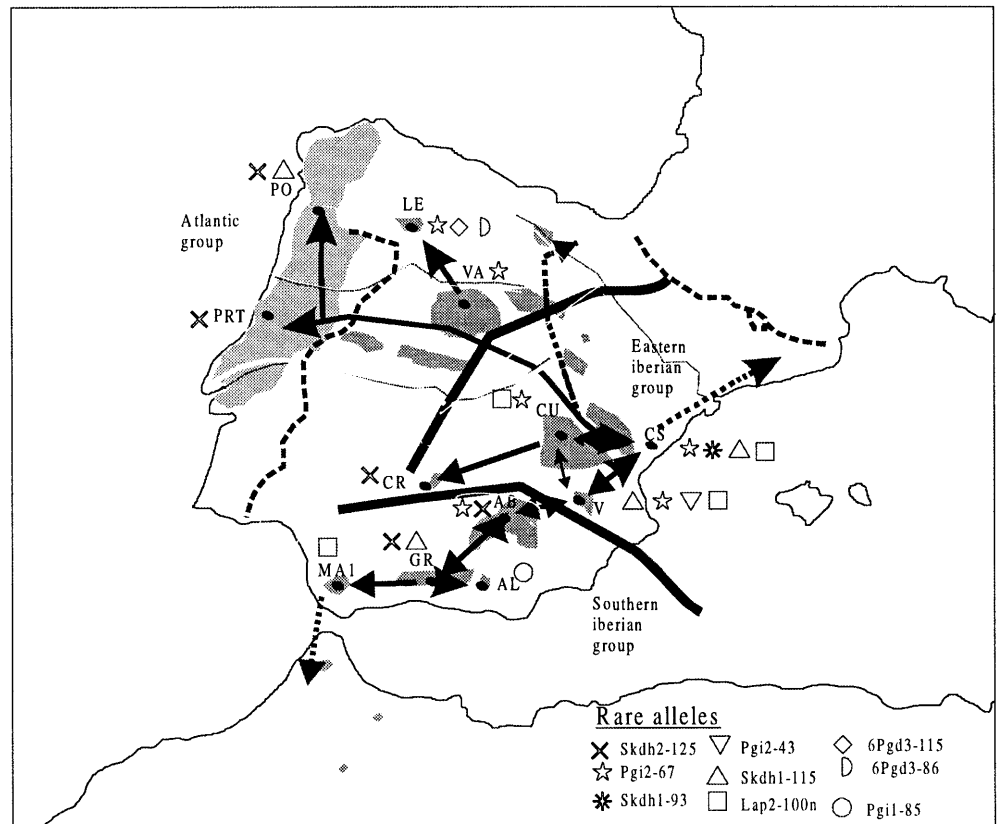
The coefficient of genetic differentiation is clearly higher than that of the Portuguese populations ( $G_{ST}=0.020$ ) (Torres de Castro 1989) and slightly higher ( $G_{ST}=0.077$ ) than the mean value for conifers (0.065) (Hamrick et al. 1992). Petit et al. (1995) mentioned a greater value for the entire range of the species using polymorphic and putative loci (0.161). In our case, there is not a clear relationship between total differentiation and the coefficient of differentiation, which could be explained by the large number of loci analysed. The gene flow is relatively high ( $N_m=2.99$ ), but 2 loci (*Lap-2*, *Dia*) have values close to 1.6, which indicates isolation among populations (Slatkin 1994), in this case from the southernmost populations of Spain with respect to the rest of the areas.

Some marginal populations (CR, PO and AL) could be defined as being relatively isolated from the main area of distribution of the species and showing a lower level of variability. These populations have different proper-



**Fig. 2** Phylogenetic tree using the Wagner method and the Cavalli-Sforza and Edwards' (1967) chord distance. The multiple addition criterion procedure of Swofford (1993) was used (Cophenetic correlation=0.862)

**Fig. 3** Rare alleles and migration pathways in three putative groups of maritime pine. *Solid lines* correspond to the populations actually studied, and *dotted lines* are hypothetical migrations



ties. Two of them (AL, CR) are small in size and isolated from the main line of migration of the species in Spain, as shown in Fig. 3. The existence of cave paintings depicting a *Pinus pinaster* tree within the region of the CR population dated approximately 5,000 years B.P. and palynological evidence in the area (Díaz-Fernández 1992) support the existence of refugia of the species in this area. The low level of variability in this population

is similar to that of the Atlantic ones. Since a direct connection with these populations does not exist, we can assume this result is due to the great reduction in the size of the CR stands that has occurred since the Middle Ages when the population was documented to be much greater (Gil 1991).

The geographic structuration of the phylogenetic tree, the existing linear relationship between geographic and



genetic distance in the eastern and southern provenances and the presence of rare alleles in the populations (Fig. 3) provide a clear picture of the genetic variation of the species in an area of study.

Our results are in general agreement with those of previous studies based on wider ranges of distribution. However, even when our data are consistent with the classification of the main groups based on various markers – terpenes, seed proteins, isozymes, etc. (Baradat and Marpeau 1989; Bahrman et al. 1994; Petit et al. 1995; Allona et al. 1996) – these earlier investigations allowed only differentiation among the populations but no clear geographical structure. Even in a recent work with chloroplast microsatellites (Vendramin et al. 1998) there is a lack of geographical structure in the results. With respect to this, it is worth while making some remarks.

Baradat and Marpeau (1988) stated that the origin of the species could be established in the southwest of the Iberian peninsula. Conversely, Vendramin et al. (1998) revealed a low level of haplotypic diversity in this area by means of chloroplast microsatellites. The Atlantic group is extremely homogeneous in terms of isozymes. This is due to a different pattern of recolonization and migration of the species after the glaciations in the western range where the species exist. Gullberg et al. (1985) showed that a low genetic differentiation exists in conifers that are at present in areas that were permanently covered by ice during glaciations. Although the Iberian peninsula was not covered by ice, the species still disappeared from most of the northern area. The population from northern Spain (PO) is located in one of the extremes of the migration to the western range of the species, close to the Portuguese population but displaying a much lower variability. This fact may be more easily linked to the strong decrease that occurred in this population before the beginnings of the Middle Ages rather than explained by an introduction from foreign seed sources, as other authors have postulated. The study of six populations in Portugal (Torres de Castro 1989) completes the sampling in this area, and his results are in accordance with those shown in this study if the same loci are considered. Comparing the two studies, we cannot support the idea of a refugium in Portugal since no special variants have been detected, in agreement with the conclusions of Vendramin et al. (1998). The earliest evidence of *Pinus pinaster* in Portugal, such as charcoal found in the Portuguese Estremadura (Figueiral 1995), is dated 33,000–22,000 years ago, which is very much prior to the Würm III. Thus, we suggest that *Pinus pinaster* disappeared from this entire area during the last glaciation.

The connection with the populations of the Landes (France) is difficult to explain via the northwestern Spanish provenances because of the low variability detected in this area. However, if we take into account the use of foreign seed sources during the last two centuries, the mating system of the species and the intense silvicultural management with low rotations, the high level of variability detected in Landes by means of chloroplast mi-

cro-satellites (Vendramin et al. 1998) is easier to understand. The phylogenetic tree shows a zone located in the Iberian mountain range (including V and CU populations) between the Atlantic and the Southern group of populations. The highest level of polymorphism is found in this area (V has the highest number of alleles) and in the southern group. The large genetic variability of the Iberian range populations may be related to a high ecological variability and the great genetic variation in growth and survival recorded in provenance tests (Alía et al. 1995). The ancient presence of maritime pine in this area is confirmed by palynological analysis from the south-eastern Iberian range (Valencia) dated 29,690 ± 560 years B.P. (Dupré 1988) and by fossils of the species dated 9,000–6,000 years B.P. detected in Soria, at the northwestern limit of this mountain area (García-Antón et al. 1995).

In discordance with the hypothesis of Vendramin et al. (1998), our results suggest the eastern Iberian group as the starting point of the recolonization process of the Atlantic group of populations. Destremau et al. (1982), following Rikly (1943), pointed out that a mountainous area in the Iberian peninsula, where the Mediterranean and Atlantic influences converge, was possibly the centre of expansion of the species.

The southern populations are clearly different from the eastern group, indicating some other refugia in this complete area. This aspect confirms the importance of southern Spain as a glacial refugium for different species, such as reported for *Pinus sylvestris* by Prus-Glowacki and Stephan (1994). The linear relationship between genetic and geographical distance clearly reflects a stepping-stone model of variation in the eastern and southern populations. The populations from Morocco (Sbay et al. 1995) do not express higher levels of diversity than those found in this study for the 7 loci analysed.

In conclusion, the data presented show the structuration of the genetic variability in the central area of distribution of *Pinus pinaster* and could contribute to the understanding of the migration processes of the species and supply information for the management of its genetic resources.

**Acknowledgements** Thanks given to Prof. C. Benito, who laboratory started this work, and Maite Seisdedos, who assisted in the laboratory work; Prof. Dr. C. Aragoncillo, who reviewed an early draft of this work; and Christina Bickart and José Climent, who corrected the English. This study has been funded by the Dirección General de Conservación de la Naturaleza.

## References

- Alía R, Gil L, Pardos JA (1995) Performance of 43 *Pinus pinaster* provenances on 5 locations in Central Spain. *Silvae Genet* 44:75–81
- Alía R, Martín S, De Miguel J, Galera R, Agúndez D, Gordo J, Salvador L, Catalán G, Gil L (1996) Las regiones de procedencia de *Pinus pinaster* Ait. DGCONA, Madrid
- Allona I, Saiz JA, Casado R, Aragoncillo C (1996) Megagametophyte salt-soluble proteins as genetic markers in *Pinus pinaster* Ait. *Silvae Genet* 45:21–24

- Bahrman N, Zivy M, Damerval C, Baradath Ph (1994) Organisation of the variability of abundant proteins in seven geographic origins of maritime pine. *Theor Appl Genet* 88:407–411
- Baradat Ph, Marpeau A (1988) Le Pin maritime *Pinus pinaster* Ait.. Biologie et génétique des terpènes pour la connaissance et l'amélioration de l'espèce. PhD thesis Université Bordeaux I, Bordeaux France
- Barbero M, Lisel R, Quezel P, Richardson DM, Romaine F (1998) Pines of the Mediterranean basin. In: Richardson DM (ed) *Ecology and biogeography of Pinus*. Cambridge University Press, Cambridge, UK, pp 153–170
- Brewer GJ, Sing CF (1970) An introduction to isozyme techniques. Academic Press, London
- Cavalli-Sforza LL, Edwards AWF (1967) Phylogenetic analysis models and estimation procedure. *Evolution* 21:550–570
- Conkle MT, Hodgkiss PD, Nunnally LB, Hunter SC (1982) Starch gel electrophoresis of conifer seeds: a laboratory manual. US Dep Agric For Serv Pac Southwest For Range Exp Stn Gen Tech Rep PSW-64
- Destremau DX, Alazard P, Chaperon H (1982) Monographic génétique de *Pinus pinaster*. *Ann For Zagreb* 9/4:125–150
- Díaz-Fernández PM (1992) Historia de la vegetación en Sierra Madrona y su entorno. Unpublished graduation thesis, Universidad Complutense de Madrid, Madrid, Spain
- Dupré M (1988) Palinología y Paleoambiente. Nuevos datos españoles. Referencias. Diputación Provincial de Valencia, Valencia, Spain
- Figueiral I (1995) Charcoal analysis and the history of *Pinus pinaster* (cluster pine) in Portugal. *Rev Palaeobot Palynol* 89:441–454
- García-Antón M, Franco F, Maldonado J, Morla C, Sainz H (1995) Una secuencia polínica en Quintana Redonda (Soria). Evolución holocena del tapiz vegetal en el Sistema Ibérico septentrional. *An Jard Bot Madrid* 52:87–195
- Gil L (1991) Consideraciones históricas sobre *Pinus pinaster* Aiton en el paisaje vegetal de la Península Ibérica. *Estud Geograf* 202:5–27
- Gil L, Gordo J, Alfá R, Catalán G, Pardos JA (1990) *Pinus pinaster* Ait. en el paisaje vegetal de la Península Iberica. *Ecologia* 1:469–495
- Gullberg U, Yazdani R, Ryman N (1985) Allozyme variation in Scots pine (*Pinus sylvestris* L.) in Sweden *Silvae Genet* 34:193–200
- Hamrick JL, Godt MJ, Sherman-Broyles SL (1992) Factors influencing levels of genetic diversity in woody plant species. *New For* 65–124
- Ledig FT (1992) Human impacts on genetic diversity in forest ecosystems. *Oikos* 63:87–108
- Nei M (1973) Analysis of gene diversity in subdivided populations. *Proc Natl Acad Sci USA* 70:3321–3323
- Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89:283–290
- Petit RJ (1988) Ressources genetiques du pin maritime: apport des marqueurs enzymatiques. DEA, Université de Paris Sud-Orsay, Paris, France
- Petit RJ, Bahrman N, Baradat Ph (1995) Comparison of genetic differentiation in maritime pine (*Pinus pinaster* Ait.) estimated using isozyme, total protein and terpenic loci. *Heredity* 75:382–389
- Prus-Glowacki W, Stephan BR (1994) Genetic variation of *Pinus sylvestris* from Spain in relation to other European populations. *Silvae Genet* 43:7–14
- Rikly M (1943) Das Pflanzenkleid der Mittelmeerländer, vol 1. Huber, Bern
- Salvador L (1997) Estudio de la variabilidad genética de *Pinus pinaster* en España usando marcadores proteicos e isoenzimáticos. PhD thesis, UPM ETSI Montes, Madrid
- Sbay H, El Alami SL, Arbez M (1995) Variabilité enzymatique de six provenances marocaines de pin maritime. *Ann Recherch For Maroc* 1994–95: 14–20
- Slatkin M (1994) Gene flow and population structure. In: Real L (ed) *Ecological genetics*. Princeton University Press, Princeton, N.J.
- Swofford DL, Selander BR (1993) BIOSYS-1. Release 1.7. Center for Biodiversity Illinois Natural History Survey
- Tanksley SD, Orton TJ (1983) Isozymes in plant genetics and breeding, part A. Elsevier Science Publ, Amsterdam
- Thirgood JV (1981) Man and the Mediterranean forest. Academic Press, London
- Torres de Castro LF (1989) Isoenzimas do *Pinus pinaster* Ait. numa perspectiva de aplicação ao melhoramento genético da espécie, Vila Real. Universidade de Trás os Montes e Alto Douro, Portugal
- Vendramin G, Anzidei G, Madaghiele A, Bucci G (1998) Distribution of genetic diversity in *Pinus pinaster* Ait. as revealed by chloroplast microsatellites. *Theor Appl Genet* 97:456–463
- Willis JK, Bennet KD, Birks HJD (1998) The late Quaternary dynamics of pines in Europe. In Richardson DM(ed) *Ecology and biogeography of Pinus*. Cambridge University Press, Cambridge, UK, pp 107–121