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Genetic diversity estimates of *Pinus pinaster* in the Iberian Peninsula: a comparison of allozymes and quantitative traits

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Abstract

The estimation of genetic diversity using molecular markers is a major component of genetic conservation programs. However, molecular data are only weakly correlated with adaptive variation, which seriously limits the value of molecular information for guiding conservation policies. In this paper, we used allozyme markers to analyse the distribution of gene diversity in the native range of *Pinus pinaster* Ait. in the Iberian Peninsula, including seven marginal populations close to the Mediterranean Basin. Then, the variability of three quantitative traits (total height, stem form and survival) was computed using data from a multisite provenance test in central Spain and the two data sets were compared. Within the general pattern of variation, marginal populations presented levels of diversity closely related to those of nearby central populations, clearly suggesting that historical factors were more important than actual population sizes in determining levels of observed diversity. A weak but nevertheless significant correlation between allozymes and quantitative variability was found in maritime pine. Environmental gradients, as measured by geographic variables, are suggested to have selective effects on quantitative traits and to influence effective population size, which might explain the weak correlation found between allozyme and adaptive variability.

Key words: molecular markers, common garden experiments, genetic variation, maritime pine, Mediterranean Basin.

Resumen

Diversidad genética de *Pinus pinaster* en la Península Ibérica: correlación entre isoenzimas y caracteres cuantitativos

Uno de los principales componentes de los programas de conservación genética es el uso de marcadores moleculares para la estimación de la diversidad genética. A pesar de ello, la variación observada en marcadores moleculares está débilmente correlacionada con la variación adaptativa, lo que limita seriamente la aplicación de estos estudios en el desarrollo de políticas de conservación. En este trabajo, se compara la distribución de la diversidad genética de *Pinus pinaster* Ait. en la Península Ibérica obtenida con marcadores moleculares (isoenzimas) y caracteres cuantitativos (altura, forma del fuste y supervivencia). El estudio incluye siete poblaciones marginales. Éstas, muestran valores de diversidad similares a los de poblaciones centrales próximas lo que sugiere una gran importancia de los procesos históricos regionales frente a efectos relacionados con el tamaño efectivo poblacional. La correlación de los marcadores moleculares utilizados con los caracteres cuantitativos medidos en los ensayos de campo es débil, aunque significativa. Esta correlación podría explicarse considerando efectos selectivos en respuesta a gradientes ambientales que afectan a su vez el tamaño efectivo de las poblaciones.

Palabras clave: marcadores moleculares, ensayos de campo, variación genética, pino negral, Cuenca Mediterránea.

Introduction

The amount and distribution of genetic diversity within and among populations in subdivided plant species are major concerns in conservation and breeding strategies (Young *et al*., 2000). In particular, within-population variation determines the ability of populations to evolve in response to environmental change and criteria based on within-population genetic diversity have been extensively used to define conservation policies for a given species (Petit *et al*., 1998). Forest species have long reproductive cycles, and large periods of time are required to perform field experiments with them. Thus, the use of molecular markers to estimate genetic variability has become common.

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Allozymes are widely used markers, and single-locus allozyme variation has been extensively studied in many temperate and tropical forest species (see Hamrick *et al*., 1992 and Loveless, 1992 for reviews). However, molecular marker data are generally weakly correlated with adaptive variation (Lynch, 1995; Hamann *et al*., 1998). The correlation between estimates of genetic diversity derived using neutral molecular markers and quantitative traits is usually poor, which limits the value of molecular data in conservation decision-making. The importance of adaptive traits in long-term conservation programmes has been highlighted in the framework of dynamic conservation strategies (Eriksson *et al*., 1993). In fact, given finite resources for conservation programs, it has been suggested that the priority is to conserve adaptive variation, that is, the genetic diversity that can respond to natural or artificial selection (Namkoong *et al*., 2000).

Maritime pine (*Pinus pinaster* Ait.), a widespread species in southwestern Europe that has been extensively planted outside its natural range, is an important forest species with high ecological and economic value. The discontinuity and high altitude of mountain ranges in southwestern Europe have led to the isolation of relatively close populations of the species. This factor, in combination with the ancient human impact in the Mediterranean basin, explains the current scattered natural distribution of the species. In addition, highly divergent ecological conditions are found across the range of *P. pinaster*, and large genetic differences among populations have been reported at regional and wide-range spatial scales using various genetic markers and common garden experiments (González Martínez *et al*., 2004 and references therein).

Within the range of *P. pinaster*, the Iberian Peninsula is one of the most important native areas. The location of various glacial refugia of maritime pine close to the Mediterranean basin, the complex genetic structure of the species in this region, and the existence of populations with well-documented native origins make this area ideal for the study of processes involved in the differentiation of the species. Analyses of quantitative traits and allozymes would be expected to show different distributions of genetic diversity, because different evolutionary forces influence these traits. Therefore, ideally both types of traits should be used to obtain estimates of genetic diversity for use in conservation programmes and sustainable forest management. Indeed, allozyme markers provide inferences about historical processes affecting gene diversity, whereas quantitative trait variation might reflect the potential of adaptation to changing environments.

In this paper, the estimates of genetic variation and the contribution of individual populations to the total diversity of maritime pine populations are analysed both for allozyme and quantitative traits, and implications of the results for the conservation of the species in the Iberian Peninsula are discussed.

Material and Methods

Plant material

A total of 32 populations were sampled from all over the range of *Pinus pinaster* Ait. in the Iberian Peninsula (Figure 1). The locations of the selected populations range from 36º 31' 05'' N to 42º 45' 04'' N latitude and from 0º 00' 46'' E to 9º 00' 00'' W longitude. A wide range of altitudes is also represented (from 200 to 1,500 m.a.s.l.). All the populations have been documented as having a native origin with the possible exception of Leiria (Alía *et al*., 1996; Gil, 1991). Leria population (Lr) is regarded as a classic example for the species in Portugal, where maritime pine covers more than one million hectares, and has been extensively used in population genetic studies. Thus, this population was also included in our study. Twenty-five populations were located in mainland areas, and the other seven were from restricted area provenances and are considered marginal populations in this study. The selection of populations covers 19 breeding areas of importance for breeding programs in Spain.

Allozyme analysis

For each population, 2-3 cones were collected from 80 trees separated from each other by at least 50 m. For 15 populations, 72-80 unordered megagametophytes (haploid tissue) per population were analysed, and for the other 17 populations ordered megagametophytes and embryos (diploid tissue) of 36-40 seeds were analysed. All 32 populations were used to estimate genetic differentiation among populations and the subset of 17 populations, where diploid tissue was also analysed, for detection of departures from Hardy-Weinberg proportions.

Horizontal starch-gel electrophoresis of nine enzymes encoded by 14 polymorphic loci was conducted. The

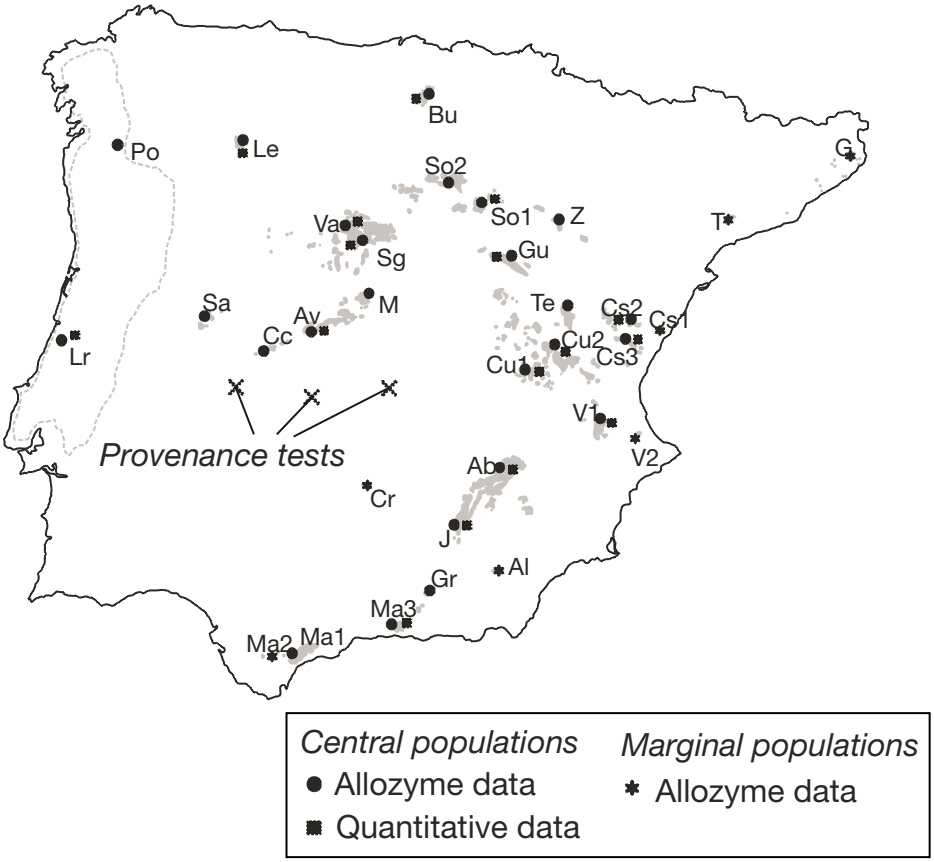

Figure 1. Populations of *Pinus pinaster* sampled in the Iberian Peninsula, and type of information available for each population. The shadowed area represents populations for which native origin has been documented; the dashed line encircles a region where populations have an uncertain origin due to extensive historical seed transfer among provenances.

enzyme systems and the loci scored were as follows: 6-phosphogluconate dehydrogenase (*6Pgd-1* and *6Pgd-2*; EC 1.1.1.44), isocitric dehydrogenase (*Idh*; EC 1.1.1.42), malic dehydrogenase (*Mdh-1*, *Mdh-2*, *Mdh-3* and *Mdh-4*; EC 1.1.1.37), phosphoglucose isomerase (*Pgi*; EC 5.3.1.9), acid phosphatase (*Acph*; EC 3.1.3.2), glutamate dehydrogenase (*Gdh*; EC 1.4.1.3), glutamate-oxaloacetate transaminase (*Got-1* and *Got-2*; EC 2.6.1.1), phosphoglucomutase (*Pgm*; EC 2.7.5.1) and leucine aminopeptidase (*Lap*; EC 3.4.11.1). Methods of enzyme extraction, staining procedures, and genetic interpretation have been described elsewhere (Conkle *et al*., 1982; Salvador, 1997). None of the loci differed significantly from the null hypothesis of neutrality in the Ewens-Watterson test (for details about this test see Hartl and Clark, 1997, pp. 298-300).

In each population, the molecular variation was studied by analysing single-locus genetic parameters, namely the percentage of polymorphic loci, gene diversity and allelic richness. Gene diversity (also known as expected heterozygosity), h, was calculated following Nei (1973). Allelic richness, i.e. the number of alleles found in a given population, is highly dependent on effective population size and it is more sensitive to past demographic changes than average heterozygosity (Nei *et al*., 1975). Consequently, the usefulness of this measure in conservation genetics has been emphazised by several authors (e.g., Petit *et al*., 1998). All 32 populations were used to compute θ, an unbiased estimate of Wright's F_{st} (Weir, 1990). A subset of 17 populations was used to calculate f, an inbreeding coefficient analogous to Wright's F_{is} (Weir, 1990). Bootstrap re-sampling over loci 10,000 times with replacement was used to estimate 95% confidence intervals. GDA software was used to conduct these analyses (Lewis and Zaykin, 2001). Finally, we tested whether there were differences in gene diversity and

allelic richness in marginal populations using a Wilcoxon signed-rank test for the difference between a given marginal population and the closest central one.

The contributions of each single population to total diversity (named *CT*) and allelic richness (named *CTR*) were computed as described by Petit *et al*. (1998). This method also splits the individual contributions of each population in two components, one related to its own diversity and another to its divergence. When contributions to allelic richness were calculated, the rarefaction method of Hurlbert (1971) was used to adjust for uneven population sample sizes (n = 72-80; rarefaction to 65). The analysis was computed using CONTRIB software (Rémy J. Petit, Laboratoire de Génétique et Amélioration des Arbres Forestiers, INRA, France; available at http://www.pierroton.inra.fr/genetics/labo/Software/Contrib/).

Quantitative traits

The quantitative data used in the analysis were measurements of height, survival and stem form (in a subjective scale from 1-good to 6-poor stem form) of trees aged 32 years in a multisite provenance test located in central Spain (covering three sites and 52 populations). The provenance field tests correspond to a Randomised Complete Block design (RCB) with four replicates and 16-tree plots. The experiments have been previously described in Alía *et al.* (1995, 1997, 2001). The multisite analysis of the selected populations follows a linear fixed model for the expected value of the general response y_{ijkl} (for height and stem form) of the l^{th} individual of the i^{th} population at the j^{th} location and k^{th} block:

$$y_{ijkl} = \mu + P_i + L_j + PL_{ij} + B(L)_{k(j)} + \varepsilon_{ijkl} \qquad [1]$$

where μ is some general mean, P and L represent the additive main effects of populations and locations, respectively, PL is the interaction effect, and B(L) is the block effect within location. The usual sum to zero constraints for identifiability of the parameters were employed, as well as the assumptions concerning constant variance, null covariances and Gaussian form of the distribution of y. Survivorship was evaluated by the percent of living trees in each plot, and the usual arcsine square-root transformation was applied. In this case, the model was simplified, and the B(L) term was not considered in the analysis.

Populations included in the analysis were a subset of 17 populations in which genetic diversity estimates obtained using molecular markers were available. Populations in both sets of data (allozyme and quantitative traits) were obtained from random samples of trees collected from the same areas and thus assumed to represent the same genetic source.

The objective of the analysis was to estimate (i) the within-population quantitative variation, and (ii) the contribution of each individual population to the total quantitative variability. Because provenance tests were selected to make the population by location interaction low, mean values for populations were used. The quantitative variability within a given population was estimated using the coefficient of variation of the phenotypic value. This value is an overestimation of the additive variance, and it can be used to estimate quantitative genetic variation within populations when no other data are available (Kremer, 1994). More accurate estimators of within-population quantitative variation, such as narrow-sense heritability (h^2), were not computed because they need combined provenance-progeny common garden experiments for their estimation, and these resources were not available for maritime pine at the moment of the study. For each trait, the contribution of each single population to the total quantitative genetic variability (*CTQ*) was computed as the contribution of the population, in percent, to the total sum of squares of the population factor.

These estimations could be biased by different factors. Firstly, we had to assume that the heritabilities of the traits under study were similar for the different populations (an assumption usually made in mult-provenance experiments; see, for instance, Kremer *et al.*, 1997). Secondly, the within-population estimates included genetic and microenvironmental factors that could not be separated. In our particular common garden experiments, the large sample size for each population (156 trees, distributed in 12 plots) may imply a similar level of microenvironmental variation in all the populations.

The relationship between quantitative and allozyme variation was analysed in two steps. First, populations were grouped according to allozyme genetic diversity using a Principal Component Analysis (two groups; see Results). Second, canonical analyses based on the previously defined groups were performed using quantitative genetic data (height, stem form and survival), and the performance of this discriminant procedure to differentiate among allozyme-defined groups, when using quantitative traits, was evaluated.

Results

Genetic diversity and allelic richness estimates

Maritime pine showed high levels of genetic diversity at the loci and populations analysed. The number of polymorphic loci, Nei's gene diversity and allelic richness (32 populations), and Weir's *f* (17 populations) are shown in Table 1. The average number of polymorphic loci was 55% and 38 alleles have been detected. The coefficient of inbreeding (*f*) showed a significant departure from Hardy-Weinberg proportions only in two cases: the marginal population V2, which displayed a slight excess of the heterozygous type (-0.089) and the central population Cs3, which showed

Table 1. Single-locus allozyme diversity, allelic richness, and Weir's estimator of the coefficient of inbreeding within populations, *f*, in *P. pinaster* populations from the Iberian Peninsula.

Code	Polymorphic loci (%)	Nei's gene diversity	Allelic richness	*f* (Low95, Up95)[a]
Central populations				
Po	40.00	0.063	21	—
Lr	53.33	0.086	23	—
Sa	53.33	0.087	24	—
Cc	46.67	0.075	22	0.062 (–0.082, 0.196)
Av	60.00	0.089	26	0.114 (–0.014, 0.304)
M	60.00	0.097	25	—
Sg	60.00	0.098	25	0.107 (–0.049, 0.206)
Va	60.00	0.088	25	—
Le	60.00	0.082	26	—
So1	66.67	0.094	25	0.018 (–0.096, 0.168)
So2	60.00	0.112	25	—
Bu	60.00	0.098	24	0.061 (–0.108, 0.260)
Z	60.00	0.089	26	0.120 (–0.059, 0.235)
Gu	53.33	0.097	24	0.105 (–0.088, 0.281)
Cu1	73.33	0.089	28	0.003 (–0.096, 0.142)
Cu2	53.33	0.112	25	—
Cs2	66.67	0.118	26	–0.025 (–0.069,0.008)
Cs3	53.33	0.110	23	0.103 (0.018, 0.180)
Te	60.00	0.122	27	0.015 (–0.063, 0.133)
V1	53.33	0.083	26	—
Gr	46.67	0.092	24	—
Ma3	53.33	0.097	26	–0.021 (–0.166, 0.146)
Ab	53.33	0.097	24	—
J	60.00	0.106	26	–0.018 (–0.056, 0.043)
Ma1	46.67	0.095	23	—
Mean	56.53	0.095	24.76	0.049 (–0.071, 0.177)
Marginal populations				
Ma2	66.67	0.110	27	–0.047 (–0.129, 0.079)
Al	46.67	0.073	22	—
Cr	46.67	0.080	22	—
V2	46.67	0.086	23	–0.089 (–0.118, –0.051)
Cs1	53.33	0.101	25	—
G	40.00	0.097	21	0.248 (–0.016, 0.399)
T	46.67	0.124	24	–0.047 (–0.131, 0.038)
Mean	49.52	0.096	23.42	0.016 (–0.099, 0.116)
Grand mean	55.00	0.095	24.47	0.042 (–0.078, 0.163)

[a] Low95 and Up95 are the lower and upper 95% confidence interval, respectively, computed using bootstrap re-sampling over loci (10,000 bootstraps).

a positive inbreeding coefficient (0.103). Population G had a high coefficient of inbreeding (0.248) but no statistical significance of this value has been found at the 95% confidence level. Differentiation among all 32 populations (θ= 0.073, CI: 0.043-0.102) is similar to that reported by Salvador *et al.* (2000). However, differentiation among populations was much lower (θ= 0.046, CI: 0.018-0.079) when marginal populations were removed from the analysis.

In central populations, Nei's genetic diversity and mean allelic richness was geographically structured (see Table 1). Genetic diversity was generally higher in eastern (Cu2, Cs2, Cs3 and Te) and southern (J) Iberia populations, and a reduction in genetic diversity from East (Bu = 0.098 and So2 = 0.112) to West (Po = 0.063 and Lr = 0.086) was found in the northwestern range of the species. Populations from the westernmost range also showed lower allelic diversity (21 alleles in Po and 23 in Lr) than the population average for mainland populations (24.76). With respect to private alleles, i.e. alleles characteristic of a particular region, three of them were found in southern Iberia: *Got-2* allele 3, *Mdh-1* allele 2 and *Pgm* allele 3, and only one in the northwestern area (Le population, *6Pgd-2* allele 3).

The gene diversity in marginal populations was not homogeneous. The highest values of diversity were found in two marginal populations near the Mediterranean coast in eastern and southern Iberia (T and Ma2, respectively). The other marginal populations showed a progressive reduction in diversity according to their distance from these high diversity spots. As expected, the pattern in allelic richness was a better indicator of marginality than the gene diversity in the Iberian maritime pine populations. With the exception of Ma2, the populations considered marginal in this study showed a reduction in allelic richness, irrespective of their level of gene diversity, when compared with the nearest central population. When removing population Ma2 from the dataset, the difference in allelic richness between central and marginal populations was significant ($p < 0.03$) as shown by a Wilcoxon signed-rank test. The reduction of allelic richness was marked in populations located at the edge of the range of the species in the Iberian Peninsula (G) and in inland populations of southeastern Spain (Cr and Al). In contrast, Ma2, a marginal population located close to the Mediterranean coast, showed high diversity and allelic richness, and one private allele (*6Pgd-1* allele 3).

Contributions of individual populations to diversity and allelic richness

The contributions of individual populations to total single-locus allozyme diversity are shown in Figure 2a. Nearly all the populations from the northwestern range of the species contributed negatively to total diversity. Contributions to total diversity can be negative, either because the diversity is lower than the mean diversity or because the population is not divergent from the rest (Petit *et al.*, 1998). In contrast, the populations from Eastern Iberia (Cu2, Cs2, Cs3 and Te) and some from the southernmost range of the species (J and Ma1) showed a high contribution to diversity. This was due mostly to the own diversity component, the divergence component of individual contributions to diversity being weak except in the case of Ma1. The results, based on the contributions to total allelic richness (Figure 2b) showed that positive values in the northwestern range of the species were generally based on the divergence component. This result highlights the presence in this area of populations with singular allelic compositions (especially Le and So2). In contrast, the positive contributions of eastern and southern populations were based on their own richness components.

Two marginal populations, G and T, contributed much more to the total diversity than the others, due to strong divergence in the case of G and to both divergence and its own diversity in T. Strikingly, the contributions of G and T (1.49% and 1.63%, respectively) were double the values found for any other population, including central ones. The patterns of allelic richness in these populations indicated a strong originality of allelic compositions in G (3.54%) and a moderate divergence contribution in T (0.51%).

Quantitative variability

A large degree of quantitative variability was found in maritime pine. The coefficient of variation, as a measure of quantitative variability for the different traits analysed, showed great differences among populations and traits. The quantitative variation within populations was higher for stem form (0.170, SD: 0.017) than for the other two traits surveyed: total height (0.159, SD: 0.025) and survival (0.127, SD: 0.029). No clear geographic structure of variability was found for any of the three quantitative traits,

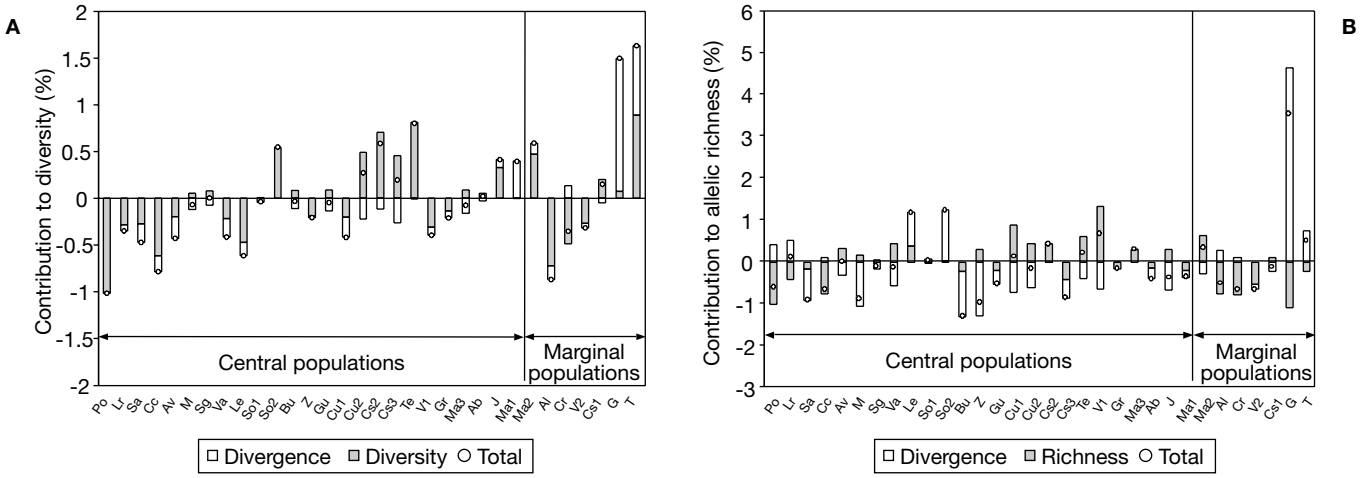

Figure 2. Individual contributions of 32 *Pinus pinaster* populations to global allozyme diversity (A) and global allelic richness (B). The contribution of each population is partitioned in two components: the contribution due to its own diversity/richness, and the contribution due to its own divergence.

although for height growth and stem form the highest values of variation were found in eastern and southern Iberia. In survival, the populations from eastern Iberia (Cs2, Cs3 and Cu2) had high variability (0.138, SD: 0.008) when compared with the rest of the range of the species (0.104, SD: 0.016 and 0.101, SD: 0.034, for the southern and northwestern ranges, respectively).

The contributions of individual populations to quantitative variability differed depending on the trait being considered (Figure 3), and we could not discern any clear geographical pattern. Local effects caused by factors affecting the general adaptability of individual populations seemed to have a strong influence. For survival, Lr and Cs3 showed the largest contributions.

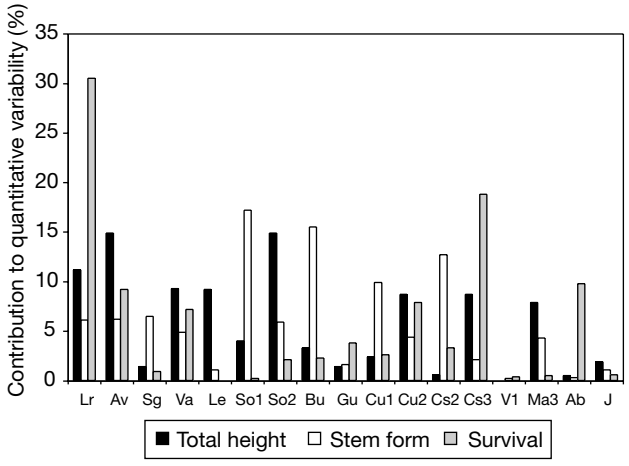

Figure 3. Individual contributions of 17 *Pinus pinaster* populations to the global variability in three quantitative traits (survival, total height and stem form) measured in a multisite provenance test in central Spain.

For total height variation, the most significant populations were Av, So2, and Lr. For stem form, the populations from So1, Bu and Cs2 gave the largest contributions. The results showed that, for quantitative traits, only a small number of populations gave large contributions to variability, and these populations differed depending on the trait. It is important to note the low contribution of some populations (namely V1, Gu and J) to total quantitative variability.

A Principal Component Analysis based on allozyme variation clearly separated northwestern maritime pine populations from the rest of the distribution of the species (data not shown). The canonical discriminant analysis computed using quantitative traits (height, stem form and survival) was able to distinguish between these two groups, revealing a weak geographical structure for quantitative traits. Indeed, the average canonical score was 0.551 and –0.735 for populations from the northwestern and the eastern/southern regions, respectively. Moreover, only two populations from the northwestern range of the species (Va and Le) overlapped (as shown by canonical scores) with eastern and southern Iberia populations.

Discussion

This paper presents new results concerning the amount and geographical distribution of both genetic diversity (estimated by allozyme data) and quantitative variability (estimated by the performance of different populations in field experiments) in maritime pine, an

important forest species extensively used for plantations in many parts of Europe, Australia, New Zealand and South Africa. The area of study is reportedly one of native origin, in which the species has been present since the last glaciations (Carrión *et al*., 2000). The importance of the area analyzed is emphazised by the strong genetic variation found here using different types of molecular markers (González Martínez *et al*., 2004 and references therein). This large degree of genetic variation is often associated with glacial refugia situated close to the Mediterranean basin. On the other hand, various adaptations of the populations to water stress, growth and pest resistance have also been reported among these populations (Tognetti *et al*., 2000; Guyon and Kremer, 1982; Alía *et al*., 1997; Harfouche *et al*., 1995).

Genetic diversity distribution, as estimated using allozymes, followed the general pattern previously described in the literature (Salvador *et al*., 2000; Ribeiro *et al*., 2001; Burban and Petit, 2003). Within this general pattern, the large number of populations included in this study allowed the identification of singular populations within regions previously described as homogenous (e.g., Le and So2 in the northwestern area). Furthermore, joint estimates of genetic variation, based on both molecular markers and quantitative variability, should improve the selection of populations to be included in conservation programs. For instance, two populations that were closely related according to allozyme data (Av and Bu) showed contrasting contributions to quantitative variability and their performance was extremely different in the multisite provenance test (data not shown).

Marginal populations presented levels of diversity closely related to those of nearby central populations, clearly reflecting the importance of historical factors in determining the current levels of diversity. Ellstrand and Elam (1993) pointed out that when there is no positive correlation between population size and genetic diversity, historical factors could have a stronger influence than the population size on genetic diversity. This is the case in maritime pine, as well as other typical Iberian species, such as *Quercus suber* L. (Jiménez *et al.*, 1999). The levels of genetic variability in quantitative traits of marginal populations are not well known. In the case of maritime pine, only a few results are available, concerning the different behaviour of this type of population in terms of growth and stem form, but the general adaptability of these populations has not usually been assessed in provenance tests.

Some populations within geographical groups that were homogenous according to allozymes differed widely in quantitative traits. This discrepancy could be explained by the fact that processes affecting the variation in these quantitative traits are related to natural selection and adaptation to ecological factors, whereas allozyme diversity is caused by historical factors (e.g., migration or genetic drift) that are more similar over large areas. Because the three traits under evaluation (height, stem form and survival) are significant for the adaptation of the species, they are likely to be severely affected by natural selection. Height growth is positively correlated to water use efficiency in maritime pine populations under controlled, mesic conditions (Tognetti *et al.*, 2000), and we could expect a larger variation for this trait among geographical regions. Stem form clearly differs between populations from flat and mountainous areas (Alía *et al.*, 1995). Survival is strongly related to drought tolerance and, therefore, Leiria (an Atlantic provenance assayed in Mediterranean conditions) gave the largest contribution to variation of all the populations. A study of the genotype per environment interaction for the species showed that rainfall of the place of origin was an important factor to explain the interaction in survival for this species (Alía *et al.*, 1997).

A weak but nevertheless significant correlation between allozyme diversity and quantitative variability was found in maritime pine. A slight concordance of morphological and allozymic variation has also been reported for other forest species with wide ranges [e.g., *Pseudotsuga menziesii* (Mirb.) Franco, El-Kassaby, 1982; *Picea abies* K., Lagerkrantz and Ryman, 1990; and *Alnus rubra* Bong., Hamann *et al.*, 1998]. It has been suggested that environmental gradients, as measured by geographic variables, may have selective effects on quantitative traits as well as influences on effective population size, explaining the weak correlation usually found among allozyme loci and quantitative traits (Hamann *et al.*, 1998). This last explanation seems plausible in maritime pine as a gradient in climatic conditions is present from south to north of the Iberian Peninsula. The lack of correlation among different quantitative traits indicates that there might be differences in the genetic control of the traits, and diverse environmental factors could differentially affect their fitness, promoting different geographical patterns of variation.

In conclusion, this paper presents new data concerning the diversity of the species, both in allozyme

and quantitative traits, and the contribution of the different populations to the total variability of maritime pine in the Iberian Peninsula. The main results, concerning the pattern of genetic variation with different types of data, and the role of marginal populations, provides useful information for the forest genetic resources conservation program of maritime pine. The results obtained from the provenance test at age 32 confirm the large genetic variation of the species and the different adaptability of the provenances to Mediterranean environments.

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