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Population genetic structure in a Mediterranean pine (*Pinus pinaster* Ait.): a comparison of allozyme markers and quantitative traits

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F-statistics were employed to analyse quantitative and allozyme variation among 19 native populations of maritime pine (*Pinus pinaster* Ait.). Fourteen polymorphic allozyme loci were used to provide an empirical basis for constructing a null hypothesis to test natural selection as a determinant of quantitative evolution in stem form, total height growth and survival at 30 years old. Hidden biases, that may result in a difference between quantitative (Q_{ST}) and allozyme (F_{ST}) differentiation which are not because of the action of natural selection, were avoided by comparing pairs of populations using linear models. All quantitative traits showed higher differentiation than allozymes. The highest divergence was found in stem form, whereas divergences in total height and survival were significantly lower. Differential adaptation to regional and local patterns of precipitation, temperature and soil type seem to be the best explanation of the different structure found in quantitative traits and allozyme loci. Possible bias in the estimation of Q_{ST} due to the level of quantitative within-population diversity and the role of adaptation of maritime pine after the last glaciation to highly diverse ecological conditions are discussed with special reference to the actual geographical structure of gene diversity in the species' native range.
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Introduction

Wright's fixation indices, or *F*-statistics, are the most widely used parameters to describe population structure. Various levels of population subdivision of molecular markers by means of *F*-statistics are known for a considerable number of species. Focusing on molecular markers has several limitations in providing information that could be useful to define conservation strategies (Lynch, 1995). Because the major aim of conservation genetics is to quantify and preserve the evolutionary potential of a species, optimal sampling of genetic diversity should include quantitative trait variability and co-adapted gene complexes (Petit *et al*, 2000). Sampling using molecular markers has been proposed as an indirect indicator of quantitative genetic variation (Marshall and Brown, 1975; Petit *et al*, 1998). However, Waldmann and Andersson (1998) indicate three major shortcomings of this approach: (1) the higher mutation rate of quantitative traits suggests shorter recovery times after a bottleneck; (2) when the non-additive variance is high, the expected loss of genetic additive variance caused by genetic drift follows a different pattern than the reduction in molecular marker heterozygosity; and (3) genetic variation is

expected to differ for both types of traits as a result of the action of different evolutionary forces.

The pattern of variation of quantitative traits is likely to be driven by environmental factors such as natural selection pressures (Lynch *et al*, 1999; Petit *et al*, 2000), whereas most molecular markers are considered effectively neutral. The combination of quantitative and molecular measures of variation allows one to test empirically hypotheses relating to different modes of phenotypic evolution in subdivided populations. Some recent papers dealing with *Drosophila* (Prout and Barker, 1993; Long and Singh, 1995), *Daphnia* (Spitze, 1993; Lynch *et al*, 1999), *Clarkia* (Podolsky and Holtsford, 1995), *Medicago* (Bonnin *et al*, 1996), *Scabiosa* (Waldmann and Andersson, 1998), *Arabidopsis* (Kuittinen *et al*, 1997), *Salix viminalis* (Lascoux *et al*, 1996), *Quercus petraea* (Kremer *et al*, 1997) and *Pinus contorta* ssp. *latifolia* (Yang *et al*, 1996) have reported joint estimates of quantitative and molecular traits by means of *F*-statistics. Lynch *et al* (1999) found a striking pattern when summing up information from such a great diversity of organisms. Because quantitative differentiation is always greater than or equal to molecular estimations, Lynch *et al* (1999) suggest that measures of subdivision at the level of molecular markers provide conservative estimates of genetic subdivision for quantitative traits (but see Petit *et al*, 2000). This pattern conforms to expectations if main evolutionary forces associated with molecular markers are gene flow, random genetic drift and mutation. Divergence of quantitative variation could be explained by local adaptation to

ecological variables. So, comparing *F*-statistics in molecular markers to those in quantitative traits seems to be a useful way of drawing conclusions on the role of natural selection in populations.

Estimates of genetic differentiation parameters for quantitative traits are not common in forest trees. The long-life cycle makes it difficult to perform complete genetic experiments using both populations and families to obtain estimates of within-population genetic variability. This introduces a bias in the literature towards organisms with short generation times (Ritland, 2000). However, common garden experiments (provenance tests) are usually range-wide established and could give valuable information on the genetic process of adaptation of trees to different environments, using some well-founded assumptions on within-population genetic variability (eg, Kremer *et al*, 1997).

Maritime pine (*Pinus pinaster* Ait.) is a wind-pollinated outcrossing conifer with a wide distribution in the Mediterranean basin, where it spreads all over the western range. The discontinuity and high altitude of mountain ranges in south-western Europe contribute to isolation of populations with relatively close neighbours. This fact, together with the ancient human impact in Mediterranean areas, explains the highly fragmented distribution of the species. Maritime pine grows in extremely variable ecological conditions, from calcareous to sandy soils and from Mediterranean to Atlantic climates. This results in several adaptations of the species in growth and survival (Alía *et al*, 1995). Studies of genetic variation have shown great population differences both with molecular markers (Baradat and Marpeau, 1988; Petit *et al*, 1995; Vendramin *et al*, 1998; Salvador *et al*, 2000; Ribeiro *et al*, 2001) and quantitative traits. A high level of genetic differentiation in adaptive traits has been reported in maritime pine for survival, adaptation to different climatic conditions, growth and growth phenology, resistance to insects and drought tolerance (Matziris, 1982; Guyon and Kremer, 1982; Kremer and Roussel, 1986; Alía *et al*, 1995, 1997; Harfouche *et al*, 1995).

The genetic variation of maritime pine has been described in 18 geographically structured races, in three main groups: Atlantic, circum-Mediterranean and Maghrebian (Baradat and Marpeau, 1988). The Iberian Peninsula could be considered the main pathway of the different groups, since all the three main groups are present, as well as eight of the geographical races. A latitudinal cline has been described in the Iberian Peninsula with an unclear geographical structure in the Atlantic region (Salvador *et al*, 2000; Ribeiro *et al*, 2001). This pattern has been explained as the result of a migration pathway from putative glacial refugia in mountain ranges near the Mediterranean basin (south-eastern Spain) to the Atlantic area of *P. pinaster*. The study of this area using both genetic markers and quantitative traits could clarify the role of selection in the adaptation of a long-lived species to Mediterranean climates after the last glaciation.

The aim of this work is to investigate the differences in population structure of allozyme markers (14 polymorphic loci) and three quantitative traits (total height, stem form and survival) in 19 native populations of maritime pine. The role of evolutionary forces in molecular and quantitative differentiation in this species is investigated to examine whether divergences among populations are caused by similar evolutionary processes.

Materials and methods

Study populations
A total of 19 populations of putative native origin were sampled basically over the western-most range of *P. pinaster* (Table 1 and Figure 1). Most populations ($n = 17$) were located in the Iberian Peninsula, where the species occurs both in the foothills of the main mountain ranges and in sandy areas either near the coast or on the plains of central Spain. Populations selected range from 33°52′ N to 42°45′ N latitude, and 9°10′ E to 8°45′ W longitude. A wide range of altitudes are also represented (from 200 to 1600 metres above sea level). Populations from all the main geographical groups defined by Baradat and Marpeau (1988) have been included in this work.

Allozyme markers
The material analysed was either 70–80 megagametophytes per population (seven populations) or megagametophytes and embryos of 35–40 seeds per population (12 populations). Diploid material (embryos) was analysed to detect departures from Hardy-Weinberg proportions. The cones were collected from trees separated from each other by at least 50 m. When individual collecting was not possible, random samples from a whole stand seed-lot were analysed (Tmj and Cg, see Figure 1). The trees sampled from each population for allozyme analysis were not necessarily the same as those for the quantitative genetic analysis. However, both sets of trees are random samples collected in the same zone and the bias from sampling error is considered negligible (Yang *et al*, 1996).

Horizontal starch-gel electrophoresis of nine enzymes encoded by 14 polymorphic loci was conducted. The enzyme systems and the loci scored were as follows: 6-phosphogluconate dehydrogenase (*6Pgd-1* and *6Pgd-2*; EC 1.1.1.44), isocitrate dehydrogenase (*Idh*; EC 1.1.1.42), malate 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). Allozyme methods, staining procedures and genetic interpretation have been described elsewhere (Salvador, 1997). All the loci included in this work were neutral when tested with Ewens-Watterson neutrality test. *F*-statistics were estimated for each locus and for overall loci following Weir (1990) and Yang *et al* (1996). Bootstrap re-sampling over loci 1000 times with replacement was used to estimate 95% confidence intervals.

Quantitative traits
Five field trials were planted in 1967 for the purpose of evaluating the performance of maritime pine provenances (see Alía *et al*, 1995, 1997, for a complete description of the experiment). Each plantation followed a randomised complete block design with 16-tree plots and four replications. Three quantitative traits with evolutionary significance were measured at age 30 in three experimental sites (Figure 1). The sites were selected for not having an overall significant genotype-by-environment interaction. The variables analysed were total height, survival and stem form. Survival was analysed in terms of percentage with respect to the experimental unit, using

Table 1 Geographical location of the studied *Pinus pinaster* populations

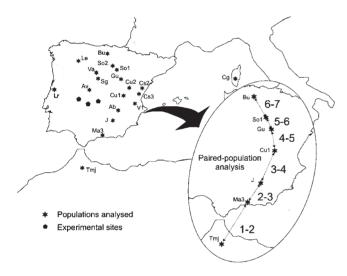| Population	Code	Latitude	Longitude	Altitude (m.a.s.l.)
Leiria	Lr	40° 00′ 00″ N	8° 45′ 00″ W	200
Arenas de San Pedro	Av	40° 11′ 19″ N	5° 07′ 13″ W	1000
Coca	Sg	41° 13′ 52″ N	4° 30′ 11″ W	795
Iscar	Va	41° 20′ 05″ N	4° 31′ 11″ W	760
Tabuyo del Monte	Le	42° 18′ 13″ N	6° 13′ 25″ W	1050
Quintana Redonda	So1	41° 35′ 56″ N	2° 35′ 56″ W	1050
San Leonardo	So2	41° 50′ 39″ N	3° 05′ 10″ W	1100
Oña	Bu	42° 45′ 04″ N	3° 31′ 10″ W	700
Mazarete	Gu	40° 55′ 35″ N	2° 10′ 30″ W	1100
Almódovar del Pinar	Cu1	39° 40′ 44″ N	1° 52′ 54″ W	1000
Boniches	Cu2	39° 59′ 18″ N	1° 39′ 03″ W	1075
Peñagolosa	Cs2	40° 15′ 04″ N	0° 21′ 11″ W	1400
Villamalur	Cs3	39° 57′ 51″ N	0° 25′ 25″ W	850
Cortes de Pallás	V1	39° 10′ 48″ N	0° 56′ 41″ W	900
Cómpeta	Ma3	36° 51′ 44″ N	3° 53′ 33″ W	1250
Riopar	Ab	38° 28′ 05″ N	2° 27′ 31″ W	1200
Cazorla	J	37° 55′ 05″ N	2° 55′ 11″ W	1150
Tamjout	Tmj	33° 52′ 15″ N	4° 02′ 40″ W	1600
Zonza	Cg	41° 44′ 15″ N	9° 10′ 35″ E	760

Figure 1 Location of *Pinus pinaster* populations and test sites used in the study of quantitative genetic variation. Numbers in the magnified area indicate the pairs of populations included in the paired-population models. The shadowed area represents the range of the species.

the angular transformation ($y^* = \arcsin (y)^{1/2}$). Stem form was measured on a subjective scale from 1 (slim trees) to 5 (crooked trees) according to stem straightness and branching. Analysis of variance was carried out using the following mixed model:

$$y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \gamma_{k(j)} + \epsilon_{ijk}$$

Where y_{ijk} is the phenotypic value of the kth block of the ith provenance at the site j; μ is the overall mean; α_i is the effect of the ith provenance; β_j is effect of the jth site; $\alpha\beta_{ij}$ is the interaction between the ith provenance and the jth site; $\gamma_{k(j)}$ is the effect of the kth block within the jth site; and ϵ_{ijk} is the experimental error. The variance components (V_α, $V_{\alpha\beta}$, V_ϵ) were estimated using the REML option of the VARCOMP procedure of SAS. When random mating ($F_{IS} = 0$) and linkage equilibrium are assumed, estimates of population genetic differentiation for quanti-

tative traits (Q_{ST}, following the notation of Spitze, 1993) based on partition of the total additive genetic variance into the among-population (σ_B^2) and the within-population (σ_w^2) components are as follows:

$$Q_{ST} = \frac{\sigma_B^2}{\sigma_B^2 + 2 \cdot \sigma_w^2} = \frac{\left(V_\alpha + \dfrac{V_{\alpha\beta}}{n}\right)}{\left(V_\alpha + \dfrac{V_{\alpha\beta}}{n}\right) + 2 \cdot (h^2 \cdot V_\epsilon)}$$

Where h^2 is the narrow-sense heritability and n is the number of experimental sites.

Inbreeding can inflate Q_{ST} even if absolute levels of among-population divergence are similar (Charlesworth *et al*, 1997; see Bonnin *et al*, 1996 Appendix, for a formulation of Q_{ST} under local inbreeding). Yang *et al* (1996) showed that estimates of Q_{ST} by partition of the additive genetic variance were conservative unless there was a very strong disassortative mating ($F_{IS} \rightarrow -1$). Outcrossing rates are high in pines usually exceeding 90% (Schemske and Lande, 1985). In our study, Hardy-Weinberg disequilibrium was only present four times over all populations (14 loci × 12 populations assayed) and in different allozyme loci (*Pgm* in Av, *Mdh-3* in Bu, *Acph* in Gu and *Pgi* in Ma3). The assumption of linkage equilibrium may not be true if severe inbreeding depression occurs even with Hardy-Weinberg proportions (Yang *et al*, 1996). When compared with other *Pinus* species, *P. pinaster* appears to be less affected by inbreeding. The evolutionary history of the species and its scattered distribution are supposed to reduce the genetic load in maritime pine (Durel *et al*, 1996). Therefore, Hardy-Weinberg and linkage equilibrium in maritime pine populations seem reasonable assumptions.

A major obstacle in Q_{ST} studies is the requirement of an experiment designed to estimate heritability. This introduces a bias towards easily reared organisms with short generation times, such as annual plants or fruit flies, being estimates of long-lived species scarce (Ritland, 2000). In our study, we first assumed a value of 0.2 for h^2, considering the narrow-sense heritabilities usually found in maritime pine (Costa and Durel, 1996; Kusnan-

dar *et al*, 1998), and bootstrap re-sampling was carried out at the level of individuals within a block to estimate 95% confidence intervals for Q_{ST} estimations (1000 samples). Then, a simulation procedure was conducted in order to evaluate the influence of heritability values (in the range 0–1) in the estimation of maritime pine quantitative differentiation (Q_{ST}).

Data analysis

The comparison of quantitative trait (Q_{ST}) and allozyme (F_{ST}) estimates of differentiation was made using the overall-loci estimate of allozymes as a null hypothesis to test quantitative differentiation divergences (Yang *et al*, 1996). To detect differences of means, a test based on the standard deviation confidence intervals for bootstrap samples was made (Manly, 1997).

Levels of differentiation in molecular markers are highly dependent on the diversity values of the loci assayed (Nei, 1987; Petit *et al*, 1995). Long and Singh (1995) showed that the choice of enzyme systems may result in a difference between quantitative and allozyme population subdivision, which is not because of the action of natural selection. To avoid this pitfall they proposed to compare pairs of populations. If quantitative and allozyme population subdivisions are not proportional over the pairs of populations compared, the action of natural selection can be inferred. The analysis of paired populations also provides information of differences in population subdivision that can be related to specific site characteristics. Seven populations situated along a south-north cline from Morocco (latitude 33°52′N) to north Spain (latitude 42°45′N) were investigated by means of differentiation estimates for adjacent populations (Figure 1). Values of F_{ST} and Q_{ST} were angular transformed ($y^* = $ arcsine $(y)^{1/2}$) to approach normality. In order to test the deviation of quantitative (Q_{ST}^*) and allozyme (F_{ST}^*) differentiation avoiding potential bias due to selection of molecular markers, the following linear model was fitted for each quantitative trait (Long and Singh, 1995):

$$Q_{ST}^*{}_{ijk} = \mu + \alpha_i + \alpha\beta_{ij} + \epsilon_{ijk}$$

Where α_i is the effect of allozyme F_{ST}^*, $\alpha\beta_{ij}$ measures the lack of fit from a model which assumes that the quantitative (Q_{ST}^*) and allozyme (F_{ST}^*) differentiation are proportional and ϵ_{ijk} is the error within a set based on 500 bootstrap replicates for each pair of populations compared.

Results

The overall estimates of genetic differentiation from 14 polymorphic allozyme loci (F_{ST}) and three quantitative traits (Q_{ST}), and the frequency distributions of 1000 bootstrap samples are shown in Table 2 and Figure 2, respectively. Judging from 95% bootstrap confidence intervals all quantitative Q_{ST} estimates were different from allozyme F_{ST}, ie the neutral expectation ($F_{ST} = 0.04795$). Tests of difference of means were highly significant ($P < 0.00$) for all the traits analysed. The highest divergences were found in stem form ($Q_{ST} = 0.97309$) and total height ($Q_{ST} = 0.79093$), survival being the lowest estimation ($Q_{ST} = 0.73229$). The differences between quantitative Q_{ST} and allozyme F_{ST} are still significant when heritability values in the range 0–1 are supposed (Figure 3). If non-

Table 2 Allozyme (F_{ST}) and quantitative trait (Q_{ST}) differentiation in 19 native populations of *Pinus pinaster*

Variable	Differentiation (F_{ST} or Q_{ST})	Standard deviation	Lower95[a]	Upper95[a]
Allozymes	0.04795	0.01458	0.02317	0.07202
Stem form	0.97309	0.01066	0.93215	0.96663
Total height	0.79093	0.03053	0.80203	0.89998
Survival	0.73229	0.05145	0.62469	0.79109

[a]Lower95 and Upper95 are the lower and upper 95% confidence interval for allozyme (F_{ST}) and quantitative trait (Q_{ST}) differentiation based on 1000 bootstrap samples, respectively.
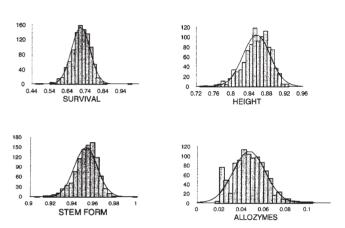
Figure 2 Frequency distributions of allozyme (F_{ST}) and quantitative trait (Q_{ST}) differentiation based on 1000 bootstrap samples.
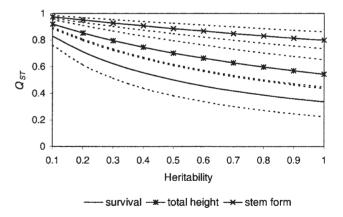
Figure 3 Estimated Q_{ST} in the 0–1 range of narrow-sense heritability. Dotted lines indicate 95% confidence intervals obtained from 1000 bootstrap replicates.

constant heritability values are assumed for each quantitative trait, Q_{ST} for stem form is always higher than Q_{ST} for survival and only equals Q_{ST} for total height when the difference in heritabilites for both traits is higher than 0.5, which is unlikely. In the case of survival and height, a difference in heritability of 0.3 would be enough to equalise quantitative differentiation estimations.
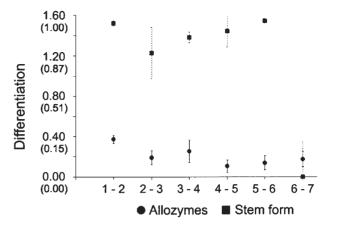
Models for paired comparisons, fitted in order to avoid potential bias due to the choice of molecular markers, are

shown in Table 3. The interaction effect ($\alpha\beta_{ij}$) reflects the lack of proportionality between allozyme ($F_{ST}*$) and quantitative ($Q_{ST}*$) differentiation. If the two measures of differentiation are proportional over the set of populations compared, the absence of selection acting on the quantitative trait is assumed and incremental effects are attributed to the different kind of data analysed (Long and Singh, 1995). Great differences in patterns of population subdivision were present for the traits analysed. Stem form and total height interaction effects were highly significant whereas survival $Q_{ST}*$ was proportional to allozyme $F_{ST}*$. Estimations of quantitative and allozyme differentiation, and bootstrap based standard deviations for each set of populations compared are shown in Figure 4. The highest divergences between paired comparisons corresponded to stem form whose pattern of population subdivision was only similar to allozymes between populations So1 and Bu (pair 6–7). The total height showed lesser divergences from allozymes than stem form, being similar in two sets: Cu1-Gu and So1-Bu (pairs 4–5 and 6–7, respectively). In contrast, survival $Q_{ST}*$ was proportional to allozymes F_{ST}, not showing interaction over the pairs of populations compared.
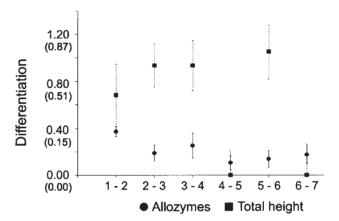
Discussion

Joint estimates of differentiation for life-history and growth-related traits and for nuclear marker loci over the past few years have shown two main patterns. Some species, such as *Daphnia pulex* (Lynch *et al*, 1999) or *Arabidopsis thaliana* (Kuittinen *et al*, 1997), have a quantitative population structure essentially identical to that for molecular markers, whereas species such as *Quercus petraea* (Kremer *et al*, 1997) or *Clarkia dudleyana* (Podolsky and Holtsford, 1995) are highly divergent. The same division is found in coniferous species. The difference between molecular (F_{ST}) and quantitative trait (Q_{ST}) differentiation is slight in *Abies concolor* and *Pinus palustris* (cited in Podolsky and Holtsford, 1995) and in *P. halepensis* (Aleppo pine) from the Iberian Peninsula (Alía *et al*, 2001). On the contrary, Yang *et al* (1996) found significant differences between allozyme ($F_{ST} = 0.019$) and quantitative genetic differentiation for specific gravity ($Q_{ST} = 0.133$), stem diameter ($Q_{ST} = 0.166$), stem height ($Q_{ST} = 0.195$) and branch length ($Q_{ST} = 0.161$) in *P. contorta* ssp. *latifolia*. In *P. pinaster* (this study), the degree of quantitative differentiation is even higher in all the traits analysed. Such differences are expected if molecular markers
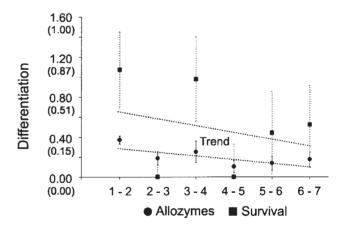

Figure 4 Estimations of allozyme ($F_{ST}*$) and quantitative ($Q_{ST}*$) differentiation and bootstrap-based standard deviations for each pair of populations compared. Codes of populations are shown in Figure 1. Numbers in parenthesis indicate a scale without angular transformation.

tend to be selectively neutral and the differentiation in quantitative traits are due to local adaptations (Lynch *et al*, 1999).

The great divergents found in this study between quantitative and allozyme differentiation supports the idea that maritime pine is a complex species with regional adaptations. Although extensive gene flow has been estimated in *P. pinaster* using molecular markers ($Nm = 3.02$; Salvador *et al*, 2000), high quantitative differ-

Table 3 Significance of the effects in the paired-population models

Model	Effect	DF	Sum of squares	P
Stem form	α_i	1	0.055	0.212
	$\alpha\beta_{ij}$	4	1.696	0.000
	ϵ_{ijk}	2994	105.525	–
Total height	α_i	1	0.112	0.141
	$\alpha\beta_{ij}$	4	1.037	0.000
	ϵ_{ijk}	2994	158.699	–
Survival	α_i	1	0.704	0.022
	$\alpha\beta_{ij}$	4	0.356	0.625
	ϵ_{ijk}	2994	404.132	–

entiation is detected in this study. Long and Singh (1995) and Lynch (1995) showed that in nature two populations can experience high gene flow and be different for a quantitative trait. As shown in other species (Bossart and Scriber, 1995), environmental heterogeneity and genotype-by-environment interaction seem to have a major role in quantitative differentiation when gene flow is high. These factors are especially important in maritime pine, in comparison to other Mediterranean coniferous species, such as Aleppo pine (Q_{ST} = 0.12 for height growth at four years; Alía *et al*, 2001), or Scots pine (*P. sylvestris*) from the Iberian Peninsula (Q_{ST} = 0.20 for height growth in provenance tests; Bastien and Alía, 2000). This result is consistent with quantitative analysis of the species and especially with those on the genotype-by-environment interaction in provenance tests (Alía *et al*, 1995, 1997). The importance of the interaction could be caused by the differential adaptability of the populations to the diverse ecological conditions where maritime pine can be found. Some important ecological factors (rainfall, altitude, soil type) explain most of the interaction present in this species (Alía *et al*, 1997). Another possible explanation is related to the levels of quantitative within-population diversity (h^2) used in the calculations. Relative measures of among-population divergence, such as Q_{ST}, are inherently dependent on the extent of within-population diversity. When low levels of diversity are found an inflated estimation of Q_{ST} is produced (Charlesworth, 1998). In the case of maritime pine, the divergences from the neutral F_{ST} estimations were significant, independent of the value of heritability adopted for obtaining Q_{ST}.

A better insight into the specific causes of the divergences observed for each trait is obtained when the wide-range estimations are combined with the paired-population analysis along the cline. Variation among traits in quantitative differentiation (Q_{ST}) appears to be associated with variation in within-population diversity (Lynch *et al*, 1999; total height and survival in this study, see Figure 3) but the action of different selective processes cannot be completely rejected (stem form in this study). In relation to stem form, great differences are found among populations of maritime pine. This species is usually described as a crooked tree. However, some of the populations have a straight stem (Corsica and several other mountain populations). Competition for light and resistance to snow and dominant winds have been described as the main causes of selection in trees for stem straightness. Differences between lowland and mountain populations in the paired-population model (pairs 3–4, 4–5, and 5–6 in Figure 4) reflect the influence of these strong selective factors. Selection in this species could act by means of the higher capacity of mountain races to reorientate the stem as has been shown in experiments of response of maritime pine families to phototropic stimuli (Sierra-de-Grado *et al*, 1997). Stem growth traits, such as total height, have been reported to be probably under selection in *P. contorta* ssp. *latifolia* because individuals must grow rapidly to escape suppression (Yang *et al*, 1996). *Pinus pinaster* grows in a great variety of sites and climatic conditions. Differential adaptation to regional and local patterns of precipitation, temperature and soil type seems to be the best explanation of the high divergence of this variable from non-selective expectations. In fact, the altitude of origin was correlated with the prov-

enances mean volume of wood and explained about a 20% of the interaction sum of squares in a factorial regression model that included environmental variables in order to examine the genotype-by-environment interaction in maritime pine (Alía *et al*, 1997). Quantitative differentiation in total height of pairs of populations along the cline only fails to show divergences in two pairs of populations (4–5 and 6–7). Both sets are located in the main post-glacial migration pathway of *P. pinaster* in the Iberian Peninsula (Salvador *et al*, 2000). Lagerkrantz and Ryman (1990) have shown that historical patterns can partly account for quantitative variation. A rapid spread from glacial refugia could explain the low amount of differentiation found among these pairs of populations.

With respect to survival, no selection acting in the populations is detected in the clinal model for paired comparisons. This means that the divergences found in molecular and quantitative differentiation for this trait could be only a consequence of the different type of data analysed. Directional selection to increase fitness has been proposed in natural populations (Lande, 1982; Spitze, 1993). Such a wide-range pattern of selection should reduce diversification among populations. The results of this work could conform to this expectation. An alternative explanation is derived from latitudinal environmental variation. Whereas allozyme variation along the cline is explained due to historical events related to migration pathways (Salvador *et al*, 2000; González-Martínez *et al*, 2001), the proportional genetic differentiation in survival could be explained as the selective pressure of parameters with latitudinal variation and not only as a result of non-selective forces. In the populations studied, mean temperature and precipitation range from 9.7°C to 14.0°C and from 550 mm to 985 mm, respectively. Since the field trials took place in Mediterranean areas, populations from humid areas (ie, badly adapted to drought) are expected to have lower survival than populations from xeric areas. In order to test this hypothesis, the correlation between Mahalanobis distance for survival and geographical distance for pairs of populations were calculated (Figure 5). The effect of altitude in climatic parameters could explain atypical cases
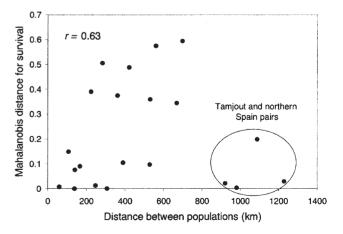

Figure 5 Correlation between Mahalanobis distance for survival and geographical distance among populations. The Pearson's correlation coefficient (*r*) when Tamjout (Tmj) and northern Spain pairs are excluded from the analysis (see further explanation in the text) is shown.

such as the similarity between Tamjout in Morocco (located at 1600 metres above sea level) and northern Spain populations (located at 700 metres above sea level). Taking this consideration into account, a latitudinal cline for survival was detected from south to north, which could reflect the latitudinal climatic variation in the studied range.

The pattern of variation previously described reflects the role of adaptation of maritime pine after the last glaciation to the highly diverse ecological conditions where it can survive. In the southern range of the species, isolation among populations has resulted in a different performance of adjacent populations in terms of survival, height growth and stem form. Description of the migration pathways of the species during the last glaciation (Salvador *et al*, 2000; Ribeiro *et al*, 2001) and the co-evolution with a specific pest, *Matsococcus feytaudi*, of maritime pine in this area (Burban *et al*, 1999) are in agreement with this hypothesis. In the northern range of the Iberian Peninsula, this adaptive process was not completed by the rapid spread of the species from its glacial refugia. Therefore, only local effects can be described.

In conclusion, this paper reflects the importance of selective factors to explain the main types of populations that have been previously described in the natural range of maritime pine. One of the main goals of conservation genetics is to use the information provided by genetic markers to implement conservation policies, and in particular to identify areas for on-site conservation. However, patterns of variation of molecular and quantitative traits are different in maritime pine and a knowledge of molecular marker information alone is insufficient for developing genetic resource conservation programmes in this species.

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