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Phenotypic plasticity and climatic adaptation in an Atlantic maritime pine breeding population

Raúl de la Mata · Jordi Voltas · Rafael Zas

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Abstract

- **Context** *Pinus pinaster* Ait. is found in the Iberian Peninsula under Mediterranean and Atlantic conditions. Both climates encounter each other in Galicia (NW Spain), where two bioclimatic regions can be differentiated: coastal and inland. A breeding program was launched in the coastal area, with two breeding and deployment areas delimited.
- **Aims** We analyse plasticity patterns across regions in a coastal breeding population to assess the suitability of current breeding areas and how genetic material will likely respond to future climate.
- **Methods** Total height at ages 3 and 8 years was assessed in 16 trials established along the coast and in inner Galicia. Clustering of environments with similar genotypic performance, family sensitivities to climatic factors and stability analyses were performed.
- **Results** Sizeable genetic variation in plasticity was found among families, and crossover genotype-by-environment

interactions were detected within and between regions. It was unfeasible to regionalize Galicia into alternative areas of stable genotypic performance. Only the cold regime was found to noticeably underlie the array of phenotypic responses to changing environmental conditions.

- **Conclusions** Results suggest that previous delimitation in two breeding areas is pointless and indicate reduced effects of a changing climate towards Mediterranean conditions on decreasing population fitness.

Keywords Genotype×environment interaction · Crossover interaction · Environmental stability · Phenotypic plasticity · Climate change · *Pinus pinaster*

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Contribution of the co-authors RM, JV and RZ conceived the paper; RM coordinated field assessments and processed the data; RM, JV and RZ performed the statistical analysis; RZ achieved the funds; RM coordinated the writing of the manuscript; RZ and JV helped in writing by subsequent revisions.

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1 Introduction

Two main bioclimatic regions can be distinguished in the Iberian Peninsula: Atlantic and Mediterranean. The Atlantic region, which occupies a narrow coastal strip in the north and the northwest (Fig. 1), is characterized by high and well-distributed precipitations and a narrow seasonal oscillation of temperature. In the Mediterranean region, precipitations are lower and concentrate in autumn and winter, summer drought being the most limiting factor for plant growth. In Galicia (NW Spain), both regions encounter each other, and the typical Atlantic coast climate is gradually transformed towards the interior, following a NW–SE gradient, into clear Mediterranean conditions (Fig. 1).

Local climate models in Galicia predict an increase in mean temperature coupled with an increasing frequency of extreme heat events in summer, milder winter cold extremes and important changes in rain distribution over the course of the year (Pérez-Muñuzuri et al. 2009). Climate in the coastal

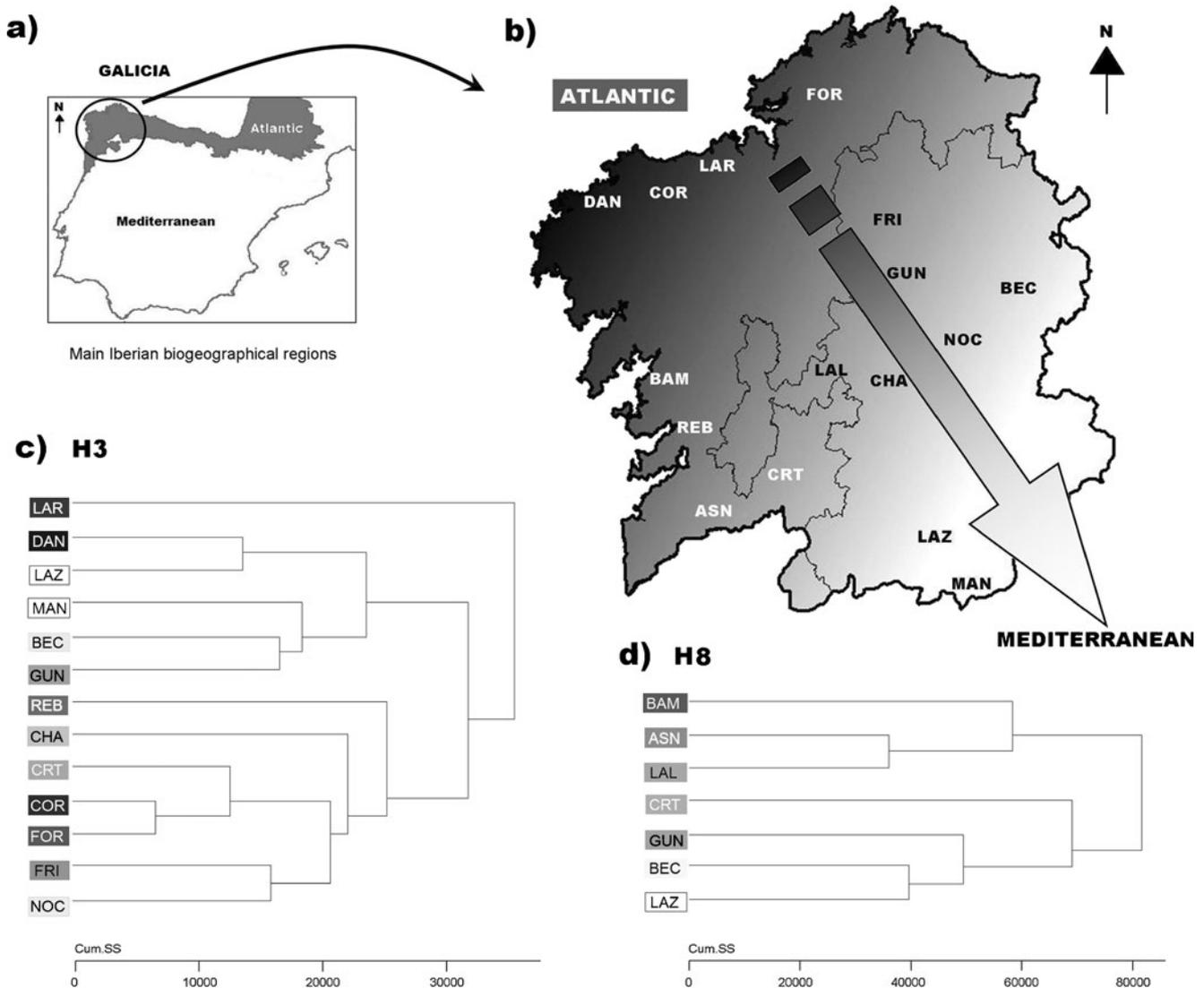


Fig. 1 **a** The two main biogeographical regions of the Iberian Peninsula: Atlantic (*grey shaded*) and Mediterranean (*white shaded*). **b** Location of the test sites across the NW–SE environmental gradient between Atlantic and Mediterranean conditions in Galicia. The *grey* pattern and the *arrow* illustrate the gradual increase of thermal oscillations and summer drought, and the decline in annual rainfall from the NW (*dark grey*) to the SE of the region (*light grey*). The *thin black line* denotes the boundary between the two bioclimatic areas currently operative for breeding and deployment

purposes. The 16 test sites are represented in the map by their respective codes (see Online Resource 1, 2 and 3). **c**, **d** Dendrograms representing the environmental cluster obtained by means of the CINTERACTION procedure for height growth at ages 3 and 8 years. Sites are *coloured* according to their location in the Atlantic–Mediterranean gradient, following a colour gradient from *dark* (true Atlantic climate) to *white* (strong Mediterranean influence)

Atlantic areas will, thus, likely evolve towards more Mediterranean conditions in the near future.

In the Iberian Peninsula, maritime pine (*Pinus pinaster* Ait.) is found both in the Atlantic and the Mediterranean regions, showing a wide array of adaptation patterns to contrasting environments. Indeed, populations have evolved to adapt to local environmental conditions (Fernandes and Rigolot 2007; González-Martínez et al. 2002; Tapias et al. 2004), leading to a high genetic differentiation among populations and a geographically highly structured intraspecific variation both in terms of quantitative traits and neutral

genetic variation (Bucci et al. 2007; González-Martínez et al. 2002). Among Mediterranean pines, *P. pinaster* has been shown to display high levels of plasticity, modulating its phenotype according to the environment where it grows (Chambel et al. 2007; Corcuera et al. 2010).

P. pinaster is particularly valuable in Galicia, where it is the dominant forest tree species in both bioclimates and also the one mostly used for reforestation. Previous studies have identified important differences in terms of growth patterns and genetic structure of *P. pinaster* stands originating from both bioclimatic regions (Alía et al. 1996; Álvarez-González

et al. 2005). While coastal stands show excellent growth and branch characteristics, and also satisfactory stem form, the stands of the inner region, mostly of unknown origin, have clearly inferior quality in relation to growth, stem form and branch habit (Alía et al. 1996). These differences have also been observed in common-garden tests (Molina 1965). The outstanding performance of the coastal stands has prompted the implementation of a classical breeding program aimed to supply genetically improved seeds for reforestation in the region. This program included phenotypic mass selection in coastal stands to make up a breeding population upon which three series of open-pollinated progeny trials were established both along the coast and in inner Galicia. This multi-environment trial (MET), which tests many genetics entries along the Atlantic–Mediterranean gradient, provides an excellent experimental device to assess how climate modulates phenotypes and whether this modulation is under genetic control. The analysis of the Genotype by Environment ($G \times E$) interaction should, thus, be valuable for understanding phenotypic responses of this Atlantic maritime pine breeding population to future climate change.

When studying $G \times E$ interaction, it is important to note that not all types of non-parallel responses have the same adaptive or breeding relevance. On the one hand, *crossover* interactions (COI), caused by changes in rank order for genotypes between environments, usually have large impacts in selection processes as the best genotypes may differ across environments (Hammer and Cooper 1996). On the other hand, *non-crossover* interactions, caused by heterogeneity of either genetic or residual variances, or both combined, are irrelevant for selection providing that the testing environments covers the range of environmental conditions where the species is prone to be used (Delacy et al. 1996). Former evaluations of the Atlantic *P. pinaster* breeding population of NW Spain, both in field trials located in the Atlantic zone (trial series 1995 as described in Online Resource 1, Zas et al. (2004)) and under controlled conditions in the greenhouse (analysing a subset of the breeding population under different water and nutrient availabilities, Zas and Fernández-López (2005)), showed that $G \times E$ interaction was sizeable, although most interaction could be attributed to a few highly interactive families (Zas et al. 2004). When analysed across bioclimatic regions (coastal and inland Galicia, trial series 2001 as described in Online Resource 1) crossover effects were observed both between and within regions, suggesting improper territorial division for stable genotypic performance (de la Mata and Zas 2010b). However, these findings demand further confirmation as they were based on a reduced number of field trials, and some of them suffered important sanitary problems.

This work takes advantage of the MET device established along the coast and in inner Galicia to (1) understand how climatic factors modulate the phenotype of different *P.*

pinaster genotypes from an Atlantic breeding population, (2) further assess the suitability of the current deployment areas for Galicia, and (3) seek, if necessary, for alternative geographic divisions that minimize the impact of $G \times E$ interaction for growth. To this end, we analysed 16 progeny trials corresponding to the whole experimental device including those nine trials belonging to previous series and seven new trials including all the 116 families of the breeding program. We used a two-stage analysis in which we first corrected the original data for spatial autocorrelation at the trial level, and then we investigated $G \times E$ interaction patterns using a restricted maximum likelihood (REML) approach implemented in mixed-model analysis (Searle et al. 1992) along with multivariate procedures.

2 Materials and methods

2.1 Genetic material, test sites and growth measurements

The genetic material consisted of 116 half-sib families obtained from plus trees replicated in a first generation clonal seed orchard (Sergude, Consellería de Medio Rural, Xunta de Galicia, 42.82° N, 8.45° W) that provides high-quality seed for reforestation in the coastal area of Galicia. Plus trees were individually selected in either natural or artificial stands located in the Atlantic region of Galicia according to their superior growth, stem form and branch habit.

Data were obtained from three series of trials installed in 1995, 2001 and 2005. A total of 16 individual trials were measured, with each trial including between 73 and 116 families of the whole collection, with an average of 20 individuals per family and site. Eight trials were established in the Atlantic region and eight trials in the inland region where Mediterranean influence is strong (Fig. 1). The number of common families between sites varied from 62 to 116 for sites of the same region and from 59 to 116 for sites of different regions. Site characteristics, experimental design, mean tree growth and mean mortality in each trial are shown in Online Resource 1, 2 and 3.

All trees from each trial (except those dead or badly suppressed) were measured for total height (H) from the root collar to the extreme of the apical bud with a pole in centimetres. Measurements were made at 3, 7 and 8 years after planting depending on the particular trial. Height at ages 7 and 8 years were considered equivalent (and referred to as H_8) since no relevant changes in family rankings are expected between both ages. Therefore, 3- (H_3) and about 8 (H_8)-year-old measurements were used for analysis. In four out of 16 trials, heights were measured at both ages; in nine trials, H_3 was only measured, and in the remaining three sites, H_8 was the only evaluation (Online Resource 3).

2.2 Statistical analyses

2.2.1 Spatial adjustments

Since spatial autocorrelation is a common concern in forest genetic trials (Magnussen 1990) and the available information belongs to different series of trials with different block designs, we first corrected the dependent variable for spatial autocorrelation at each site. Spatial adjustments were done using the iterative spatial analysis method (Zas 2006). This method uses geostatistics tools (variography and kriging) to model the spatial variation of the residuals of height data adjusted for genetic effects. Residuals are obtained assuming a complete randomised design and disregarding block effects to prevent the occurrence of artificial boundaries that might hinder the modelisation of the spatial structure. Then, the spatial model of the residual variation is used to adjust the original variable for its spatial autocorrelation (see details in Zas 2006). Spatially adjusted data from each trial were then jointly analysed to study the relevance and patterns of $G \times E$ interaction. Because the joint modelling of spatial variation and genetic variance–covariance structures across sites is computationally very demanding, this two-stage approach is an attractive alternative for the analysis of large data sets (Cappa et al. 2009; de la Mata and Zas 2010b).

2.2.2 Age-to-age correlation

The genetic correlation between ages 3 and 8 years was estimated using a mixed multivariate repeated measures analysis (Holland 2006) for those sites in which height was measured at both ages.

2.2.3 Relevance of $G \times E$ interaction

As a first assessment of the relevance of $G \times E$ interaction, we fitted a simple two-way linear mixed model for the spatially adjusted dataset:

$$Y_{ijk} = \mu + G_i + S_j + G \times S_{ij} + \varepsilon_{ijk} \quad (1)$$

where Y_{ijk} is the observation of the k th tree of the i th family in the j th site, μ is the overall mean, G_i is the random effect of the family i , S_j is the fixed effect of the site j , $G \times S_{ij}$ is the random effect of interaction between the family i and the site j , and ε_{ijk} is the random tree effect of the k th individual or error term. The within-site block effect was not included in the model since the dependent variable was already free of spatial correlation. The mixed model was fitted using the MIXED procedure of SAS (Littell et al. 2006), and variance components were estimated using the REML method. The fitted model also allowed for heterogeneity of error

variances in order to account for heterogeneous standard errors of genotype–environment means, as large differences in height growth were observed among sites.

2.2.4 Geographic patterns of $G \times E$ interaction

To determine whether $G \times E$ interaction was caused mainly by differential family performance across main climatic regions, we tested whether COI was relevant only at the regional level. To this end, we used a log-likelihood ratio test to compare the fit of a model with no restrictions in the family variance–covariance matrix (full model, unconstrained genetic correlations across sites) with a reduced model in which family covariances among sites of the same region are constrained to $R_G = 1$ (i.e. no family rank changes are allowed within regions) (de la Mata and Zas 2010b; Yang 2002). Both models are extensions of the more general model (Eq. 1) in which alternative variance–covariance structures are tested to accommodate heterogeneity of variances and covariances at the level of the random term $G \times S_{ij}$. Under the null hypothesis, the difference in minus two times the log-likelihood of the reduced and the full models is χ^2 -distributed, with degrees of freedom given by the difference in the number of covariance parameters of both models (Fry 2004; Yang 2002).

2.2.5 Environmental clustering

Because relevant COI occurred within regions (see “Results”), a method was implemented for seeking for clusters of sites minimizing $G \times E$ interaction at the within-group level while simultaneously maximising the dissimilarity between groups for $G \times E$ interaction. To this end, the CINTERACTION procedure of GenStat (Payne et al. 2006), which is based on the agglomerative hierarchical clustering method defined by Corsten and Denis (1990), was applied to the dataset. This procedure sequentially groups genotypes and environments in successive steps so that most of $G \times E$ interaction is explained between groups of genotypes and environments at the end of the merging process, minimising the residual $G \times E$ interaction. This analysis was done using family means per site for those families represented in all sites at each age (76 and 57 families at ages 3 and 8 years, respectively). The mean error variance across sites and its degrees of freedom were used as starting parameters of the procedure.

2.2.6 Relevance of environmental factors explaining $G \times E$ interaction

The exploration of environmental factors underlying $G \times E$ interaction was performed using factorial regression models (Denis 1988). Factorial regression analysis allows quantifying the relative merit of explicit environmental factors on

the explanation of $G \times E$ interaction. In particular, the amount of interaction between the genotype effect and an environmental covariate is related to the relevance of the genotypic variation in sensitivity (i.e. slope of response) to that explicit factor. Biologically, factorial regression models are informative on the extent by which differences across sites in environmental characteristics can explain differential genotypic performance. In other words, with these models, we can quantify the genetic variation in phenotypic plasticity attributable to specific environmental parameters.

In this study, the original $G \times S$ term in (Eq. 1) was replaced by single effects accounting for the random interaction of genotypes with specific environmental variables; a residual variance component was also estimated reflecting the variation that remained after each single explanatory source was removed from the original $G \times S$ term (Frensham et al. 1998):

$$Y_{ijk} = \mu + G_i + S_j + GxS_{ij} + GxCov_{ij} + \varepsilon_{ijk} \quad (2)$$

Where the terms are the same than in model (Eq. 1) except $G \times Cov_{ij}$, which refers to the random interaction of genotypes with a single environmental covariate and $G \times S_{ij}$ that now denotes the residual variance of the interaction. Models were fitted with the MIXED procedure of SAS.

The environmental factors considered included altitude, annual precipitation, summer precipitation (from June to August), the Gaussen Index as an indicator of the intensity of summer drought ($GI = \sum(2T - P)$ for each month where $2T > P$, where T is the mean temperature and P the monthly precipitation), annual mean temperature, number of frost days ($T_{\min} < 0^\circ\text{C}$) per year, absolute minimum and maximum temperature, and mean of daily minimum temperatures of the coldest month, and of the maximum temperatures of the warmest month. Climatic factors were obtained from the closest meteorological stations to each test site for the corresponding 3 or 8 years growth period.

2.2.7 Genotypic stability

We also quantified the particular contribution of each family to the $G \times E$ interaction. If the interaction is mainly caused by just a reduced group of interactive families, then its relevance can be minimised by removing these families from the breeding population. Shukla's stability variance (Shukla 1972) was chosen as a biologically intuitive method to estimate variability in family stability across environments. The model was implemented with the MIXED procedure of SAS using family means per site (Piepho 1999), including the maximum number of families that allowed model convergence (100 and 68 families at age 3 and 8 years, respectively). In Shukla's stability variance, a

separate variance (or genotypic stability) is assigned to each genotype. The model is fitted using a variance-covariance matrix with a banded main diagonal structure at the level of the random $G \times S$ term where the variances of the diagonal are interpreted as the environmental stability of each genotype (Piepho 1999). Larger stability variance values correspond to more interactive genotypes. Shukla's stability variance is linearly related to the classical ecovalence value (Wricke 1962), which quantifies the contribution of a genotype to the interaction sum of squares.

3 Results

Mean height growth was highly variable across sites, ranging from 78.2 to 155.4 cm at age 3 years and from 294.9 to 558.1 cm at age 8 years (Online Resource 3). On average, height growth was 21% and 38% higher in the coastal sites than in the inner sites at ages 3 and 8 years, respectively. Average mortality in the test sites was generally low and below the usual thresholds for forest plantations in Galicia (Online Resource 3). Several sites, however, showed abnormally high mortality due to the incidence of different unexpected problems such as insect herbivory (Daneiro), fungal diseases (Laracha), water logging conditions (Chantada) or high post-transplanting stress (Becerreá). Because the causes underlying these high mortality rates were beyond the ordinary environmental variation found among sites, survival was not further considered in the analyses.

Residuals after subtracting family effects revealed pronounced non-random spatial structures at both ages in all sites (Online Resource 4). Either spherical, exponential or linear theoretical semivariograms fitted well the residuals of each trial ($r^2 > 0.87$, $P < 0.001$). The percentage of total residual variation explained by the spatial pattern (intensity of the spatial dependence) varied from 22% to 59% at age 3 years and from 41% to 74% at age 8 years (Online Resource 4).

3.1 Geographical pattern of $G \times E$ interaction

The ratio of estimated variance components for $G \times S$ interaction and family effects ($\sigma_{G \times S}^2 / \sigma_G^2$) was larger than unity (1.34 and 1.11 at ages 3 and 8 years, respectively), indicating a high relevance of the interaction as compared with the genotype main effect. The genetic correlation between both ages was 0.708 ± 0.085 , reasonably high to assume that a 3-year assessment is representative of tree performance at age 8 years.

The model that assumes absence of COI within regions (i.e. assuming perfect genetic correlations, $R_G = 1$, between all pair of sites belonging to the same region) showed a

significantly worse goodness of fit (i.e. larger -2RLL in Table 1) than the full model with unrestricted genetic correlations, suggesting that COI existed both within and between bioclimatic regions (see also Online Resource 5). In agreement with this result, genetic correlations between sites from a particular bioclimatic region were not significantly larger than those involving sites of different regions ($F_{1,19}=3.35$, $P=0.083$ for age 3 years and $F_{1,76}=0.03$, $P=0.853$ for age 8 years; Table 2).

Overall, the clustering procedure tended to separate Atlantic from Mediterranean sites (Fig. 1), although both main groups also included sites from the alternative climatic region, and some few sites were classified independently from these main groups. Nevertheless, COI was still present within these optimal clusters, as models assuming absence of ranking change for genotypes between sites of the same cluster showed a significantly worse goodness of fit than the full, unconstrained model (Table 1). Therefore, grouping sites into regions with negligible $G \times E$ interaction was unfeasible.

3.2 Environmental factors underlying $G \times E$ interaction

Most factorial regression models showed a marginal or lack of significance for most environmental variables at age 3 years. The mean daily minimum temperature of the coldest month was the only parameter that was able to significantly reduce the original $G \times S$ term (a reduction of 8.8% decrease in variance component). At age 8 years, annual precipitation and annual mean temperature also played some role in the explanation of $G \times S$ interaction, but the mean daily minimum temperature of the coldest month was again the most relevant parameter underlying genotypic responses to changing environmental conditions (Table 3).

3.3 Stability analysis

Shukla's stability variances of families followed a unimodal but positively skewed distribution with a few families showing relatively high values (about two to three times larger than the average stability) (Fig. 2). Therefore, not all families contributed equally to the total $G \times E$ interaction. Sequentially removing the most interactive families from the analyses progressively reduced the relative importance of the interaction term, but too many families had to be removed to achieve a negligible interaction (Fig. 2).

4 Discussion

4.1 Genetic variation in phenotypic plasticity

The large variability in height growth among test sites is indicative of the relevance of plastic responses in *P. pinaster*. Tree performance was better in the Atlantic area, where the genotypes were originally selected and the environmental conditions are also more favourable for growth. The high ratio of $G \times E$ interaction to family variance components ($\sigma_{G \times S}^2 / \sigma_G^2$) suggests the presence of contrasting adaptation patterns within the Atlantic breeding population when tested across the Galician territory and confirms that genetic variation in plasticity does exist for this population. These results agreed with previous findings upon the same breeding population both within the Atlantic region (Zas et al. 2004) and across the two bioclimatic regions of Galicia (de la Mata and Zas 2010b). The existence of significant crossover interactions both within and between regions suggests a large impact of $G \times E$ interaction on achieving progress from selection.

Table 1 Log-likelihood ratios testing for crossover interactions appearing either within and across regions or only across regions

Model	AGE 3				AGE 8			
	-2RLL	LLR	DF ^a	$p > \chi^2$ ^b	-2RLL	LLR	DF ^a	$p > \chi^2$ ^b
Full	178980	–	–	–	137566	–	–	–
Atl-Medit	179144	163.6	36	<0.001	137640	74.5	11	<0.001
2 Groups	179088	108.1	36	<0.001	137629	63.5	9	<0.001
3 Groups	179085	104.9	31	<0.001	137610	44.2	6	<0.001

The table shows the restricted log-likelihood (*RLL*) value of the full model (unconstrained variance–covariance matrix) and of different reduced models in which genetic covariances across sites of the same region were constrained to unity. The corresponding log-likelihood ratio ($LLR = -2(RLL_{\text{reduced model}} - RLL_{\text{full model}})$), associated degrees of freedom (*DF*) and probability levels are presented. Sites were grouped according to the bioclimatic region (Atlantic or Mediterranean regions), or according to the optimal clustering obtained by the CINTERACTION procedure (see Fig. 1), assuming the first two levels of subdivision in two or three groups

^a LLR is distributed approximately as χ^2 with degrees of freedom given by the difference between the number of covariance parameter specifying the full model and the reduced model (Fry 2004)

^b Significance of χ^2 test for LLR. *P* values lower than 0.05 indicate that the reduced model fits significantly worse than the full model

Table 2 REML estimates of genetic correlations (and approximate standard errors within brackets) between pairs of sites for height growth at ages 3 and 8 years

Age 3 years		Mediterranean											
Atlantic		FOR	LAR	REB	BEC	CHA	FRI	GUN	LAZ	MAN	NOC		
COR	0.72±0.15 ***	1±0.00 ***	0.18±0.23	1±0.00 ***	-0.32±0.24	0.69±0.21 ***	0.79±0.24**	0.46±0.19 *	0.35±0.18	0.80±0.26 **	0.80±0.20 ***		
CRT	0.63±0.14 ***	0.75±0.14 ***	0.51±0.17 **	0.64±0.15 ***	0.28±0.19	0.58±0.17 ***	0.64±0.18 ***	0.40±0.15 **	0.46±0.13 ***	0.31±0.22	0.51±0.18 **		
DAN		0.43±0.21 *	0.43±0.21 *	0.01±0.22	0.63±0.19 ***	0.26±0.23	0.38±0.24	0.24±0.18	0.84±0.11 ***	0.60±0.24 *	-0.01±0.24		
FOR			0.78±0.19 ***	0.87±0.17 ***	0.25±0.23	0.78±0.20 ***	0.56±0.24 *	0.37±0.19 *	0.55±0.16 ***	0.31±0.27	0.86±0.19 ***		
LAR				0.34±0.23	0.41±0.23	0.64±0.21 **	0.30±0.25	0.62±0.16 ***	0.55±0.16 ***	0.25±0.27	0.14±0.25		
REB					0.34±0.23	1±0.00 ***	0.55±0.24 *	0.12±0.19	0.07±0.19	0.12±0.26	1±0.00 ***		
BEC						0.41±0.23	-0.12±0.26	0.59±0.17 ***	0.56±0.16 ***	0.40±0.26	0.03±0.25		
CHA							0.52±0.26 *	0.52±0.18 **	0.38±0.18 *	0.86±0.25 ***	0.74±0.21 ***		
FRI								0.21±0.21	0.22±0.20	0.46±0.29	0.78±0.24 **		
GUN									0.41±0.14 **	0.29±0.22	0.32±0.20		
LAZ										0.54±0.20 **	0.20±0.19		
MAN											0.09±0.29		
Age 8 years													
Atlantic		Mediterranean											
BAM													
ASN	0.67±0.12 ***												
BAM													
CRT													
BEC													
GUN													
LAL													

Test sites are grouped according to bioclimatic regions (Atlantic and Mediterranean). Bolded values denote correlations between pair of sites of the same region
 *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$ significance levels

Table 3 Relative merit of explicit climatic factors on the explanation of G×E interaction at age 8 years

Climatic variables	Simple model	Extended models		
	$\sigma_{G \times S}^2$	$\sigma_{G \times COV}^2$	$\sigma_{G \times S}^2$	Reduction in original $\sigma_{G \times S}^2$ (%)
	105.84 (13.64)			
Annual precipitation (mm)		4.30 E ⁻⁵ (1.40 E ⁻⁵)	93.09 (13.28)	12.05
Summer precipitation (mm)		4.99 E ⁻³ (3.10 E ⁻³)	102.35 (13.59)	3.30
Annual mean temperature (°C)		0.64 (0.12)	98.89 (13.25)	6.57
Average min temperature ^a (°C)		4.64 (1.47)	81.09 (12.90)	23.38

The variance component (and approximate standard errors within brackets) of the original G×S term was obtained from a simple two-way linear mixed model (Eq. 1 in “Materials and methods”). In the extended models (Eq. 2 in “Materials and methods”), the G×S term was replaced by a covariate term accounting for the interaction of genotypes with specific climatic variables plus a G×S residual reflecting the variation that remained after each single explanatory source was removed from the original G×S term. Only those factors explaining a relevant amount of the original G×S are presented

^a Mean of daily minimum of the month of lowest average

Remarkably, the relevance of G×E interaction in the studied breeding population seems higher than that observed in

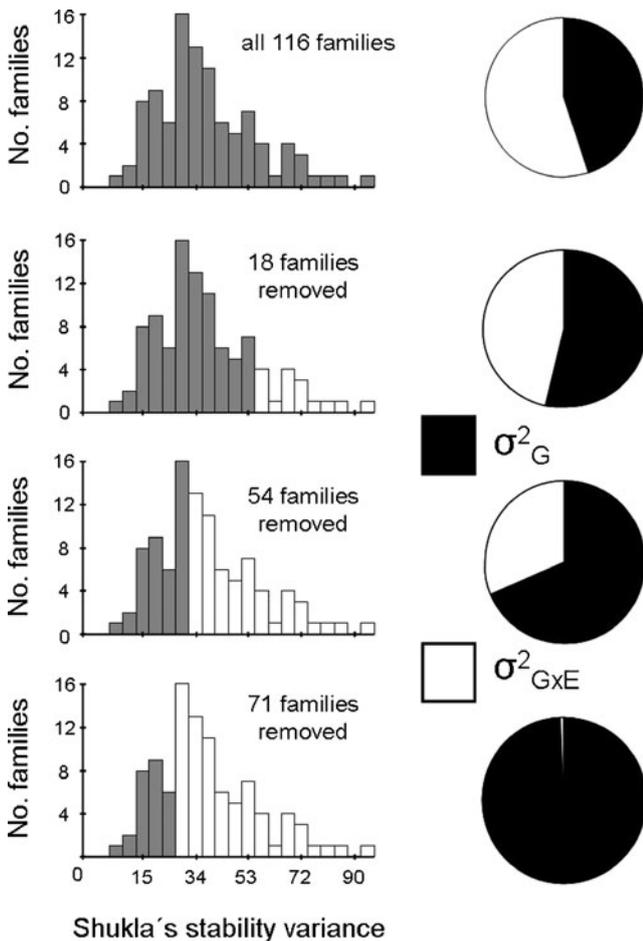


Fig. 2 Frequency histogram of Shukla's stability variance for height growth at age 3 years and magnitude of the G×E interaction in relation to genotypic variation as the most interactive families were sequentially eliminated from the analysis. The grey and white bars in the histograms represent families included and excluded, respectively

provenance MET data for this species (Guyon and Kremer 1982; de la Mata and Zas 2010a; Matziris 1982). This is somewhat surprising given the small size of the targeted area and the fact that genotypes were selected for the same phenotypic traits within a rather homogeneous region of origin (Atlantic Galicia). The family×site interaction variance has been shown, however, to be much higher than the provenance×site interaction variance in provenance MET analysis with family structure (Corcuera et al. 2010). In this regard, the sizeable amount of genetic variation in plasticity detected in the present study may have facilitated local adaptation of individuals to transitional climates. This possibility has also been suggested for *P. pinaster* Atlantic populations by other authors (Alía et al. 1997; Chambel et al. 2007).

It should be noted that the relatively early evaluation ages (3 and 8 years) may underlie the large impact of G×E interactions on height growth and, hence, may not completely reflect definitive adaptation patterns of the target population to different environments. Although the relative magnitude of G×E interaction (expressed as ratio of G×E to G variance components) was reduced from age 3 to 8 years, such reduction was nevertheless small, and genetic correlations between both ages were relatively high, suggesting a quite uniform genotypic performance across ages. Previous studies reporting age–age correlations in *P. pinaster* revealed that selection can be reasonably effective when trees are about 8 to 12 years old (Danjon 1994; Kremer 1992). In the present study, the assessment at ages 7–8 years can be thus considered quite effective for achieving a reliable understanding of adaptation patterns to local environmental conditions.

4.2 Geographical pattern of plastic responses

Both the estimation of genetic correlations across sites and the likelihood analysis on the relevance of crossover

interactions indicated that family performance varied as much within as between bioclimatic regions. We can conclude, thus, that there is not enough evidence to subdivide Galicia into the two currently operative deployment areas. An optimal clustering of sites minimizing $G \times E$ interaction at the within-group level provided no clear geographical pattern of site grouping. Although Atlantic and Mediterranean sites tended to group apart in the clustering history, this trend was spoiled by some sites that were not grouped as could be expected. These were, however, sites either with anomalous growth due to the incidence of pests (Daneiro) or diseases (Laracha) (see de la Mata and Zas 2010b), or located in the central part of Galicia relatively close to the boundary between the two deployment areas (Chantada, Cortegada, Friol, Lalín and Noceda). Altogether, these results suggest that there is not a clear delimited limit between both bioclimatic regions, but rather a diffuse gradient between coastal areas, with a mild Atlantic climate, and the inland zone, characterised by more extreme climatic conditions. Within each bioclimatic region, adjacent sites did not appear as the closer in the clustering history, evidencing the irregularity of this transitional gradient, probably originated by the strong geographic heterogeneity of the region, which also generates large environmental variability at intermediate scales (Martínez et al. 1999).

4.3 Variation in climate sensitivity

Factorial regression models pointed to the thermal regime, especially winter cold, as the climate factor better explaining the $G \times E$ interaction patterns for the studied breeding population. Therefore, regional differences in thermal regime probably underlie the observed divergence in phenotypic plasticity among genotypes. Contrary to what could be expected, our results indicate that both annual and summer rainfall play a reduced role on the explanation of adaptive patterns in the studied population. Overall, this population appears to be well adapted in terms of growth rate to the intensity and duration of drought events typically found in the Mediterranean region of Galicia. Therefore, the variation in precipitation patterns over the targeted regions does not seem to be responsible of changes in family ranking whereas the cold regime arises as key determinant of genetic variation in phenotypic plasticity in this Atlantic breeding population of maritime pine. The Landes provenance (SW France), another Atlantic origin present in non-water-limiting areas, has also shown a high tolerance to drought that has been attributed to adaptation to windy and sandy areas (Corcuera et al. 2010). The Galician coastal provenance could have experienced a similar process, and, thus the acquisition of some degree of drought tolerance would allow using this material in regions with relatively lower rainfall, but where winds and water soil retention are not so

limiting. By contrast, the results indicate a high relevance of the thermal regime as important driver of relative changes in performance among coastal breeding material. This observation agrees with results on young seedlings of less thermophilic species such as *Pinus sylvestris* (Sonesson and Eriksson 2000) or *Pseudotsuga menziesii* (Campbell and Sorensen 1978), but differs from the performance of other thermophilic pines such as *Pinus taeda*, for which rainfall regime was the factor that better explained the $G \times E$ interaction (e.g. Cannell et al. 1978; Waxler and van-Buijtenen 1981).

Local climate models in NW Spain predict an increase in temperatures, especially in summer, and important changes in seasonal precipitation distribution, with a progressive evolution towards more Mediterranean conditions (Pérez-Muñuzuri et al. 2009). Our results suggest, thus, a relatively low impact of future climate conditions on the relative performance of the Atlantic breeding population. Because little changes in extreme colds are expected, this population would be well-adapted to the predicted evolution towards conditions similar to the actual climate of inner Galicia.

4.4 Genetic variation in stability

The stability analysis showed that families varied largely in their sensitivity to changing environmental conditions, with some progenies being much more influenced than others. A few especially interactive families were therefore particularly responsible for $G \times E$ interaction effects. These results are consistent with those previously obtained for the same breeding population in the field (Zas et al. 2004) and under controlled conditions (Zas and Fernández-López 2005). Indeed, this behaviour is common to many other pines (e.g. McKeand et al. 2006; Sonesson and Eriksson 2000).

Removing unstable families is an alternative to reduce the impact of $G \times E$ interaction in breeding populations. Many authors have suggested considering the genotypic stability across sites as a screening trait in selection processes (e.g. Johnson and Burdon 1990). Selection based on stability parameters is also a safeguard decision regarding the current global change scenarios. Under this uncertain situation, selection for specific adaptation at present may lead more easily to future adaptation concerns in the deployed material (Ledig and Kitzmiller 1992). Removing the most interactive families indeed reduced $G \times E$ interaction, but achieving near-complete stability in the breeding population would require roguing up to 70% of the initial material. This would imply a too large reduction in genetic variability which is by far not the best decision from a sustainability point of view. Applying a low intensity selection for stability, that is, removing around one third of the most interactive families should be the option of choice as it may substantially reduce COI effects while probably keeping a sufficient genetic pool for future activities in the breeding program.

5 Conclusions

We found a sizeable $G \times E$ interaction for early height growth that suggests the presence of contrasting adaptation patterns among families in the studied maritime pine population. The large $G \times E$ interaction did not follow any clear geographical pattern, with COI effects appearing both within and across bioclimatic regions. Some climatic variables, however, significantly influenced the relative performance of the pine families. Particularly, winter cold rather than drought appeared to be the most relevant modulator of phenotypic plasticity, being the climatic factor that had a greater effect on changes in family rankings. Both the large genetic variation in phenotypic plasticity of the studied Atlantic population and its low sensitivity to summer drought suggest reduced effects of predicted climate change, so a good overall adaptation is expected to future Mediterranean-like climatic conditions in the region. Accordingly, the observed $G \times E$ patterns indicate that there is not enough evidence to subdivide Galicia into the two currently operative deployment areas for improved material.

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