

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

3

4 **Abstract**

- 5 • Context: *Pinus pinaster* Ait. is found in the Iberian Peninsula under
6 Mediterranean and Atlantic conditions. Both climates encounter each other in
7 Galicia (NW Spain), where two bioclimatic regions can be differentiated: coastal
8 and inland. A breeding program was launched in the coastal area, with two
9 breeding and deployment areas delimited.
- 10 • Aims: We analyse plasticity patterns across regions in a coastal breeding
11 population to assess the suitability of current breeding areas and how genetic
12 material will likely respond to future climate.
- 13 • Methods: Total height at age three and eight was assessed in 16 trials
14 established along the coast and in inner Galicia. Clustering of environments with
15 similar genotypic performance, family sensitivities to climatic factors and stability
16 analyses were performed.
- 17 • Results: Sizeable genetic variation in plasticity was found among families, and
18 crossover genotype-by-environment interactions were detected within and
19 between regions. Therefore, it was unfeasible to regionalize Galicia into
20 alternative areas of stable genotypic performance. Only the cold regime was found
21 to noticeably underlie the array of phenotypic responses to changing
22 environmental conditions.

23 • Conclusions: Results suggest that previous delimitation in two breeding areas is
24 pointless, and indicate reduced effects of a changing climate towards
25 Mediterranean conditions on decreasing population fitness.

26

27 **Keywords**

28 Genotype × environment interaction; crossover interaction; environmental
29 stability; phenotypic plasticity; climate change; *Pinus pinaster*

30

31 **1. Introduction**

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

41 Local climate models in Galicia predict an increase in mean temperature
42 coupled with an increasing frequency of extreme heat events in summer, milder
43 winter cold extremes and important changes in rain distribution over the course of
44 the year (Pérez-Muñuzuri et al. 2009). Climate in the coastal Atlantic areas will,
45 thus, likely evolve towards more Mediterranean conditions in the near future.

46 In the Iberian Peninsula maritime pine (*Pinus pinaster* Ait.) is found both
47 in the Atlantic and the Mediterranean regions, showing a wide array of adaptation
48 patterns to contrasting environments. Indeed, populations have evolved to adapt to
49 local environmental conditions (Fernandes and Rigolot 2007; González-Martínez
50 et al. 2002; Tapias et al. 2004), leading to a high genetic differentiation among
51 populations and a geographically highly-structured intraspecific variation both in
52 terms of Q_{st} (genetic differentiation of quantitative traits) and F_{st} (genetic
53 differentiation for genetic loci) values (Bucci et al. 2007; González-Martínez et al.

54 2002). *P. pinaster* also displays a high level of plasticity throughout its natural
55 geographical distribution in growth (Alia et al., 1995) and physiological
56 parameters (Corcuera et al., 2010), modulating its phenotype according to the
57 environment where it grows.

58 *P. pinaster* is particularly valuable in Galicia, where is the dominant forest
59 tree species in both bioclimates, and also the one mostly used for reforestation. In
60 situ measurements in coastal stands show excellent growth and branch
61 characteristics, and also satisfactory stem form, while the stands of the inner
62 region, mostly of unknown origin, have clearly inferior quality in relation to
63 growth, stem form and branch habit (Alía et al. 1996). The outstanding
64 performance of the coastal stands has prompted the implementation of a classical
65 breeding program aimed to supply genetically improved seeds for reforestation in
66 the region. This program included phenotypic mass selection in coastal stands to
67 make up a breeding population upon which three series of open-pollinated
68 progeny trials were established both along the coast and in inner Galicia. This
69 multienvironment trial (MET), which tests many genetics entries along the
70 Atlantic-Mediterranean gradient, provides an excellent experimental device to
71 assess how climate modulates phenotypes and whether this modulation is under
72 genetic control. The analysis of the Genotype by Environment (G×E) interaction
73 should, thus, be valuable for understanding phenotypic responses of this Atlantic
74 maritime pine breeding population to future climate change.

75 When studying G×E interaction, it is important to note that not all types of
76 non-parallel responses have the same adaptive or breeding relevance. On the one

77 hand, *crossover* interactions (COI), caused by changes in rank order for genotypes
78 between environments, usually have large impacts in selection processes as the
79 best genotypes may differ across environments (Hammer and Cooper 1996). On
80 the other hand, *non-crossover* interactions, caused by heterogeneity of either
81 genetic or residual variances, or both combined, are irrelevant for selection
82 (Delacy et al. 1996). Former evaluations of the Atlantic *P. pinaster* breeding
83 population of NW Spain, both in field trials located in the Atlantic zone (trial
84 series 1995 as described in Online Resource 1, Zas et al. 2004) and under
85 controlled conditions in the greenhouse (analyzing a subset of the breeding
86 population under different water and nutrient availabilities, Zas and Fernández-
87 López 2005), showed that G×E interaction was sizeable, although most interaction
88 could be attributed to a few highly interactive families (Zas et al. 2004). When
89 analyzed across bioclimatic regions (coastal and inland Galicia, trial series 2001
90 as described in Online Resource 1), crossover effects were observed both between
91 and within regions, suggesting improper territorial division for stable genotypic
92 performance (de la Mata and Zas 2010b). However, these findings demand further
93 confirmation as they were based on a reduced number of field trials, and some of
94 them suffered important sanitary problems.

95 This work takes advantage of the MET device established along the coast
96 and in inner Galicia to i) understand how climatic factors modulate the phenotype
97 of different *P. pinaster* genotypes from an Atlantic breeding population, ii) further
98 assess the suitability of the current deployment areas for Galicia, and iii) seek, if
99 necessary, for alternative geographic divisions that minimize the impact of G×E

100 interaction for growth. To this end, we analyzed 16 progeny trials corresponding
101 to the whole experimental device including those nine trials belonging to previous
102 series and seven new trials including all the 116 families of the breeding program,
103 using a two-stage analysis in which we first corrected the original data for spatial
104 autocorrelation at the trial level, and then we investigated G×E interaction patterns
105 using the REML (Restricted Maximum Likelihood) approach implemented in
106 mixed model analysis (Searle et al. 1992) along with multivariate procedures.

107

108 **2. Material and methods**

109 **2.1. Genetic material, test sites and growth measurements**

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

116 Data were obtained from three series of trials installed in 1995, 2001 and
117 2005. A total of 16 individual trials were measured, with each trial including
118 between 73 and 116 families of the whole collection, with an average of 20
119 individuals per family per site. Eight trials were established in the Atlantic region
120 and eight trials in the inland region where Mediterranean influence is strong (Fig.
121 1). The number of common families between sites varied from xx to xx for sites
122 of the same region and from xx to xx for sites of different regions. Site

123 characteristics, experimental design, mean tree growth and mean mortality in each
124 trial are shown in Online Resource 1.

125 All trees from each trial (except those dead or badly suppressed) were
126 measured for total height (H) from the root collar to the extreme of the apical bud
127 with a pole in centimetres. Measurements were made at 3, 7 and 8 years after
128 planting depending on the particular trial. Height at ages 7 and 8 were considered
129 equivalent (and referred to as H8) since no relevant changes in family rankings
130 are expected between both ages. Therefore, three (H3) and about eight (H8) year
131 old measurements were used for analysis. In four out of 16 trials, heights were
132 measured at both ages; in nine trials only H3 was measured and in the remaining
133 three sites H8 was the only evaluation (Online Resource 1).

134

135 **2.2. Statistical analyses**

136 ***Spatial adjustments***

137 Since spatial autocorrelation is a common concern in forest genetic trials
138 (Magnussen 1990) and the available information belongs to different series of
139 trials with different block designs, we first corrected the dependent variable for
140 spatial autocorrelation at each site. Spatial adjustments were done using the
141 Iterative Spatial Analysis method (ISA) (Zas 2006). This method uses
142 geostatistics tools (variography and kriging) to model the spatial variation of the
143 residuals of height data adjusted for genetic effects, considering a completely
144 randomized design and disregarding block effects to prevent the occurrence of
145 artificial boundaries that might hinder modelling the spatial structure. Then, this

146 spatial model is used to adjust the original variable for its spatial autocorrelation
147 (see details in Zas 2006). Spatially-adjusted data from each trial were then jointly
148 analyzed to study the relevance and patterns of G×E interaction. Because the joint
149 modelling of spatial variation and genetic variance-covariance structures across
150 sites is computationally very demanding, this two-stage approach is an attractive
151 alternative for the analysis of large data sets (Cappa et al. 2009; de la Mata and
152 Zas 2010b).

153

154 *Age to age correlation*

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

158

159 *Relevance of G×E interaction*

160 As a first assessment of the relevance of G×E interaction we fitted a
161 simple two-way linear mixed model for the spatially-adjusted dataset:

162

$$163 \quad Y_{ijk} = \mu + G_i + S_j + G \times S_{ij} + \varepsilon_{ijk} \quad [1]$$

164

165 where Y_{ijk} is the observation of the k th tree of the i th family in the j th site, μ is the
166 overall mean, G_i is the random effect of the family i , S_j is the fixed effect of the
167 site j , $G \times S_{ij}$ is the random effect of interaction between the family i and the site j ,
168 and ε_{ijk} is the random tree effect of the k th individual or error term. The within-site

169 block effect was not included in the model since the dependent variable was
170 already free of spatial correlation. The mixed model was fitted using the MIXED
171 procedure of SAS (Littell et al. 2006), and variance components were estimated
172 using the REML method. As large differences in height growth were obtained
173 across sites, the fitted model also allowed for heterogeneity of residual variances,
174 obtaining a different value for each site.

175

176 ***Geographic patterns of G×E interaction***

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

191

192 ***Environmental clustering***

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

206

207 ***Relevance of environmental factors explaining G×E interaction***

208 The exploration of environmental factors underlying G×E interaction was
209 performed using factorial regression models (Denis 1988). Factorial regression
210 analysis allows quantifying the relative merit of explicit environmental factors on
211 the explanation of G×E interaction. In particular, the amount of interaction
212 between the genotype effect and an environmental covariate is related to the
213 relevance of the genotypic variation in sensitivity (i.e. slope of response) to that
214 explicit factor. Biologically, factorial regression models are informative on the

215 extent by which differences across sites in environmental characteristics can
216 explain differential genotypic performance. In other words, with these models we
217 can quantify the genetic variation in phenotypic plasticity attributable to specific
218 environmental parameters.

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

224

$$225 \quad Y_{ijk} = \mu + G_i + S_j + GxS_{ij} + GxCov_{ij} + \varepsilon_{ijk} \quad [2]$$

226

227 where the terms are the same than in model [1] except GxS_{ij} that performs as the
228 residual variance of the interaction and $GxCov_{ij}$ as the random interaction of
229 genotypes with a single environmental covariate. Models were fitted with the
230 MIXED procedure of SAS.

231 The environmental factors considered included altitude, annual
232 precipitation, summer precipitation (from June to August), the Gausson Index as
233 an indicator of the intensity of summer drought ($GI = \Sigma(2T-P)$ for each month
234 where $2T > P$, where T is the mean temperature and P the monthly precipitation),
235 annual mean temperature, number of frost days ($T_{\min} < 0 \text{ } ^\circ\text{C}$) per year, absolute
236 minimum and maximum temperature, and mean of daily minimum temperatures
237 of the coldest month, and of the maximum temperatures of the warmest month.

238 Climatic factors were obtained from the closest meteorological stations to each
239 test site for the corresponding 3 or 8 years growth period.

240

241 *Genotypic stability*

242 We also quantified the particular contribution of each family to the G×E
243 interaction. If the interaction is mainly caused by just a reduced group of
244 interactive families, then its relevance can be minimized by removing these
245 families from the breeding population. Shukla's stability variance (Shukla 1972)
246 was chosen as a biologically intuitive method to estimate variability in family
247 stability across environments. The model was implemented with the MIXED
248 procedure of SAS using family means per site (Piepho 1999) and including the
249 maximum number of families that allowed model convergence (100 and 68
250 families at age 3 and 8 respectively). In Shukla's stability variance, a separate
251 variance (or genotypic stability) is assigned to each genotype. The model is fitted
252 using a variance-covariance matrix with a banded main diagonal structure at the
253 level of the random G×S term where the variances of the diagonal are interpreted
254 as the environmental stability of each genotype (Piepho 1999). Larger stability
255 variance values correspond to more interactive genotypes. Shukla's stability
256 variance is linearly related to the classical ecovalence value (Wricke 1962) and,
257 expressed in percent, the two values will be identical for each genetic entry (Hill
258 et al. 1998). The ecovalence values can thus be tested for statistical significance
259 using the method described by Shukla (1972).

260

261 **3. Results**

262 Mean height growth was highly variable across sites, ranging from 78.2
263 cm to 155.4 cm at age 3, and from 294.9 cm to 558.1 cm at age 8 (Online
264 Resource 1). On average, height growth was 21 and 38% higher in the coastal
265 sites than in the inner sites at ages 3 and 8 respectively.

266 Average mortality in test sites was low and below the usual thresholds for
267 forest plantations in Galicia at age 3 ($\approx 20\%$), with the exception of several sites
268 where there have been some extraordinary problems beyond the assessed
269 environmental variation (Online Resource 1).

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

276 The ratio of estimated variance components for G×S interaction and family
277 effects ($\sigma^2_{G \times S} / \sigma^2_G$) was larger than unity (1.34 and 1.11 at ages 3 and 8,
278 respectively), indicating a high relevance of the interaction as compared with the
279 genotype main effect. The genetic correlation between both ages was $0.708 \pm$
280 0.085 , reasonably high to assume that a 3-yr assessment is representative of tree
281 performance at age 8.

282

283 ***Geographical pattern of G×E interaction***

284 The model that assumes absence of COI within regions (i.e. assuming
285 perfect genetic correlations, $R_G = 1$, between all pair of sites belonging to the
286 same region) showed a significantly worse goodness of fit (i.e. larger -2RLL in
287 Tab. 1) than the full model with unrestricted genetic correlations, suggesting that
288 COI existed both within and between bioclimatic regions (see also Online
289 Resource 3). In agreement with this result, genetic correlations between sites from
290 a particular bioclimatic region were not significantly larger than those involving
291 sites of different regions ($F_{1,19} = 3.35$, $p = 0.083$ for age 3 and $F_{1,76} = 0.03$, $p =$
292 0.853 for age 8; Tab. 2).

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

301

302 ***Environmental factors underlying G×E interaction***

303 Most factorial regression models showed a marginal or lack of significance
304 for the most environmental variables at age 3, being the mean daily minimum
305 temperature of the coldest month the only parameter that was able to significantly
306 reduce the original G×S term (a reduction of 8.8 % decrease in variance

307 component). At age 8, annual precipitation and annual mean temperature also
308 played some role in the explanation of G×S interaction, but the mean daily
309 minimum temperature of the coldest month was again the most relevant parameter
310 underlying genotypic responses to changing environmental conditions (Tab. 3).

311

312 *Stability analysis*

313 Shukla's stability variances of families followed a unimodal but positively
314 skewed distribution with a few families showing relatively high values (about 2-3
315 times larger than the average stability) (Fig. 2). Therefore, not all families
316 contributed equally to the total G×E interaction. Sequentially removing the most
317 interactive families from the analyses progressively reduced the relative
318 importance of the interaction term, but too many families had to be removed to
319 achieve a negligible interaction (Fig. 2).

320

321 **4. Discussion**

322 *Genetic variation in phenotypic plasticity*

323 The large variability in height growth among test sites is indicative of the
324 relevance of plastic responses in *P. pinaster*. Tree performance was better in the
325 Atlantic area, where the genotypes were originally selected and the environmental
326 conditions are also more favourable for growth. The high ratio of G×E interaction
327 to family variance components ($\sigma^2_{G \times S} / \sigma^2_G$) suggests the presence of contrasting
328 adaptation patterns within the Atlantic breeding population when tested across the
329 Galician territory and confirms that genetic variation in plasticity does exist for

330 this population. These results agreed with previous findings upon the same
331 breeding population both within the Atlantic region (Zas et al. 2004) and across
332 the two bioclimatic regions of Galicia (de la Mata and Zas 2010b). The existence
333 of significant crossover interactions both within and between regions suggests a
334 large impact of G×E interaction on achieving progress from selection.

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

347 It should be noted that the relatively early evaluation ages (3 and 8 years)
348 may underlie the large impact of G×E interactions on height growth and, hence,
349 may not completely reflect definite adaptation patterns of the target population to
350 different environments. Although the overall variance of G×E interaction term
351 was reduced from age 3 to age 8, such reduction was nevertheless small and
352 genetic correlations between both ages were relatively high, suggesting a quite

353 uniform genotypic performance across ages. Previous studies reporting age-age
354 correlations in *P. pinaster* revealed that selection can be reasonably effective
355 when trees are about eight to 12 years old (Danjon 1994; Kremer 1992). In the
356 present study, the assessment at ages 7-8 can be thus considered quite effective for
357 achieving a reliable understanding of adaptation patterns to local environmental
358 conditions.

359

360 ***Geographical pattern of plastic responses***

361 Both the estimation of genetic correlations across sites and the likelihood
362 analysis on the relevance of crossover interactions indicated that family
363 performance varied as much within as between bioclimatic regions. We can
364 conclude, thus, that there is not enough evidence to subdivide Galicia into the two
365 currently operative deployment areas. An optimal clustering of sites minimizing
366 G×E interaction at the within-group level provided no clear geographical pattern
367 of site grouping. Although Atlantic and Mediterranean sites tended to group apart
368 in the clustering history, this trend was spoilt by some sites that were not grouped
369 as could be expected. These were, however, sites either with anomalous growth
370 due to the incidence of pests (Daneiro) or diseases (Laracha) (see de la Mata and
371 Zas 2010b), or located in the central part of Galicia relatively close to the
372 boundary between the two deployment areas (Chantada, Cortegada, Friol, Lalín
373 and Noceda). Altogether, these results suggest that there is not a clear delimited
374 limit between both bioclimatic regions, but rather a diffuse gradient between
375 coastal areas, with a mild Atlantic climate, and the inland zone, characterized by

376 more extreme climatic conditions. Within each bioclimatic region, adjacent sites
377 did not appear as the closer in the clustering history, evidencing the irregularity of
378 this transitional gradient, probably originated by the strong geographic
379 heterogeneity of region, which also generates large environmental variability at
380 intermediate scales (Martínez et al. 1999).

381

382 *Variation in climate sensitivity*

383 Factorial regression models pointed to the thermal regime, especially
384 winter cold, as the climate factor better explaining the G×E interaction patterns for
385 the studied breeding population. Therefore, regional differences in thermal regime
386 probably underlie the observed divergence in phenotypic plasticity among
387 genotypes. Contrary to what could be expected, our results indicate that both
388 annual and summer rainfall play a reduced role on the explanation of adaptive
389 patterns in the studied population. Overall and based on the growth rate, this
390 population appears to be well adapted to the intensity and duration of drought
391 events typically found in the Mediterranean region of Galicia. Therefore, the
392 variation in precipitation patterns over the targeted regions does not seem to be
393 responsible of changes in family ranking whereas the cold regime arises as key
394 determinant of genetic variation in phenotypic plasticity in this Atlantic breeding
395 population of maritime pine. The Landes provenance (SW France), another
396 Atlantic origin present in non water limiting areas, has also shown a high
397 tolerance to drought that has been attributed to adaptation to windy and sandy
398 areas (Corcuera et al. 2010). The Galician coastal provenance could have

399 experienced a similar process and, thus the acquisition of some degree of drought
400 tolerance would allow using this material in regions with relatively lower rainfall
401 but where winds and water soil retention are not so limiting. By contrast, the
402 results indicate a high relevance of the thermal regime as important driver of
403 relative changes in performance among coastal breeding material. This
404 observation agrees with results on young seedlings of less thermophilic species
405 such as *P. sylvestris* (Sonesson and Eriksson 2000) or *Pseudotsuga menziesii*
406 (Campbell and Sorensen 1978), but differs from the performance of other
407 thermophilic pines such as *P. taeda*, for which rainfall regime was the factor that
408 better explained the G×E interaction (e.g. Cannell et al. 1978; Waxler and van-
409 Buijtenen 1981).

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

418

419 ***Genetic variation in stability***

420 The stability analysis showed that families varied largely in their
421 sensitivity to changing environmental conditions, with some progenies being

422 much more influenced than others. A few especially interactive families were
423 therefore particularly responsible for G×E interaction effects. These results are
424 consistent with those previously obtained for the same breeding population in the
425 field (Zas et al. 2004) and under controlled conditions (Zas and Fernández-López
426 2005). Indeed this behaviour is common to many other pines (e.g. McKeand et al.
427 2006; Sonesson and Eriksson 2000).

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

443

444 **5. Conclusions**

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

459

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477

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601

602 **Table 1** Log-likelihood ratios testing for crossover interactions appearing either
603 within and across regions or only across regions. The table shows the Restricted
604 Log-Likelihood (RLL) value of the full model (unconstrained variance-covariance
605 matrix) and of different reduced models in which genetic covariances across sites
606 of the same region were constrained to unity. The corresponding Log-Likelihood
607 Ratio ($LLR = -2(RLL_{\text{reduced model}} - RLL_{\text{full model}})$), associated degrees of freedom
608 (DF) and probability levels are presented. Sites were grouped according to the
609 bioclimatic region (Atlantic or Mediterranean regions), or according to the
610 optimal clustering obtained by the CINTERACTION procedure (see Fig. 1), assuming
611 the first two levels of subdivision in two or three groups
612

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

613

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

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

619

620 **Table 2** REML estimates of genetic correlations between pairs of sites for height
621 growth at ages 3 and 8. Test sites are grouped according to bioclimatic regions
622 (Atlantic and Mediterranean). Shaded cells denote correlations between pair of
623 sites of the same region
624

AGE 3													
ATLANTIC						MEDITERRANEAN							
	COR	CRT	DAN	FOR	LAR	REB	BEC	CHA	FRI	GUN	LAZ	MAN	NOC
COR		0.72***	0.42	1***	0.18	1***	-0.32	0.69***	0.79**	0.46*	0.35	0.80***	0.80***
CRT			0.63***	0.75***	0.51**	0.64***	0.28	0.58***	0.64***	0.40**	0.46***	0.31	0.51**
DAN				0.43*	0.43*	0.01	0.63***	0.26	0.38	0.24	0.84***	0.60*	-0.01
FOR					0.78***	0.87***	0.25	0.78***	0.56*	0.37*	0.55***	0.31	0.86***
LAR						0.34	0.41	0.64**	0.30	0.62***	0.55***	0.25	0.14
REB							0.34	1***	0.55*	0.12	0.07	0.12	1***
BEC								0.41	-0.12	0.59***	0.56***	0.40	0.03
CHA			0.22±.32	0.22 (0.32)					0.52*	0.52**	0.38*	0.86***	0.74***
FRI										0.21	0.22	0.46	0.78**
GUN											0.41**	0.29	0.32
LAZ												0.54**	0.20
MAN													0.09

AGE 8							
ATLANTIC			MEDITERRANEAN				
	ASN	BAM	CRT	BEC	GUN	LAL	LAZ
ASN		0.67***	0.14	0.30	0.14	0.48**	0.47**
BAM			0.46*	0.21	0.31	0.50***	0.28
CRT				0.01	0.40	0.37	0.15
BEC					0.60*	0.16	0.70**
GUN						0.25	0.79***
LAL							0.37

625

626 ¹ Significance levels: *** = p<0.001; ** = p<0.01; * = p<0.05

627

628 **Table 3** Relative merit of explicit climatic factors on the explanation of G×E
629 interaction at age 8. The variance component (and approximate standard errors
630 within brackets) of the original G×S term was obtained from a simple two-way
631 linear mixed model (equation [1] in Material and methods). In the extended
632 models (equation [2] in Material and methods), the G×S term was replaced by a
633 covariate term accounting for the interaction of genotypes with specific climatic
634 variables plus a G×S residual reflecting the variation that remained after each
635 single explanatory source was removed from the original G×S term. Only those
636 factors explaining a relevant amount of the original G×S are presented.

Climatic variables	Simple model	Extended models		
	$\sigma^2_{G \times S}$	$\sigma^2_{G \times COV}$	$\sigma^2_{G \times S}$	Reduction in original $\sigma^2_{G \times S}$ (%)
	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 ¹ (°C)		4.64 (1.47)	81.09 (12.90)	23.38

637 ¹ Mean of daily minimum of the month of lowest average

638

Figure Captions

639

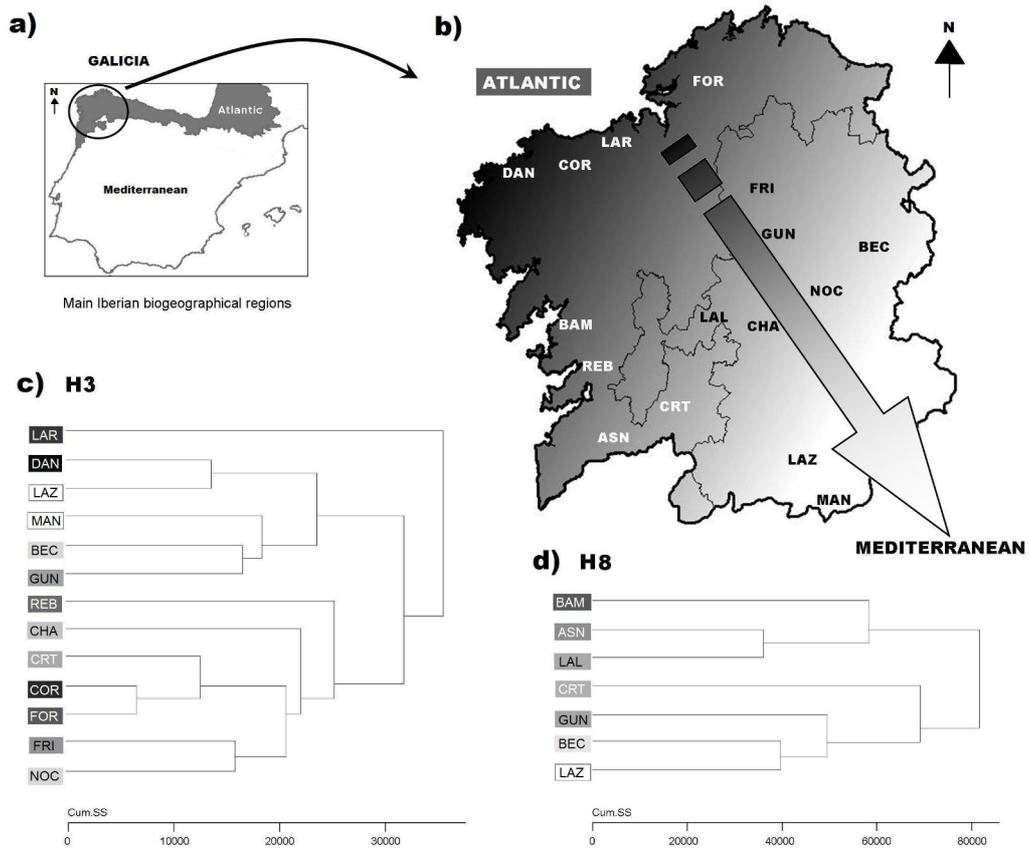
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641 **Fig. 1** a) The two main biogeographical regions of the Iberian Peninsula: Atlantic
642 (grey shaded) and Mediterranean (white shaded). b) Location of the test sites
643 across the NW-SE environmental gradient between Atlantic and Mediterranean
644 conditions in Galicia. The grey pattern and the arrow illustrates the gradual
645 increase of thermal oscillations and summer drought, and the decline in annual
646 rainfall from the NW (dark grey) to the SE of the region (light grey). The thin
647 black line denotes the boundary between the two bioclimatic areas currently
648 operative for breeding and deployment purposes. The 16 test sites are represented
649 in the map by their respective codes (see Online Resource 1). c) and d)
650 Dendrograms representing the environmental cluster obtained by means of the
651 CINTERACTION procedure for height growth at ages 3 and 8. Sites are coloured
652 according to their location in the Atlantic-Mediterranean gradient, following a
653 colour gradient from dark (true Atlantic climate) to white (strong Mediterranean
654 influence)

655

656 **Fig. 2** Frequency histogram of Shukla's stability variance for height growth at age
657 3 and magnitude of the G×E interaction in relation to genotypic variation as the
658 most interactive families were sequentially eliminated from the analysis. The grey
659 and white bars in the histograms represent families included and excluded
660 respectively

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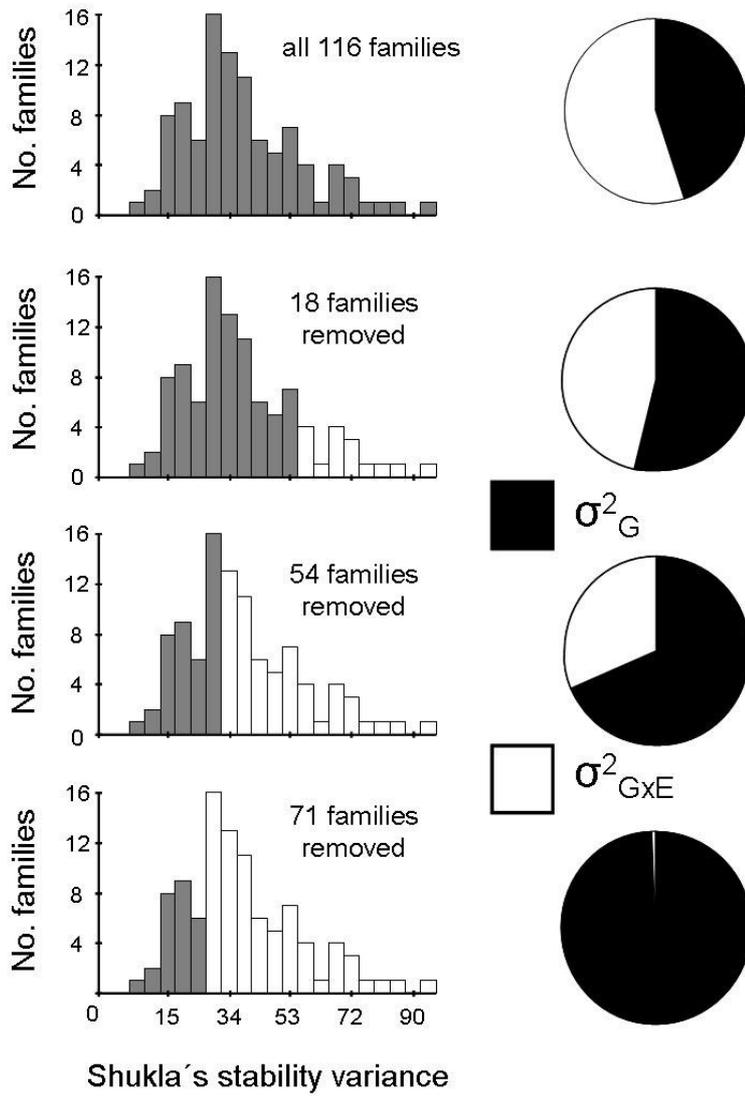
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670 **Fig. 1**

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679 **Fig. 2**

680 **Online Resource 1. Table 1.** Location and experimental design of the 16 test sites
 681

	Code	Planting Year	Spacing (m)	Longitude (W)	Latitude (N)	Altitude (m)	Aspect	Slope (%)	No. families	Exp. Design ¹	No. reps	No. plants per plot	
<i>Trial series 1995</i>													
	As Neves	ASN	1995	3x3	8.407°	42.140°	525	E	25	87	RCB	10	5
	Bamio	BAM	1995	3x3	8.740°	42.627°	300	N	27	78	RCB	10	5
	Cortegada	CRT	1994	3x3	8.114°	42.210°	530	S	5	101	RCB	10	5
	Lalín	LAL	1994	3x3	7.995°	42.641°	700	S	10	94	RCB	10	5
<i>Trial series 2001</i>													
	Becerreá	BEC	2001	3x2	7.175°	42.837°	900	S	30	111	RCB	25	1
	Daneiro	DAN	2001	3x2	8.918°	43.160°	210	SEE	0	111	RCB	23	1
	Guntín	GUN	2001	3x2	7.678°	42.900°	550	W	3	111	RCB	25	1
	Laracha	LAR	2001	3x2	8.528°	43.202°	252	SW	2	111	RCB	25	1
	Laza	LAZ	2001	2x2.5	7.492°	42.040°	770	NE	18	111	RCB	25	1
<i>Trial series 2005</i>													
	Coristanco	COR	2005	3x2	8.732°	43.160°	300	NE	0	116	IB	8	3
	Chantada	CHA	2005	3x2	7.839°	42.619°	680	E	0	94	IB	8	3
	Forgoselo	FOR	2005	3x2	7.989°	43.461°	550	W	30	116	IB	8	3
	Friol	FRI	2005	3x2	7.719°	43.070°	520	NW	3	116	IB	8	3
	Mandín	MAN	2005	3x2	7.394°	41.860°	600	SW	12	116	IB	8	3
	Noceda	NOC	2005	3x2	7.448°	42.674°	550	SSW	14	116	IB	8	3
	Rebordelo	REB	2005	3x2	8.476°	42.463°	350	NW	7	116	IB	8	3

682

683

684

685

¹ RCB: Randomized complete blocks, IB: Incomplete blocks;

686

687 **Online Resource 1. Table 2.** Average climatic characteristics during the period

688 where trees were growing in the 16 test sites

689

	Code	Period	Annual precipitation (mm)	Summer precipitation (mm)	Gausсен Index ¹	Annual mean T (°C)	No. frost days ²	Absolute minimum T (°C)	Mean minimum T (°C) ³	
Trial series 1995										
	As Neves	ASN	1995-2003	1850	131	36.8	14.9	4	-1.4	4.4
	Bamio	BAM	1995-2003	1875	151	29.9	14.3	0	0.2	6.6
	Cortegada	CRT	1994-2002	1106	118	19.7	12.6	13	-3.4	2.9
	Lalín	LAL	1994-2002	1136	107	38.3	11.4	63	-8	0
Trial series 2001										
	Becerreá	BEC	2001-2008	1408	170	22.3	8.5	63	-7.8	-0.2
	Daneiro	DAN	2001-2004	1164	134	26.3	13.4	16	-4.2	4.4
	Guntín	GUN	2001-2008	851	110	34.9	11.7	39	-7.1	0.5
	Laracha	LAR	2001-2004	1185	134	26.3	13.4	16	-4.2	3.8
	Laza	LAZ	2001-2008	706	100	66.5	13	29	-4.7	1.8
Trial series 2005										
	Coristanco	COR	2005-2008	2050	187	23.5	12.2	9	-3	3
	Chantada	CHA	2005-2008	1216	120	47.2	9.1	31	-7	1.1
	Forgoselo	FOR	2005-2008	1321	147	13.4	10.8	14	-3.3	2.5
	Friol	FRI	2005-2008	980	99	25.7	10.3	19	-4.4	1.5
	Mandín	MAN	2005-2008	661	102	67.9	13.1	36	-6	0.7
	Noceda	NOC	2005-2008	561	86	57.9	12.1	28	-5	1.4
	Rebordelo	REB	2005-2008	1518	169	48.5	13.6	2	-0.9	4.3

690 Gausсен Index = $\sum(2T-P)$ for each month where $2T > P$ (P: monthly precipitation (mm), T: monthly mean temperature

691)

692 Number of frost days ($T_{min} < 0$ °C) per year

693 Mean of daily minimum of the month of lowest average (°C)

694

695 **Online Resource 1. Table 3.** Overall mean (\pm standard deviation) of height growth
 696 and overall mortality at ages 3 and 8 in the 16 test sites.
 697

	Code	Age 3		Age 8	
		Height (cm)	Mortality (%)	Height (cm)	Mortality (%)
Trial series 1995					
As Neves	ASN			514.6 \pm 69.9	15
Bamio	BAM			492.1 \pm 66.4	21
Cortegada	CRT	134.2 \pm 24.9	3	558.1 \pm 73.9	8
Lalín	LAL			440.8 \pm 49.0	11
Trial series 2001					
Becerreá	BEC	109.1 \pm 18.3	36	294.9 \pm 44.8	41
Daneiro	DAN	78.6 \pm 16.5	60		
Guntín	GUN	105.3 \pm 16.5	5	347.8 \pm 52.8	7
Laracha	LAR	134.1 \pm 26.3	64		
Laza	LAZ	87.2 \pm 15.8	5	295.0 \pm 48.0	7
Trial series 2005					
Coristanco	COR	113.8 \pm 19.1	12		
Chantada	CHA	105.1 \pm 21.6	32		
Forgoselo	FOR	78.2 \pm 19.2	10		
Friol	FRI	91.4 \pm 21.4	21		
Mandín	MAN	83.4 \pm 20.9	15		
Noceda	NOC	89.9 \pm 20.0	9		
Rebordelo	REB	155.4 \pm 29.3	13		

698