

Vulnerability to cavitation, hydraulic efficiency, growth and survival in an insular pine (*Pinus canariensis*)

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Received: 15 January 2013 Revision requested: 5 February 2013 Accepted: 26 February 2013 Published electronically: 3 May 2013

- **Background and Aims** It is widely accepted that hydraulic failure due to xylem embolism is a key factor contributing to drought-induced mortality in trees. In the present study, an attempt is made to disentangle phenotypic plasticity from genetic variation in hydraulic traits across the entire distribution area of a tree species to detect adaptation to local environments.
- **Methods** A series of traits related to hydraulics (vulnerability to cavitation and hydraulic conductivity in branches), growth performance and leaf mass per area were assessed in eight *Pinus canariensis* populations growing in two common gardens under contrasting environments. In addition, the neutral genetic variability (F_{ST}) and the genetic differentiation of phenotypic variation (Q_{ST}) were compared in order to identify the evolutionary forces acting on these traits.
- **Key Results** The variability for hydraulic traits was largely due to phenotypic plasticity. Nevertheless, the vulnerability to cavitation displayed a significant genetic variability (approx. 5 % of the explained variation), and a significant genetic \times environment interaction (between 5 and 19 % of the explained variation). The strong correlation between vulnerability to cavitation and survival in the xeric common garden ($r = -0.81$; $P < 0.05$) suggests a role for the former in the adaptation to xeric environments. Populations from drier sites and higher temperature seasonality were less vulnerable to cavitation than those growing at mesic sites. No trade-off between xylem safety and efficiency was detected. Q_{ST} of parameters of the vulnerability curve (0.365 for P_{50} and the slope of the vulnerability curve and 0.452 for P_{88}) differed substantially from F_{ST} (0.091), indicating divergent selection. In contrast, genetic drift alone was found to be sufficient to explain patterns of differentiation for xylem efficiency and growth.
- **Conclusions** The ability of *P. canariensis* to inhabit a wide range of ecosystems seemed to be associated with high phenotypic plasticity and some degree of local adaptations of xylem and leaf traits. Resistance to cavitation conferred adaptive potential for this species to adapt successfully to xeric conditions.

Key words: vulnerability to cavitation, *Pinus canariensis*, common garden, drought, genetic differentiation, hydraulic conductivity, phenotypic plasticity, fitness, selection, trade-off.

INTRODUCTION

The responses of long-lived plant species to changes in environmental conditions are determined by the capacity of individuals to alter their structure and function (i.e. phenotypic plasticity) to novel biotic or abiotic environments, adapt through natural selection or migrate (Nicotra *et al.*, 2010). The whole-plant acclimation to water deficit requires maximizing gas exchange while avoiding hydraulic failure. Above-ground water flow through higher plants in steady state can be described by the following equation (Zimmermann, 1983):

$$Dg_s = \frac{A_s}{A_l} K_s \Delta \Psi = K_l \Delta \Psi \quad (1)$$

where D is the vapour pressure deficit of the atmosphere; g_s is the stomatal conductance; A_s is the cross-sectional sapwood

area; A_l is the leaf area; K_s and K_l are the sapwood-specific and leaf-specific hydraulic conductivity, respectively; and $\Delta \Psi$ is the water potential gradient through the system.

The adjustment of the hydraulic system to deal with climate dryness (greater D) involves: (1) stronger stomatal control to limit water loss; (2) decreasing leaf to sapwood area ratio ($A_l:A_s$), thus altering the above-ground allocation pattern between water-conducting and transpiring tissues; (3) increasing the efficiency of the conducting elements, i.e. increasing the hydraulic conductivity; and/or (4) decreasing the vulnerability to xylem embolism to limit the risk of hydraulic failure. Furthermore, trees can alter the below-ground hydraulic properties by modifying the depth of the roots, root/leaf area ratio or axial and radial hydraulic traits (Steudle, 1994; Sperry *et al.*, 1998). The combination of these strategies is possible, and different types of hydraulic adjustments have been described (Chaves *et al.*, 2002; Bréda *et al.*, 2006), although

with some restrictions. A trade-off between xylem safety (i.e. resistance to embolism) and xylem transport efficiency at the tissue level has been reported (Martínez-Vilalta *et al.*, 2002; Hacke *et al.*, 2006, 2009) as a consequence of mechanical constraints (Pittermann *et al.*, 2006) and protection from air-seeding or from freezing-induced cavitation (Sperry *et al.*, 2008).

Most of our current understanding about the variability and interaction of these hydraulic traits comes from interspecific comparisons (Hacke and Sperry, 2001; Maherali *et al.*, 2004; Jacobsen *et al.*, 2007; Pittermann *et al.*, 2010). However, information about variation within species, and to what extent genotypes are plastic for hydraulic traits, remains scarce (but see Ewers *et al.*, 2000 about the effect of nutrient and water availability in root xylem hydraulics of *Pinus taeda*). Likewise, analyses of interactions and trade-offs among hydraulic traits and their role in adaptation are based on interspecific rather than intraspecific comparisons.

Genetic differentiation within species is viewed as a key factor to adaptation. A major goal of population genetic analysis is to identify the genetic basis of adaptive phenotypic differentiation and the action of selection on this variation (González-Martínez *et al.*, 2007). Two categories of evolutionary forces determine population differentiation. The first category includes neutral evolutionary processes. The second is related to natural selection under distinct ecological environments (Still *et al.*, 2005). To discern between the influence of both categories, the comparison of differentiation of neutral markers (as reflected in F_{ST} ; Wright, 1951) and quantitative trait divergence (as reflected in Q_{ST} ; Spitze, 1993) is widely used. Both statistics quantify the proportion of total variation that occurs between populations. Any significant difference between F_{ST} and Q_{ST} (assuming that populations are in drift–migration equilibrium) is held to be evidence for natural selection (Merilä and Crnokrak, 2001). Furthermore, the more Q_{ST} differs from F_{ST} , the stronger is the evidence for local adaptation for a given trait (Merilä and Crnokrak, 2001; Latta and McKay, 2002).

Island ecosystems are natural laboratories for exploring adaptive differentiation (Emerson, 2002). In oceanic islands, volcanic and erosional activities are common, creating extremely diverse habitats that may exert varying selective pressures (Emerson, 2002). The archipelago comprising the Canary Islands is such an example, being the result of the active volcanism during the past 20 million years, with islands differing dramatically in their age (Juan *et al.*, 2000). Isolation, contrasting habitats, complex colonization–extinction processes derived from volcanic activity (Navascués *et al.*, 2006) and human activity in the last millennia (de Nascimento *et al.*, 2009) have contributed to the biology and ecology of the Canary Island flora and fauna.

Pinus canariensis is the only endemic pine of the Canary Islands. Current environmental conditions are very different from those in which this species evolved under a much wetter climate even during the late Holocene (de Nascimento *et al.*, 2009). Nowadays, despite its small distribution area, the species grows across a wide climate envelope: from xeric conditions, with barely 300 mm of rain in south-western slopes, to mixed forest with the *monteverde* in north-eastern slopes, influenced by the humid trade winds, and from close to sea level to 2400 m altitude (Climent *et al.*, 2002). As is

the case for most pines, *P. canariensis* is outcrossing, and gene flow by seed and pollen is extensive (Navascués *et al.*, 2006; Vaxevanidou *et al.*, 2006; Navascués and Emerson, 2007), particularly in open forests resulting from disturbed pinewoods or at early stages of colonization (López de Heredia *et al.*, 2010). The dispersal ability of *P. canariensis* may have consequences on the degree of adaptation of the species to local environmental conditions and to promote plasticity. Long-distance gene flow by seed and pollen can promote adaptive evolution in novel environments by increasing genetic variation for fitness (Kremer *et al.*, 2012) and enhancing plastic responses (Alpert and Simms, 2002).

Pines exhibited nearly isohydric behaviour, maintaining rather constant leaf water potential in soils with low water status and/or under high evaporative demand (Martínez-Vilalta *et al.*, 2004). Modifications of $A_l:A_s$ and stomatal control seemed to be the general adjustments of their hydraulic system, whereas anatomical traits or vulnerability to cavitation showed little plasticity in the genus (Maherali and DeLucia, 2000; Martínez-Vilalta and Piñol, 2002; Martínez-Vilalta *et al.*, 2004, 2009), despite the high variability found among conifers (Piñol and Sala, 2000; Maherali *et al.*, 2004; Martínez-Vilalta *et al.*, 2004; Brodribb and Cochard, 2009; Delzon *et al.*, 2010).

In this study, we compared the intraspecific variation and the relative contribution of plasticity and/or genetic adaptation for branch-level hydraulic properties and growth in eight populations of Canary Island pine growing in two common gardens. In addition, we aimed to find evidence of local adaptation in Canary Island pine populations by comparing neutral differentiation (F_{ST}) from neutral nuclear genes with phenotypic differentiation (Q_{ST}) from trait measures in the common gardens. Specifically, we hypothesized that trees would respond to an increase in climate dryness by increasing leaf-specific hydraulic conductivity by means of decreasing the branch leaf:sapwood area ratio. We assumed that water limitation would have been a powerful agent of natural selection, and populations from drier sites, besides adjusted branch $A_l:A_s$, would be less vulnerable to cavitation and would survive better in the xeric common garden. Conversely, the construction of a safer xylem, and due to the limit plasticity of cavitation resistance found in pines, would result in lower growth in the mesic common garden, reflecting a potential trade-off between these traits.

MATERIALS AND METHODS

Plant material and common garden experiments

Trees of *P. canariensis* from eight populations, representing the eight ecological regions of the Canary Island pine (Climent *et al.*, 2004), growing in two common garden experiments were selected for this study (Fig. 1). To establish the common gardens, cones were collected from 25 trees spaced at least 100 m apart. Cones were oven-dried to extract seeds, and, within each population, seeds were pooled across parent trees. Both common gardens were within the range of potential pine forest but they differed significantly in water availability, exposure and soil type. The most humid and productive common garden was located in the north of Tenerife at 1575 m on the windward slope of the Teide volcano and is

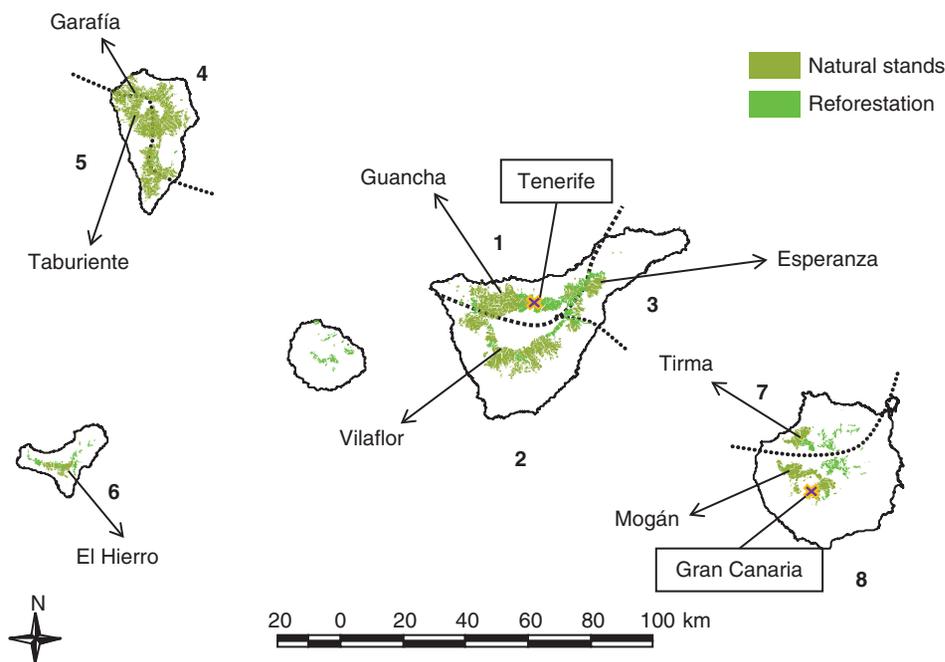


FIG. 1. Location of the sampled population of *Pinus canariensis* and common gardens in the Canary Islands. Dotted lines are the limits of the ecological regions numbered) described in Climent *et al.* (2004).

TABLE 1. Ecological regions and climatic characterization of the studied *Pinus canariensis* populations and the common garden experiments

| Population | Elevation | Pa (mm) | T (°C) | Tr (°C) | Dp (months) | ETo (mm d ⁻¹) | | | | |
|-------------------|-------------------------|---------|--------|---------|-------------|---------------------------|------|------|------|------|
| | | | | | | sp | sum | aut | win | |
| Ecological region | | | | | | | | | | |
| 1 | Guancha | 700 | 939.9 | 12.7 | 14.4 | 3.6 | 3.56 | 4.41 | 2.34 | 2.39 |
| 2 | Vilaflor | 1900 | 505 | 13.2 | 22.2 | 5.36 | 4.15 | 4.77 | 2.73 | 2.84 |
| 3 | Esperanza | 1100 | 629.7 | 14.7 | 17.4 | 4.79 | 3.74 | 4.47 | 2.41 | 2.50 |
| 4 | Garafía | 1500 | 1015.1 | 16.5 | 19.1 | 5.12 | 3.61 | 4.34 | 2.45 | 2.46 |
| 5 | Taburiente | 1000 | 719.9 | 14 | 18 | 5.12 | 3.8 | 4.44 | 2.61 | 2.64 |
| 6 | El Hierro | 1000 | 450.1 | 16.4 | 15.7 | 6.66 | 4.2 | 4.80 | 2.76 | 2.90 |
| 7 | Tirma | 850 | 379.6 | 18 | 20.6 | 6.83 | 4.37 | 4.84 | 2.85 | 2.99 |
| 8 | Mogán | 900 | 334.7 | 17.6 | 21.9 | 7.52 | 4.37 | 4.84 | 3.00 | 3.00 |
| Common gardens | | | | | | | | | | |
| 1 | Realejos (Tenerife) | 1575 | 795 | 14.3 | 21.1 | 4.07 | 3.70 | 4.46 | 2.39 | 2.47 |
| 8 | Tirajana (Gran Canaria) | 1259 | 320 | 17.8 | 20.3 | 7.68 | 4.36 | 4.90 | 3.00 | 3.01 |

Pa, annual precipitation; T, mean annual temperature; Tr, annual temperature range; Dp, drought period; ETo, evapotranspiration calculated with the Penman–Monteith equation (sp, spring; sum, summer; aut, autumn; win, winter).

under the direct influence of the humid trade winds (approx. 800 mm of annual precipitation). The common garden in the south of Gran Canaria combines an arid environment, approx. 300 mm of annual precipitation and periodic gusts of the extreme dry Saharan wind, with a very compact and stony soil (Table 1; for more details see López *et al.*, 2007). Survival, height and basal diameter were measured during the first 6 years after the establishment of the common gardens (for more detail, see López *et al.*, 2007). For the objectives of the present study, we used the height and basal diameter of the same trees used for hydraulics.

Vulnerability curves

One branch exposed to the sun, longer than 40 cm and with a maximum diameter of 1 cm, was sampled from 8–14 trees per population in each common garden. Trees were 11 years old and sampled branches corresponded to the previous year's growth unit in the mesic site and to the last 2–3 years' growth units in the dry site. Needles were removed and branches were wrapped in a black plastic bag with moist paper towels, to prevent dehydration, and sent to the laboratory in Clermont-Ferrand, France, where they were kept in a cold chamber at 4 °C. Prior to measurement, bark was removed

and sample ends were cut in water with a razor blade so as to have a length of exactly 28 cm. The bottom and top diameters of each sample were measured with a caliper. Xylem cavitation was assessed with the Cavitron technique (Cochard *et al.*, 2005). The principle of the technique is to use centrifugal force to increase the water tension in the stem segment while measuring the decrease of its hydraulic conductance. Maximal conductance of each sample (k_{\max}) was determined at a xylem pressure of -0.1 MPa, measuring the flux of a degassed ionic solution (10 mM KCl and 1 mM CaCl_2 in deionized water). Xylem pressure was then lowered stepwise by increasing the rotational velocity, and sample conductance (k) was determined again. In each step, k was measured three times, and the average was used to compute the percentage loss of conductance as $\text{PLC} = 100 \times (1 - k/k_{\max})$. The procedure was repeated until the PLC reached approx. 90%. The observed curve was fitted to a logistic function (Pammenter and Van de Willigen, 1998):

$$\text{PLC} (\%) = 100 / (1 + e^{s/[25 \times (P - P_{50})]}) \quad (2)$$

where P_{50} is the pressure inducing 50% loss of xylem conductance and s is the slope of the vulnerability curve at this point. In addition, xylem pressure at 12% loss of conductance (P_{12}), an estimate of the xylem water potential at which embolism begins, and xylem pressure at 88% loss of conductance (P_{88}), a proxy of the xylem water potential at full embolism (Domec and Gartner, 2001), were calculated as:

$$P_{12} = P_{50} + 50/s \quad (3)$$

$$P_{88} = P_{50} - 50/s \quad (4)$$

Hydraulic efficiency

Shoot specific conductivity (K_s) was assessed by dividing k_{\max} by sample basal wood area and multiplying by shoot length. Leaf specific conductivity (K_l), a measure of the hydraulic capacity of the shoot to supply water to leaves, was calculated as the ratio of shoot specific conductivity to leaf area (Tyree and Zimmermann, 2002). The projected area of 12 needles removed from the shoots used to construct vulnerability curves was obtained with a scanner and analysed with the program WinFOLIA (Regent Instruments). Then they were dried at 60°C for 3 d to determine leaf dry mass and leaf mass per area (LMA). The rest of the needles were dried as previously described and total leaf area was calculated dividing the total needle mass by LMA.

Quantitative genetic differentiation

The quantitative variability within a given population was estimated using the coefficient of variation for the phenotypic value (CV). The CVs were obtained from population means and within-common garden standard deviations.

To determine the proportion of total variation that occurs between populations, the statistic Q_{ST} was calculated for

all quantitative traits partitioning the total additive genetic variance into the among-population (σ_B) and the within-population (σ_W) components:

$$Q_{ST} = \frac{\sigma_B^2}{\sigma_B^2 + 2\sigma_W^2} = \frac{V_\alpha + \frac{V_{\alpha\beta}}{n}}{(V_\alpha + \frac{V_{\alpha\beta}}{n}) + 2h^2V_\varepsilon} \quad (5)$$

where h^2 is the narrow-sense heritability and n the number of common gardens. Since there are not any published values of heritability for *P. canariensis*, a value of 0.2 was assumed, taking into account the narrow-sense heritability found in other pines (see González-Martínez *et al.*, 2002 for a similar procedure). A simulation procedure was conducted to evaluate the influence of heritability values (in the range 0.2–0.8) on the Q_{ST} value. The variance components: variance of the population (V_α), variance of the interaction population \times common garden ($V_{\alpha\beta}$) and residual variance (V_ε) were estimated using the residual maximum likelihood option (REML) of the VARCOMP procedure in SAS 9.1 (SAS/STAT Software, SAS Institute) following the model:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon \quad (6)$$

where Y_{ij} is the phenotypic value of the i th population at the common garden j ; μ is the overall mean, α_i is the effect of the i th population, β_j is the effect of the j th common garden; $\alpha\beta_{ij}$ is the interaction between the i th population and the j th common garden and ε is the experimental error.

To assess the significance of random effects, mixed models with the factors described above were fitted using REML, and likelihood ratio tests were performed. Common garden was always treated as a fixed effect. Population was treated as random. Analyses were performed on individual-tree data. Residuals were examined for normality. Wald tests and F -statistics were used to evaluate the significance of fixed effects.

Survival was analysed with a linear logistic model (GLZ). A binomial distribution of the data was assumed and a logit function was used as the link function. The factors included in the model were as described before: common garden, population and the interaction common garden \times population.

We estimated the plasticity of each population for each hydraulic and field trait with a log response ratio, $L = \ln(\bar{X}_{GC}) - \ln(\bar{X}_{TF})$ (Hedges *et al.*, 1999), where \bar{X}_{GC} represents the population mean in Gran Canaria, and \bar{X}_{TF} the population mean in Tenerife. Approximate 95% confidence intervals for the individual log response ratios were calculated as $L - 1.96 \sqrt{v} \leq L \leq L + 1.96 \sqrt{v}$ where \sqrt{v} is the variance of L computed as:

$$v = \frac{(SD_{GC})^2}{n_{GC} + \bar{X}_{GC}} + \frac{(SD_{TF})^2}{n_{TF} + \bar{X}_{TF}} \quad (7)$$

where n and SD are the sample size and standard deviation and mean, respectively in Gran Canaria (GC) and Tenerife (TF). Populations were considered to be plastic for a specific trait when 95% confidence intervals of L for that specific trait

did not overlap zero. Differences between populations in plasticity for a trait were tested by Duncan's multiple-range test if the interaction population \times common garden in Equation 6 was significant. The overall plasticity for each trait was computed as the weighted mean of the log response ratio and its statistical significance was evaluated with the GLM of Equation 6:

$$\bar{L}^* = \frac{\sum \frac{1}{v_i} L_i}{\sum \frac{1}{v_i}} \quad (8)$$

where L_i is the log response ratio of the i th population and v_i is the variance of L_i .

Correlations between traits were evaluated by calculating Pearson's coefficient on the population Best Linear Unbiased Estimator (BLUE). In addition, Spearman's correlation coefficients were determined between the climatic conditions at origin, the BLUEs of hydraulic and growth traits of each population and their plasticity.

Molecular genetic analysis

In order to estimate neutral variation between populations, amplicons from three nuclear genes were sequenced using previously published primer pairs transferred from *Pinus pinaster* (Grivet *et al.*, 2011), the water stress-inducible protein *lp3-3* gene, and from *Pinus taeda* (Brown *et al.*, 2004), *CCoAOMT* (cell wall reinforcement) and *cad* (end of the monolignol biosynthetic pathway) (Table 2). Genomic DNA was extracted from needles from 21–24 trees collected in each of the eight populations using the Invitex kit (Invisorb[®] Spin Plant Mini Kit). Primer information and amplification conditions are described in Supplementary Data Table S1. The PCR product was directly sequenced using the Secugen S.L. sequencing service (Madrid, Spain).

Multiple sequence alignments were obtained using CLUSTALW on BIOEDIT software (<http://www.mbio.ncsu.edu/BioEdit/page2.html>). Because the data for these three loci were obtained from diploid individuals, they often include multiple heterozygous positions. The program PHASE (Stephens *et al.*, 2001) was used to estimate two haplotypes for each individual at each locus. Hence, 42–48 haplotype sequences per population were scored.

Standard molecular diversity statistics were computed for each gene and population with DnaSP 5.1 (Librado and Rozas, 2009). Departure from neutrality for each locus was evaluated with Tajima's D (Tajima, 1989), Fu's D and F (Fu

and Li, 1993) and Fu's F_S (Fu, 1997) obtained with DnaSP 5.1. In addition, we used a recently developed statistical test to detect positive selection (MFDM) that has proven analytically and empirically free from the confounding effect of varying population size, including bottlenecks and recent expansions (Li, 2011), which is a realistic scenario for *P. canariensis* (Navascués *et al.*, 2006). The test uses the maximum frequency of derived mutations within the sample to detect the presence of an unbalanced tree at a locus, which implies that a nearby locus may have experienced positive selection. A matrix of pairwise F_{ST} values was computed with Arlequin 3.11 (Excoffier *et al.*, 2005) considering the substitution model for each locus estimated with jModeltest 0.1.1 (Posada, 2008).

Comparison of F_{ST} and Q_{ST}

Confidence intervals and distribution for Q_{ST} estimations were assessed with a parametric bootstrap procedure (1000 samples) outlined in Whitlock (2008) with replacement at the individual level within a population. In addition, confidence intervals for F_{ST} were determined by bootstrapping over loci. For each bootstrap replicate, the mean F_{ST} value was calculated from the neutral loci sampled, and from that the predicted χ^2 distribution of F_{ST} was determined from the Lewontin–Krauer approach. Q_{ST} was considered to be statistically different from F_{ST} when the 95 % confidence intervals of Q_{ST} did not overlap the 95 % confidence intervals of F_{ST} (Sahli *et al.*, 2008).

RESULTS

Quantitative genetic variability between and within populations

Embolism began at a similar water potential in all populations in each common garden ($P_{12} = -1.69 \pm 0.12$ MPa in Tenerife and $P_{12} = -2.80 \pm 0.13$ MPa in Gran Canaria) but progressed differently, and slope, P_{50} and P_{88} differed among populations (Table 3). Although the maximum difference in P_{50} between populations was approx. 1.4 MPa in both common gardens, the population ranking differed (Table 4). In the mesic common garden, populations from La Palma and the leeward slopes of Tenerife and Gran Canaria were less vulnerable to cavitation, whereas in the xeric common garden, populations from La Palma were among the most vulnerable (Fig. 2; Table 4). No significant population effect on traits related to hydraulic efficiency or growth was observed (Table 3). However, plants from sites with a longer drought period

TABLE 2. Description of the three nuclear genes: GeneBank accession number, putative functional category, total number of haploid sequences (n) and coding/non-coding length screened in bp

| Gene ID | GenBank accession no. | Functional category | n | Length screened (bp) | | |
|----------------|-----------------------|--|-----|----------------------|---------------|-------------------|
| | | | | Total | Coding region | Non-coding region |
| <i>cad</i> | JX088746–JX088937 | Lignin biosynthesis (end of the monolignol biosynthetic pathway) | 384 | 412 | 301 | 111 |
| <i>lp3-3</i> | JX089129–JX089315 | Drought stress (belong to the ASR gene family) | 374 | 404 | 237 | 167 |
| <i>CCoAOMT</i> | JX088938–JX089128 | Lignin biosynthesis (cell wall reinforcement) | 382 | 511 | 264 | 247 |

TABLE 3. Percentage of the explained variation and significance values due to common garden, population and the interaction between common garden and population from the GLM and the GLZ for survival (Equation 6); coefficient of variation between (CV_{bp}) and within (CV_{wp}) populations, population differentiation for quantitative traits (Q_{ST}) and log response ratio of the trait (\bar{L}^*) for eight *Pinus canariensis* populations growing in two common gardens

| Trait | Com Gard | Pop | Com Gard × Pop | CV_{bp} | CV_{wp} | Q_{ST} | \bar{L}^* |
|-----------------------------|----------|---------|----------------|-----------|-----------|--------------|--------------|
| Hydraulic efficiency | | | | | | | |
| K_s | 15.33*** | 1.45 | 0 | 10.06 | 76.09 | 0.076 (0.10) | -0.42 (0.10) |
| K_l | 10.53*** | 3.58 | 1.28 | 20.72 | 79.11 | 0.089 (0.11) | 0.40 (0.16) |
| Vulnerability to cavitation | | | | | | | |
| P_{12} | 34.35*** | 0 | 4.96 | 6.60 | 46.13 | 0.093 (0.08) | 0.48 (0.15) |
| P_{50} | 53.11*** | 5.48*** | 5.50* | 10.21 | 17.37 | 0.365 (0.09) | 0.31 (0.12) |
| P_{88} | 42.75*** | 9.00*** | 8.39** | 11.41 | 16.22 | 0.452 (0.08) | 0.25 (0.13) |
| Slope | 7.83*** | 5.80*** | 19.31** | 17.80 | 31.66 | 0.365 (0.09) | -0.16 (0.15) |
| Biomass allocation | | | | | | | |
| $A_l:A_s$ | 40.26*** | 0.35 | 1.26 | 3.71 | 47.99 | 0.062 (0.09) | -0.61 (0.15) |
| Stem morphology | | | | | | | |
| H | 94.12*** | 0.11 | 0 | 2.88 | 21.13 | 0.044 (0.09) | -0.92 (0.15) |
| Db | 59.96*** | 2.35 | 0 | 9.43 | 37.79 | 0.135 (0.09) | -0.72 (0.12) |
| Needle morphology | | | | | | | |
| LMA | 73.16*** | 4.31* | 4.34* | 9.85 | 25.61 | 0.157 (0.09) | 0.35 (0.10) |
| Surv | 17.87*** | 4.80*** | 2.21 | | | | |

The standard deviation is given in parentheses.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

K_s , specific hydraulic conductivity; K_l , leaf specific hydraulic conductivity; P_{12} , P_{50} , P_{88} , pressure causing 12, 50 and 88 % loss of conductance, respectively; slope, slope at the inflection point of the vulnerability curve; $A_l:A_s$, branch leaf-to-sapwood area ratio; H, height 6 years after planting; Db, basal diameter 6 years after planting; LMA: leaf mass per area; Surv, survival.

showed higher survival rates and produced thicker needles in both common gardens (Table 4). This genetic differentiation for both survival and LMA was more pronounced in the xeric common garden where survival rates of populations from Gran Canaria were double the survival rates of populations from the north of Tenerife, and the LMA differed in 46.6 g m^{-2} (Table 4).

Genetic variability in phenotypic plasticity

All traits were strongly influenced by common garden (Table 3). As expected, in Tenerife, more plants survived and grew taller, branches were more vulnerable to cavitation (higher values of P_{12} , P_{50} and P_{88}) and branch $A_l:A_s$ was higher. Conversely, higher values of K_l were found in the xeric common garden, mostly due to the low leaf area of trees growing in this location, which only retained leaves formed in the current year. All populations exhibited significant plasticity (the confidence interval around L did not overlap zero) for height, diameter and LMA, and were equally plastic (the interaction common garden × population in Equation 6 was not significant). The only differences in plasticity between populations were for parameters of the vulnerability curve (Fig. 3). Noticeably, both populations from La Palma were the least plastic for P_{50} [no plasticity of population 4 and $L(P_{50}) = 0.17$ of population 5].

Relationships among field performance, hydraulic traits and climate conditions of origin

The parameters of the vulnerability curve were positively correlated with each other (r between slope and $P_{50} = 0.43$). There was also a positive correlation between variables measuring hydraulic efficiency ($r = 0.52$). No relationship was

found between both sets of traits, indicating the absence of a trade-off between hydraulic efficiency and safety.

Parameters of the vulnerability curve were related to field performance and climate of origin of the populations only in the xeric common garden. Survival was strongly correlated with population mean values of P_{88} ($r = -0.81$) and slope ($r = -0.71$) (Fig. 4). P_{88} was negatively related to the temperature range ($r = -0.76$) and positively to the mean annual precipitation, if we excluded the population from El Hierro ($r = 0.96$) (Fig. 5). Populations occurring in forests with a longer drought period showed higher LMA ($r = 0.76$). No relationship was found between plasticity and fitness traits.

Molecular genetic analysis

The partial sequences of the three nuclear genes (GeneBank accession nos: *cad*, JX088746–JX088937; *CCoAOMT*, JX088938–JX089128; and *lp3-3*, JX089129–JX089315) covered coding and non-coding regions (Supplementary Data Table S2). The number of segregating sites was similar for all three genes (10–11) when the whole range of the species was considered but was variable for each population, from a low of two in El Hierro to a high of eight in Esperanza for *cad*. The number of haplotypes was variable between populations and genes (Supplementary Data Table S2). While haplotypes were shared across all populations, private haplotypes were scored in almost all the populations for the three candidate genes. Neither standard neutrality tests (Tajima's D, Fu's D and F, and Fu's F_S) nor the MFDM test generated significant values for any of the genes when all ecological regions were considered or when they were analysed alone (Supplementary Data Table S2).

TABLE 4. Mean values (\pm s.e.) of survival, hydraulic efficiency and safety traits, field performance and leaf mass per area of eight populations of *Pinus canariensis* growing in two common gardens of contrasted environmental conditions (Tenerife and Gran Canaria)

| Common garden | Ecol. region | Hydraulic efficiency | | Vulnerability to cavitation | | | | Biomass allocation $A_1:A_s$ ($m^2 cm^{-2}$) | Field performance | | | Needle morphology LMA ($g m^{-2}$) |
|---------------|--------------|---------------------------------------|---|-----------------------------|----------------|----------------|------------|--|-------------------|----------|-----------|---|
| | | K_s ($kg m^{-1} s^{-1} MPa^{-1}$) | $K_l \times 10^4$ ($kg m^{-1} s^{-1} MPa^{-1}$) | P_{12} (MPa) | P_{50} (MPa) | P_{88} (MPa) | Slope | | Surv (%) | H (cm) | Db (cm) | |
| Tenerife | 1 | 32.8 (5.2) | 3.22 (0.69) | -1.54 (0.21) | -3.77 (0.16) | -6.01 (0.29) | 23.4 (1.6) | 0.11 (0.02) | 64.3 (9.0) | 168 (13) | 4.9 (0.4) | 142 (6.7) |
| | 2 | 45.1 (5.5) | 3.86 (0.62) | -1.84 (0.35) | -4.61 (0.42) | -7.38 (0.67) | 20.4 (2.9) | 0.14 (0.02) | 82.0 (7.1) | 127 (10) | 4.3 (0.2) | 158 (7.3) |
| | 3 | 39.2 (10.2) | 3.51 (0.47) | -1.64 (0.32) | -3.16 (0.33) | -4.69 (0.40) | 34.9 (3.2) | 0.11 (0.02) | 64.3 (9.2) | 149 (9) | 4.9 (0.4) | 148 (6.0) |
| | 4 | 73.2 (11.9) | 6.47 (0.15) | -2.00 (0.16) | -4.61 (0.29) | -7.21 (0.54) | 20.7 (2.4) | 0.12 (0.02) | 96.7 (3.1) | 140 (11) | 4.6 (0.2) | 143.4 (6.0) |
| | 5 | 83.2 (18.9) | 6.12 (0.21) | -1.60 (0.36) | -4.47 (0.33) | -7.33 (0.47) | 19.2 (2.9) | 0.13 (0.01) | 81.9 (7.2) | 139 (10) | 4.7 (0.3) | 149.8 (5.9) |
| | 6 | 62.8 (11.4) | 4.82 (0.75) | -1.52 (0.27) | -3.13 (0.32) | -4.73 (0.45) | 34.6 (4.3) | 0.13 (0.01) | 85.9 (7.1) | 151 (15) | 4.1 (0.3) | 139.8 (5.7) |
| | 7 | 62.4 (12.6) | 5.89 (1.37) | -1.70 (0.38) | -3.80 (0.26) | -5.90 (0.30) | 26.2 (2.8) | 0.11 (0.01) | 99.9 (1.0) | 144 (10) | 5.0 (0.3) | 163.1 (7.0) |
| | 8 | 78.6 (18.6) | 6.52 (1.22) | -1.68 (0.19) | -4.32 (0.15) | -6.97 (0.26) | 20.2 (1.4) | 0.11 (0.01) | 78.6 (8.5) | 126 (9) | 4.2 (0.3) | 159.7 (6.3) |
| Gran Canaria | 1 | 32.2 (4.6) | 5.77 (1.77) | -3.75 (0.49) | -5.73 (0.41) | -7.71 (0.46) | 27.1 (2.2) | 0.09 (0.02) | 32.1 (9.8) | 59 (5) | 0.7 (0.1) | 181.5 (7.2) |
| | 2 | 39.0 (8.5) | 4.26 (0.94) | -3.70 (0.21) | -6.05 (0.24) | -8.40 (0.29) | 21.5 (0.9) | 0.11 (0.02) | 55.3 (9.3) | 51 (4) | 0.8 (0.2) | 228.1 (7.6) |
| | 3 | 37.1 (7.4) | 9.31 (2.84) | -2.62 (0.53) | -5.44 (0.39) | -8.26 (0.40) | 18.8 (2.0) | 0.09 (0.02) | 42.9 (9.3) | 57(3) | 0.8 (0.1) | 210.6 (7.0) |
| | 4 | 18.9 (6.8) | 3.13 (0.51) | -1.86 (0.17) | -4.76 (0.48) | -7.67 (0.43) | 18.5 (2.2) | 0.06 (0.02) | 50.7 (9.8) | 49 (3) | 0.7 (0.2) | 210.5 (6.9) |
| | 5 | 37.9 (5.0) | 10.10 (1.70) | -2.49 (0.29) | -5.29 (0.22) | -8.08 (0.30) | 18.9 (1.3) | 0.05 (0.01) | 64.3 (9.2) | 59 (3) | 1.1 (0.1) | 228.1 (7.5) |
| | 6 | 33.8 (4.5) | 9.11 (2.15) | -2.17 (0.34) | -4.62 (0.20) | -7.07 (0.26) | 22.9 (2.0) | 0.05 (0.01) | 28.6 (9.4) | 48 (3) | 0.6 (0.1) | 227.1 (7.5) |
| | 7 | 38.2 (6.8) | 12.33 (3.78) | -3.17 (0.42) | -5.74 (0.26) | -8.30 (0.22) | 20.3 (1.6) | 0.05 (0.01) | 64.3 (9.2) | 57(3) | 0.8 (0.2) | 235.1 (8.3) |
| | 8 | 43.5 (6.3) | 8.64 (1.38) | -2.62 (0.32) | -5.65 (0.18) | -8.68 (0.29) | 18.4 (1.5) | 0.06 (0.01) | 71.4 (9.1) | 75 (2) | 1.3 (0.1) | 234.2 (8.1) |

K_s , specific hydraulic conductivity; K_l , leaf specific hydraulic conductivity; P_{12} , P_{50} , P_{88} , pressure causing 12, 50 and 88 % loss of conductance, respectively; slope, slope at the inflection point of the vulnerability curve; $A_1:A_s$, branch leaf-to-sapwood area ratio; Surv, survival 6 years after planting; H, height 6 years after planting; Db, basal diameter 6 years after planting; LMA, leaf mass per area.

Sample size was $n = 144$ for all traits except for survival, $n = 448$.

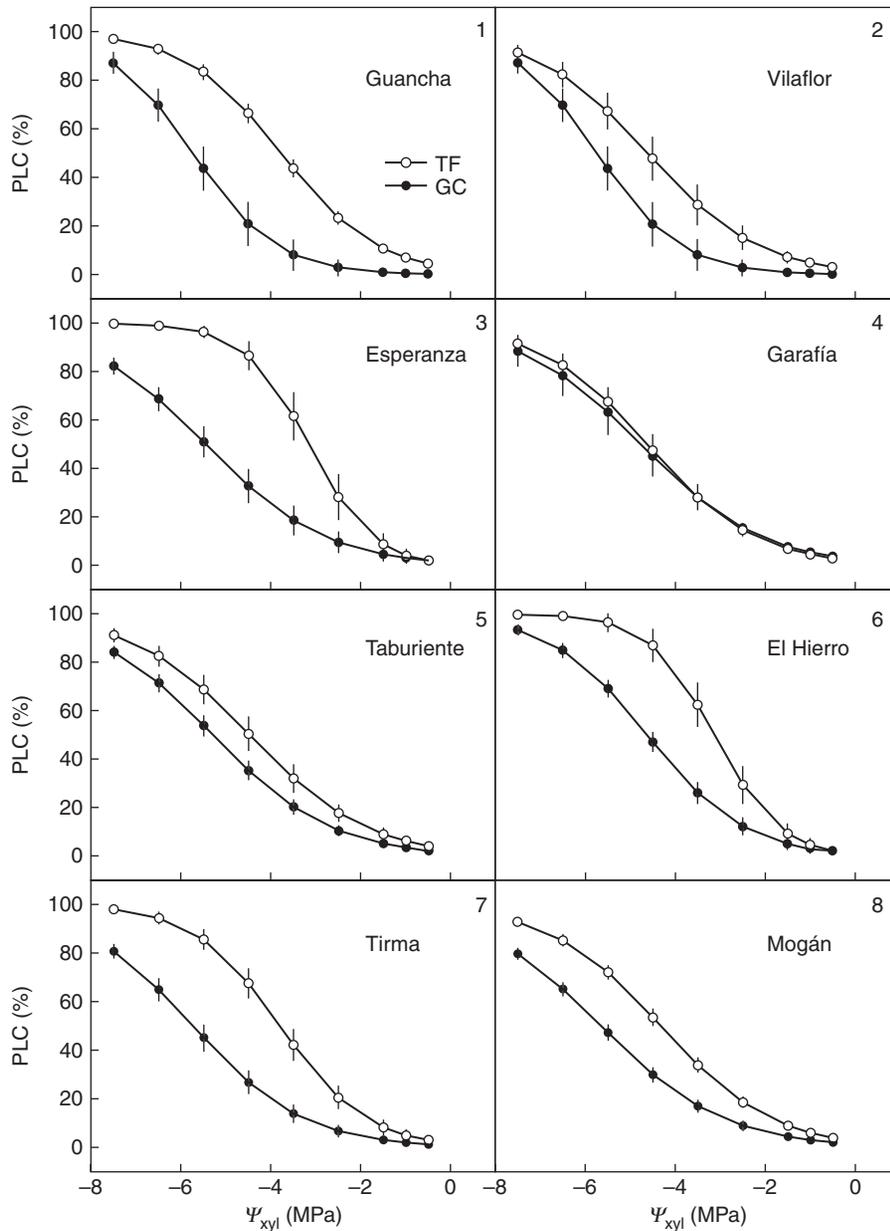


FIG. 2. Xylem vulnerability curves for shoots of *Pinus canariensis* from populations of the eight ecological regions described for the species (numbered in the graphs) planted in two common gardens (TF, Tenerife; GC, Gran Canaria). Error bars represent the s.e. Ψ_{xyl} , xylem water potential; PLC, percentage loss of conductance.

Neutral vs. adaptive differentiation

F_{ST} differentiation was low for neutral genes. The overall estimate of genetic differentiation for *cad* F_{ST} was 0.091 (0.053, $P < 0.01$), for *CCoAOMT* 0.066 (0.062, $P < 0.01$) and for *lp3-3* 0.061 (0.048, $P < 0.01$). Q_{ST} values ranged between 0.044 (0.09; $P < 0.001$) for height and 0.452 (0.08; $P < 0.001$) for P_{88} (Table 3). For height and hydraulic efficiency traits, Q_{ST} and F_{ST} values did not differ significantly. Conversely, for parameters of the vulnerability curve, the average Q_{ST} value was significantly higher than F_{ST} (Fig. 6), suggesting that populations displayed more differentiation than would be expected by drift alone. The differences

between quantitative Q_{ST} and neutral F_{ST} were still significant when heritability values in the range 0.2–0.8 were assumed (Fig. 6).

DISCUSSION

Genetic variation of vulnerability to cavitation and phenotypic plasticity of hydraulic traits

Canary Island pine populations from a wide range of ecological conditions showed evidence for climate-driven divergence for branch vulnerability to cavitation under xeric conditions. According to our expectations, and with the exception of the

population from El Hierro, populations did differ consistently in their vulnerability to water stress-induced cavitation in the xeric common garden: the dry populations were the least vulnerable and the mesic populations the most susceptible (Fig. 5). The construction of a safer xylem in drier habitats has been commonly found in interspecific comparisons (Pockman and Sperry, 2000; Choat *et al.*, 2007), but this is the first time it has been assessed in conifers at the intraspecific level. Until now, studies evaluating populations of several pines had reported little or no difference between populations for this trait, even considering a wide range of climates

(Maherali and DeLucia, 2000; Martínez-Vilalta *et al.*, 2009; Lamy *et al.*, 2011). However, the high intrapopulation variability for vulnerability to cavitation in *P. canariensis* ($CV_{wp} = 17\%$) compared with other pines (Martínez-Vilalta *et al.*, 2009; Lamy *et al.*, 2011) suggested the evolvability, i.e. ability to respond to selection, of this trait in this species (Houle, 1992) as reflected by the comparisons between neutral and quantitative differentiation (Fig. 6).

Hydraulic efficiency, biomass allocation and, in particular, growth were especially sensitive to changes in environmental conditions (higher values of the log response ratio; Table 3) and, interestingly, all populations performed similarly in both common gardens, as reflected by the non-significant population effect and negligible population \times common garden interaction (Table 3). Most of the variability of these traits resided within rather than between populations (Table 3). The general response to overcome low water availability was producing stiffer needles to reduce transpiration (Cernusak *et al.*, 2011) and reducing branch $A_1:A_s$ by means of a dramatic reduction of leaf area, resulting in higher K_l to guarantee water supply to leaves despite the reduction of K_s (Sperry and Pockman, 1993). Substituting the relative differences between common gardens in Equation 1, a 35% decrease in K_s was offset by a 71% decrease in $A_1:A_s$ without any adjustment in $\Delta\Psi$. In contrast, plasticity for vulnerability to cavitation was genotype dependent, contrary to our initial hypothesis of a limitation in plasticity of P_{50} . Only vulnerability curves of populations from La Palma almost coincided (Fig. 2), more in accordance with the lack of plasticity previously reported for pines (Martínez-Vilalta *et al.*, 2004). However, populations from the windward slopes of the main islands were highly plastic; P_{50} increased almost 50% in the mesic common garden, where the development of unnecessary drought-tolerant tissues could compromise competitive ability (Pockman and Sperry, 2000). Higher cavitation resistance has been linked to increased wood density to sustain the compressive forces generated by lower negative pressures (Hacke *et al.*, 2001). Construction of denser wood may correspond to slower growth rates (Enquist *et al.*, 1999), further decreasing fitness of cavitation-resistant genotypes when water is readily available. Nevertheless, we have not found such a trade-off in *P. canariensis*, at least in

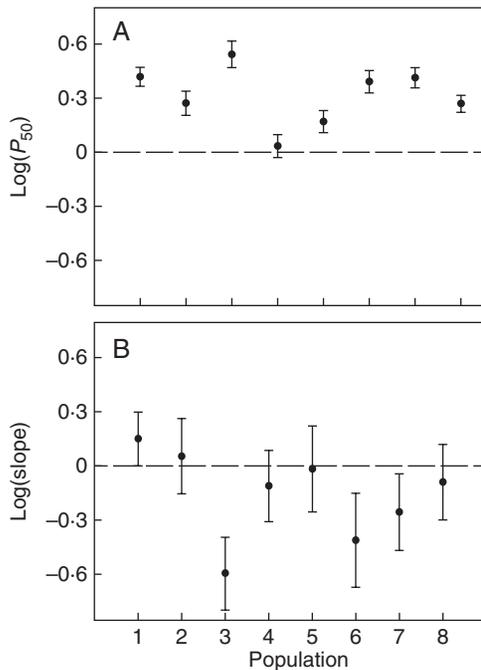


FIG. 3. (A) Log response ratio \pm 95% confidence interval of the pressure causing 50% loss of conductance (P_{50}) and (B) the slope at the inflection point of the vulnerability curve of eight populations of *Pinus canariensis* growing in two common garden experiments. If the 95% confidence intervals are above (below) zero, this indicates a significant increase (decrease) in the trait in Tenerife (mesic common garden); if the confidence intervals cross the line, then there is no significant effect of the common garden.

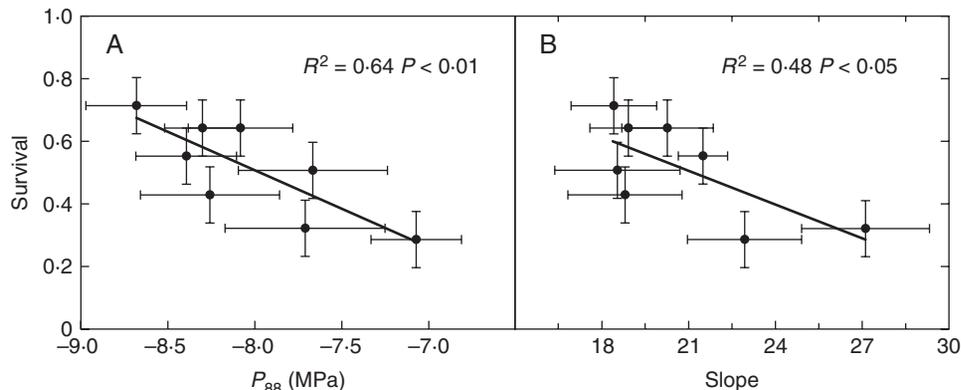


FIG. 4. Correlations between mean values of survival of *Pinus canariensis* populations growing at the xeric common garden in Gran Canaria and two parameters obtained from the vulnerability curves: (A) the pressure causing 88% loss of conductance (P_{88}) and (B) the slope at the inflection point.

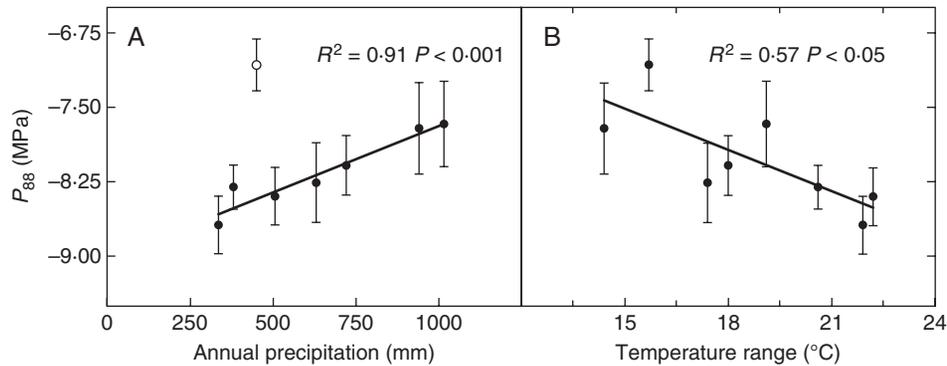


FIG. 5. Relationship of (A) mean annual precipitation and (B) temperature range with the pressure causing 88 % loss of conductance in the xeric common garden in Gran Canaria (P_{88}). The population from El Hierro (open circle) is excluded in (A).

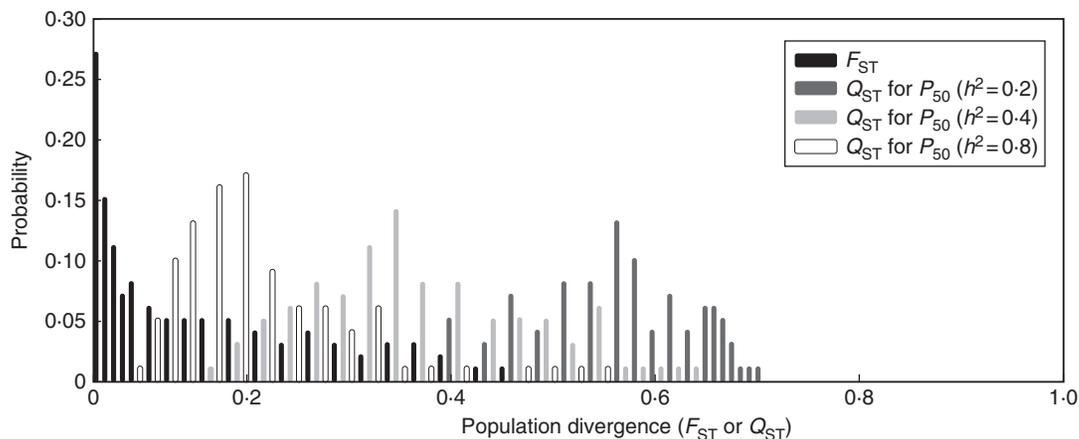


FIG. 6. Frequency distributions of the *cad* gene (F_{ST}) and quantitative trait for P_{50} (Q_{ST}) differentiation based on 1000 bootstrap samples. Estimated Q_{ST} considering different values of narrow-sense heritability (h^2).

above-ground growth, and other traits related to below-ground properties or fructification could be involved.

A possible shortcoming of the present study was that hydraulic properties were compared on 1-year-old branches in the mesic common garden and in 2- to 3-year-old branches in the xeric garden. However, a previous study with *Fagus sylvatica* did not find any significant age effect in P_{50} even comparing 1-year-old with 6-year-old stems (Herbette *et al.*, 2010). Moreover, the youngest branches tended to be less vulnerable to cavitation than the largest branches (Cochard, 1992). Thus if there was a bias, we probably would have underestimated the difference in P_{50} between the two common gardens. Another limitation was that conductance in branches was only a small fraction of total conductance. Other features such as below-ground properties can affect transpiration; for example, root K_s may have been higher in xeric populations, or may be more plastic in populations that are less plastic in P_{50} . Genetic differences in biomass allocation to roots in *P. canariensis* seedlings subjected to water deficit in hydroponic culture have been observed previously (López *et al.*, 2009).

Vulnerability to cavitation and fitness traits

We used survival and growth as the best available fitness proxies, and the results were conclusive for the adaptive

value of decreasing vulnerability to cavitation in dry conditions. We found a strong correlation between survival in the xeric common garden in Gran Canaria, and both the xylem water potential at full embolism (P_{88}) and the slope of the vulnerability curve (Fig. 4). The strong correlation between P_{50} and annual precipitation for conifers and evergreen angiosperms had already suggested the adaptive significance of decreasing vulnerability to cavitation as a mechanism of drought tolerance at the interspecific level (Brodribb and Hill, 1999; Maherali *et al.*, 2004), and our results also pointed in this direction at the intraspecific level when trees were growing in xeric conditions. Additionally, the resistance of the hydraulic system appeared to be the key factor for survival and posterior gas exchange recovery from drought (Brodribb and Cochard, 2009), and to achieve higher midday stomatal conductance to water vapour in soils with low water retention or under water deficit conditions (Holste *et al.*, 2006; Beikircher and Mayr, 2009), and thus to maintain a favourable carbon balance.

Although an overall trade-off among hydraulic conductivity and growth with P_{50} was found when pooling data of the two common gardens, this trade-off was not so evident within sites, and trees which constructed a safer xylem were not necessarily those exhibiting lower growth or less efficiency in terms of water transport. This result, when trees grew in the same

environment, was consistent with a growing body of evidence suggesting a lack of a trade-off between xylem safety and efficiency in conifers (Willson *et al.*, 2008; Martínez-Vilalta *et al.*, 2009; Peguero-Pina *et al.*, 2011; but see Cochard, 1992; Kavanagh *et al.*, 1999; Piñol and Sala, 2000; Domec and Gartner, 2002, 2003). The exact mechanism by which xylem vulnerability to cavitation acclimates to soil water deficit remains to be explained, but it should be related to changes in size, permeability or stability of the torus of the pits and wall reinforcement (Hacke and Sperry, 2001; Hacke *et al.*, 2001) rather than to conduit diameter.

Effect of natural selection on population differentiation

Our results indicated that natural selection has shaped the observed genetic differentiation in vulnerability to cavitation and LMA across the natural range of *P. canariensis*. The comparison of Q_{ST} values of P_{50} , P_{88} , slope of the vulnerability curve and LMA between populations far exceeded that expected through random drift and gene flow alone, as estimated from F_{ST} . These measurements of among-population divergence, i.e. values of Q_{ST} , are inherently dependent on the heritability of the character, but even with high heritability values the distribution of Q_{ST} differed significantly from F_{ST} (Fig. 6), consistent with divergent selection acting on drought resistance across the geographic range of this species. In fact, population differentiation could have been more pronounced if mortality had affected the most vulnerable genotypes. Slope and P_{50} could be underestimated then, particularly in populations with the lowest survival rates. The Q_{ST} value of 0.452 for P_{88} and 0.365 for P_{50} and slope is close to the upper range of values reported for drought-related traits in *Quercus suber* (Ramírez-Valiente *et al.*, 2009) but it differed widely from the low value of differentiation among populations of *P. pinaster* planted in a mesic environment (Lamy *et al.*, 2011). In this latter study, uniform selection for vulnerability to cavitation in *P. pinaster* was suggested as a consequence of canalization to buffer against genetic or environmental disturbances. In our case, and despite evidence for extensive gene flow among islands (Navascués *et al.*, 2006) and along elevational transects (Navascués *et al.*, 2008), the broad range of environments and historical perturbations could have exerted strong selection pressures leading to both phenotypically plastic genotypes and local adaptation. The occurrence of shared and private haplotypes in all the populations found for chloroplast microsatellites (Vaxevanidou *et al.*, 2006) and for nuclear genes in the present study is compatible with a scenario of substantial gene flow that, rather than counteracting local adaptation, enhances it, as in other wind-dispersed species (Kremer *et al.*, 2012). Quantitative trait genetic differentiation in cavitation (the present study), vegetative phase change (Climent *et al.*, 2006), biomass allocation and osmotic adjustment (López *et al.*, 2009), and foliar morphology and anatomy (López *et al.*, 2010) strengthen the idea of divergent selection.

A second line of evidence that natural selection has driven genetic diversification in vulnerability to cavitation was the significant correlation between survival and parameters of the vulnerability curve. Moreover, increased resistance to drought and water use efficiency appear to have evolved in

populations subjected to greater temperature seasonality and lower precipitation as in populations of *Eucalyptus globulus* (Dutkowski and Potts, 2012), *Pinus halepensis* (Voltas *et al.*, 2008) and *Cordia alliodora* (Choat *et al.*, 2007). In contrast, the Q_{ST} of xylem efficiency and growth is consistent with genetic drift alone arising from founder effects.

Conclusions

Phenotypic variability for branch hydraulic traits in *P. canariensis* was largely the result of phenotypic plasticity. Acclimation of the hydraulic system to xeric conditions implied modifications of $A_1:A_s$ and changes in the vulnerability to cavitation. We inferred that divergent selection must have acted in the past on xylem vulnerability to cavitation more evidently than in other traits sensitive to water deficit such as growth or hydraulic efficiency. Our results strongly support the adaptive role of cavitation resistance in xeric environments.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Table S1: primers and amplification conditions for the three candidate genes in the study. Table S2: diversity parameters for the three candidate genes.

ACKNOWLEDGEMENTS

We are grateful to the Canary Islands Government, the Cabildos of Tenerife and Gran Canaria and the National Park of Caldera de Taburiente for long-standing support in the study of Canary Island pine. We thank all people involved in the plantation and measurements of the common gardens and to Christian Bodet and Pierre Conchon for their assistance with the Cavitron. R.L. was supported by a González Esparcia fellowship during her stay in Clermont-Ferrand. This work was supported by the Spanish Ministry of Science in the Project AGL2009-10606 (VULCAN).

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