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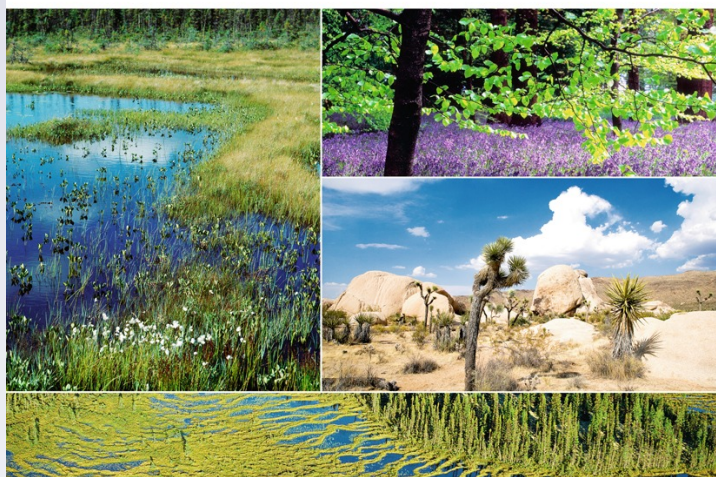
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Micro-evolutionary patterns of juvenile wood density in a pine species

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Abstract Wood density can be considered an adaptive trait, because it ensures the safe and efficient transport of water from the roots to the leaves, mechanical support for the body of the plant and the storage of biological chemicals. Its variability has been extensively described in narrow genetic backgrounds and in wide ranges of forest tree species, but little is known about the extent of natural genetic and phenotypic variability within species. This information is essential to our understanding of the evolutionary forces that have shaped this trait, and for the evaluation of its inclusion in breeding programs. We assessed juvenile wood density, leaf area, total aboveground biomass, and growth in six *Pinus pinaster* populations of different geographic origins

(France, Spain, and Morocco) growing in a provenance-progeny trial. No genetic differentiation was found for wood density, whereas all other traits significantly differed between populations. Heritability of this trait was moderate, with a low additive genetic variance. For retrospective identification of the evolutionary forces acting on juvenile wood density, we compared the distribution of neutral markers (F_{ST}) and quantitative genetic differentiation (Q_{ST}). We found that Q_{ST} was significantly lower than F_{ST} , suggesting evolutionary stasis. Furthermore, we did not detect any relationship between juvenile wood density and drought tolerance (resistance to cavitation), suggesting that this trait could not be used as a proxy for drought tolerance at the intraspecific level.

Keywords Canalization · Heritability · Q_{ST}/F_{ST} comparison · Pine · Evolutionary stasis · Juvenile wood density

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Introduction

Recent ecological theories have included wood density (mass of wood per unit volume) as key dimension of the variation between woody plant strategies (Westoby et al. 2002; Westoby and Wright 2006; Chave et al. 2009; Zanne et al. 2010). Wood density describes the proportion of cell walls in a given woody tissue. This proportion reflects three functions: the safe

and efficient transport of water from the roots to the leaves, mechanical support for the body of the plant, and the storage of biological chemicals. Wood density is strongly constrained by a trade-off between these functions (Chave et al. 2009) that has influenced the evolution of woody plants over millions of years. It is possible to extrapolate some of the features of the tree, such as growth rate and hydraulic strategy, from the wood density of the species concerned (McCulloh et al. 2011). Species with high wood density are expected to grow more slowly and to have a lower hydraulic efficiency and a higher resistance to embolism than species with a lower wood density (Poorter et al. 2010; Markesteijn et al. 2011). Wood density is also thought to be a good proxy for drought tolerance (i.e., survival of extreme drought events) (Hacke and Sperry 2001; Brodribb and Cochard 2009). For example, after the 2003 heatwave in Europe, *Pseudotsuga menziesii* trees with denser wood and a higher proportion of late wood had higher survival rates than trees with a lower wood density (Martinez-Meier et al. 2008). In *Pseudotsuga menziesii* and *Picea abies* clones, Rosner et al. (2008) and Dalla-Salda et al. (2011) found that wood density was negatively correlated with resistance to cavitation.

Chave et al. (2006) countered the widespread belief that wood density is determined principally by environmental conditions, by suggesting that there is a strong phylogenetic signal controlling interspecific wood density variation. However, in temperate region, wood density may vary considerably in the environment of an individual plant (increasing from the early to the late wood), but the variation between individuals of a given species remains limited and, more importantly, the mean wood density for a given species is generally highly conserved. Many studies have investigated the intraspecific variability of wood density, dissecting the phenotypic variation of wood density into genetic and environmental components (and their interaction) for most commercial forest tree species (Aguiar et al. 2003; Bouffier et al. 2008; Martinez-Meier et al. 2011; Apiolaza 2011). The phenotypic coefficient of variation for wood density is generally low, as is the genetic additive coefficient of variation (these conclusions do not apply to interspecific crosses, such as *Eucalyptus* sp.) (Apiolaza 2011). However, these quantitative estimators have often been measured in narrow genetic backgrounds (or hybrids), without reference to the natural variation of

wood density within the species concerned. Even in studies in which wood density was measured in several populations, efforts were rarely made to determine whether the observed variation between populations was caused by genetic drift or past selective events, even though knowledge about the mechanisms shaping the variability of this trait would be useful if this trait was to be incorporated into a breeding program. Some traits are known to be robust to genetic alterations (e.g., wing shape in *Drosophila* sp., mammalian body temperature), such as mutation, drift, or recombination events, and these traits generally have a low variability (phenotypic and/or genetic) (Gilchrist and Partridge 2001; Hansen and Houle 2004). In a breeding context, it is difficult to manipulate these traits: genetic gains at each generation are limited by the low additive genetic variance. However, such “canalized” traits provide a guarantee of a certain level of trait constancy in diverse environmental conditions (Knap 2005; Mormede et al. 2011).

Most of the previous studies dealt with wood density measured on mature trees and very little work has been devoted to juvenile wood density from an evolutionary ecology point of view. The seedling stage is the woody plant life stage with the greatest mortality (the strongest selection pressure) (Petit and Hampe 2006). Young seedling performance may be more important than mature individual performance in determining species distributions and evolution (Figuroa and Lusk 2001). This study had three main objectives: (i) to assess between- and within-population variation of juvenile wood density, using genetic material originating from contrasting climatic environments, (ii) to determine the main evolutionary forces responsible for shaping the genetic variability of juvenile wood density between populations (i.e., contributing to population differentiation), and (iii) to assess the strength of the correlation between juvenile wood density and drought tolerance (resistance to cavitation). We carried out a case study in maritime pine (*Pinus pinaster* Ait.), a species with a fragmented distribution in the western part of the Mediterranean region. The scattered distribution of this species may have prevented or limited gene flow between different groups of populations, promoting high levels of genetic divergence between ecotypes, due to genetic drift (Ribeiro et al. 2002) and/or natural selection (Quezel and Barbero 1998 in Richardson 1998).

Materials and methods

Plant material

We used the same material described in a recent paper by Lamy et al. (2011). Briefly, a provenance-progeny trial was established in December 2003 at the INRA forestry station (Cestas, France, 44°44'N, 00°46'W), with 1-year-old seedlings from open-pollinated seeds collected from 24 natural populations (or ecotypes) in France, Spain, Morocco, and Tunisia, to cover the fragmented distribution of *Pinus pinaster* (see Lamy et al. 2011). Each population was represented by 20–30 half-sib families. The trial was arranged in a randomized block design (15 blocks in total) with single-tree plots. Each block contained at least one tree from each half-sib family. A selective sampling of 6-year-old saplings was carried out as described by Lamy et al. (2011), leading to the selection of six populations representative of the maritime pine climatic envelope. Climate and location of the studied population are presented in Table 1. We sampled eight families with one half-sib in each block (6 populations/8 families/5 blocks = 240 genotypes) at random for further analysis.

Assessment of wood density

Wood density (D , g cm^{-3}) was measured on a section of dry branch. The samples corresponded to the 2007 and 2008 growth units on the 2007 whorl when possible. Sampled branches were fully exposed to the sun, more than 40 cm long and had a diameter of 0.3–1 cm. The number of trees for which wood density data were available per cambial year was as follows:

10 for 2 years old, 151 for 3 years old, 76 for 4 years old, and nine for 5 years old. No difference of branch age was found between populations ($F_{1/221} = 0.07$ and $P = 0.79$), reducing the risk of sampling procedure bias. Mean wood density was measured for 240 genotypes. For each sample, we used a double-bladed saw to cut a transverse section with a constant thickness of 2 mm. Wood density was measured with an indirect-reading X-ray densitometer (Polge 1966). Two orthogonal (longest and shortest axes) radial density profiles were obtained by analyzing the scanned images with WinDENDRO software (Guay et al. 1992). Ring limits were determined automatically, checked manually, and then corrected with this software. For each genotype, we derived three parameters from the distribution of wood density values (after removing the values corresponding to the pith), the mean value (D_{mean}), the 10th percentile (D_{min}) and the 90th percentile (D_{max}). D_{min} was strongly correlated with the mean density of earlywood, whereas D_{max} was correlated with the mean density of latewood (data not shown, $r > 0.8$). We carried out the analysis on both radial density profiles separately to check the impact of compression and normal wood on the variance estimation. Similar patterns were observed for both profiles (not in absolute values, but in terms of significance) because the sampled branches were orthotropic, so we pooled the data.

Biomass, collar diameter, leaf area, and cavitation resistance measurement

At the age of 3 years, all the saplings in six blocks (blocks 1–6) were cut at the collar position. Total

Table 1 Climatic data, location, and elevation of the studied maritime pine populations

Sampling location	Longitude (°)	Latitude (°)	n	Elevation (m)	P_i (mm)	T_m (°C)	VPD _{max} (hPa)	ETP (mm)
Bayubas de Abajo (Central Spain)	−2.87	41.52	39	955	561	10.5	11.42	882.9
Coca (Central Spain)	−4.08	41.37	40	788	452	11.9	14.23	718.7
Mimizan (South-western France)	−1.30	44.13	40	37	1176	13.2	7.26	751.59
Oria (South-eastern Spain)	−2.62	37.87	40	1232	451	13.4	14.29	922.59
San Cipriano de Ribarteme (Northern Spain)	−8.70	42.13	40	310	1625	13.8	8.54	721.91
Tamrabta (Southern Morocco)	−5.02	33.66	40	1760	550	15.1	18.56	976.54

n number of sampled individuals for wood density measurements, P_i mean annual precipitation, T_m , mean annual air temperature, VPD_{max} maximum water vapor pressure deficit (in July for all provenances), ETP annual sum of potential evapotranspiration

above-ground biomass (W_a , g), including the main stem, branches, and needles, was determined by weighing after the material had been dried in an oven at 55 °C for 72 h (Eveno 2008). Needle biomass (W_{needle} , g) was also determined separately. For this study, we used a subset of data corresponding to the selected populations.

On the other blocks (blocks 7–12), collar diameter (ϕ , mm) was measured on the selected populations and families only at the age of 6 years, in 2007. Specific leaf area (SLA, in $\text{m}^2 \text{kg}^{-1}$) was also determined for 20 needles per genotype. Total leaf area (A_{Leaf} in m^2) was estimated retrospectively, by multiplying total W_{needle} (blocks 1–6) by family mean of SLA (blocks 7–12). Resistance to cavitation was determined in a previous study (Lamy et al. 2011).

Quantitative genetic analysis

Genetic analysis was conducted with the following mixed model:

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{Z}_1\mathbf{pop} + \mathbf{Z}_2\mathbf{f} + \boldsymbol{\varepsilon}, \quad (1)$$

where \mathbf{y} is the vector of observation for a trait, \mathbf{b} is the vector (number of blocks) of fixed block effects, \mathbf{pop} is the vector (number of populations) of random population effects, \mathbf{f} is the vector (number of mother trees) of the random genetic effects of mother tree within the population, $\boldsymbol{\varepsilon}$ is the vector (number of individuals \times 1) of residuals, \mathbf{X} is called the design matrix, \mathbf{Z}_1 and \mathbf{Z}_2 are the incidence matrices linking the observations to the effects. A variance was fitted for each random effect: σ_{pop}^2 is the genetic variance between populations, $\sigma_{f(\text{pop})}^2$ is the genetic variance between mother trees nested within a population and σ_{ε}^2 is the residual variance for repeated analyses. As the density record for each year constitutes a new measurement for the same tree (Littell et al. 2000; Apiolaza and Garrick 2001), there is an autocorrelation between measurements, which was taken into account using a special variance–covariance \mathbf{R} matrix structure for repeated analysis. Several covariance structures were considered for specification of the \mathbf{R} matrix in the mixed model (autoregressive, heterogeneous autoregressive, first-order autoregressive moving-average, toeplitz, banded correlation). On the basis of Akaike's information criterion and Bayes' information criterion, a banded correlation structure for additive genetic

effects was found to be the most appropriate (data not shown). We also ran the analysis with the seed mass as covariate to control potential maternal effect, but it did not change the observed patterns.

Variance and covariance components were estimated by the restricted maximum likelihood (REML) method, assuming a normal distribution of the random effects. The significance of variance components was assessed in log-likelihood ratio tests. We included population as a random effect, for inference at the species level and to obtain an unbiased estimate of heritability and genetic population differentiation (Wilson 2008). The normality, identity, and independence of the residuals of each trait were checked graphically by plotting studentized marginal and conditional residuals. These plots confirmed that the data conformed to the assumptions of the mixed model. We estimated narrow-sense heritability as follows: $h_{\text{ns}}^2 = 4\sigma_{f(\text{pop})}^2 / (\sigma_{\varepsilon}^2 + \sigma_{f(\text{pop})}^2)$. In our study, σ_A^2 was estimated as $\sigma_A^2 = 4\sigma_{f(\text{pop})}^2$ because trees from the same family were presumed to be half-sibs (open-pollinated seeds). We did not include the population effect in the heritability calculation, because natural selection appeared to occur within each population (Visscher et al. 2008). The standard deviation of heritability was calculated with the delta method (Lynch and Walsh 1998). Phenotypic differentiation between populations, Q_{ST} (Spitze 1993), was estimated as $Q_{\text{ST}} = \sigma_{\text{pop}}^2 / (\sigma_{\text{pop}}^2 + 2\sigma_A^2)$. Variance components were standardized with the trait mean (Houle 1992) as follows: $\text{CV} = \frac{\sqrt{\sigma_{\text{trait}}^2}}{X_{\text{trait}}} 100$, where CV is the coefficient of variation. Each variance component was expressed with a CV (CV_A: additive coefficient of variation; CV_{BP} (V_{BP} = σ_{pop}^2): between-population coefficient of variation; CV_P: phenotypic coefficient of variation; CV_R: residual coefficient of variation). The variance of each component was extracted from the asymptotic covariance matrix. The significance of mean population differences was estimated with model (#1), with the GLM procedure and Student–Newman–Keuls post hoc tests.

Correlation between traits and climatic variable

Genetic correlations between traits were evaluated by calculating Pearson's coefficients for the family best

linear unbiased predictor (BLUP) estimate. These correlations are referred to hereafter as genetic correlations. Phenotypic correlations were evaluated by calculating Pearson's coefficients for the family BLUP plus the population BLUP and the grand-mean. Data from a previous study (Lamy et al. 2011) were also included, to assess the correlation between density parameters and cavitation resistance (estimated from P_{50} , the xylem pressure at which a sample lost 50 % of its conductance).

We also explore relationships between climatic variables from population origin and the population performance in the provenance-progeny trial. The first PCA axis was used as an aridity index because soil water deficit and evapotranspiration are the main contributors.

Q_{ST} and F_{ST} comparison

We investigated the contribution of selection to the differentiation of phenotypic traits, as previously described (Lamy et al. 2011) by comparing the distribution of phenotypic differentiation (Q_{ST}) with the distribution of genetic differentiation (F_{ST}) assessed with molecular markers assumed to be neutral. Briefly, the F_{ST} distribution (F_{ST}^*) was constructed from a dataset for eight neutral nuclear microsatellites previously genotyped in the populations studied (Eveno et al. 2008). Neutral expectations were simulated by randomly resampling 10^3 times with replacement between loci, to estimate the sampling variance of F_{ST} . Each F_{ST} replicate value was multiplied by a random number drawn from the (Lewontin and Krakauer 1973) distribution, which accounts for demographic deviations from the neutral model (Whitlock 2008; Whitlock and Guillaume 2009). Q_{ST} distributions (Q_{ST}^*) were constructed by performing a parametric bootstrap resampling procedure 10^3 times (O'Hara and Merilä 2005), making it possible to estimate the distribution of each variance component (σ_A^2 , σ_{pop}^2) by Satterthwaite's approximation (Satterthwaite 1946). Finally, the two resulting distributions were compared in a non-parametric test on the 2.5 and 97.5 quantiles (Kosorok 1999). A more classical Whitlock test was also performed. All analyses were performed with SAS software (2008, version 9.2 SAS Institute, Cary, NC, USA). Codes are available on request.

Results

Between-population variation

No significant difference between populations (V_{BP}) was detected for wood density (D_{mean} , D_{min} , and D_{max}), whereas significant differences were found for W_a , A_{Leaf} , and ϕ (Fig. 1; Table 2). Wood density-related traits had much lower coefficients of variation than W_a (Table 2), particularly at the between-population level ($CV_{BP} = 1\%$ and 19% for D_{mean} and W_a , respectively). The fixed block effect was significant for all the traits studied; indicating that environmental variations were significant and taken into account by the model.

The populations from the wettest areas (Mimizan and San Cipriano) had the highest A_{Leaf} and ϕ values (Fig. 1), whereas the Tamrabta population (from Morocco) had the lowest values. Iberian populations from the central and southern part of Spain (Coca, Bayubas, Oria) had intermediate values, with no detectable trend in terms of environmental aridity.

Within-population variation

Heritabilities and normalized measurements of trait dispersion (i.e., CVs) were estimated to evaluate within-population additive variance (through the analysis of CV_A) and micro-environmental sensitivity (through the analysis of CV_R) (Table 2). The values of narrow-sense heritability (h_{ns}^2) for D_{mean} (0.38 ± 0.20) and D_{max} (0.51 ± 0.20) indicated that wood density was moderately genetically determined, although the standard error was high, due to the small number of progenies per mother tree analyzed for this trait. The CVs of wood density and growth traits presented contrasting patterns, with a lower coefficient of additive variation ($CV_A \leq 7\%$) for wood density than for other traits ($CV_A > 20\%$), suggesting that evolvability is limited for wood density.

Evolutionary forces driving population differentiation

Comparisons of Q_{ST} and F_{ST} may have three possible outcomes (Spitze 1993): (i) if $Q_{ST} > F_{ST}$, the degree of differentiation for quantitative traits exceeds that attainable by genetic drift alone, (ii) if Q_{ST} and F_{ST} are not significantly different, the observed degree of

Table 2 Variance components (V_P , V_{BP} , V_A , V_R), narrow-sense heritability ($h_{ns}^2 \pm SE$), coefficient of variation (CV_P , CV_A , CV_{BP} , CV_R) and population differentiation (Q_{ST})

Traits	V_P	V_{BP}	V_A	V_R	h_{ns}^2	CV_P	CV_A	CV_{BP}	CV_R	Q_{ST}^a
D_{mean}	0.001	$1.9 \cdot 10^{-5}$ ns	$0.5 \cdot 10^{-3}$ *	0.001	0.38 ± 0.20	11	7	1	10	0.02*/**
D_{max}	0.001	$1.7 \cdot 10^{-5}$ ns	$0.7 \cdot 10^{-3}$ *	0.001	0.49 ± 0.20	10	7	1	9	0.01 */**
D_{min}	0.001	$1.6 \cdot 10^{-5}$ ns	$0.2 \cdot 10^{-3}$ ns	0.001	0.22 ± 0.21	12	5	1	11	0.03 */**
ϕ_{2007}	50.63	31.92***	17.55*	46.24	0.34 ± 0.12	37	22	29	36	0.47 ***/****
B_{tot}	16124	3246***	9207***	13822	0.57 ± 0.08	43	32	19	40	0.14 ns/ns
A_{Leaf}	0.427	0.104***	0.1817**	0.382	0.425 ± 0.14	37	24	35	35	0.22 */ns

h_{ns}^2 narrow-sense heritability and SE is the standard error of heritability, V_P phenotypic variance, V_A additive genetic variance, V_{BP} between-population variance, V_R residual variance, CV_A additive variation coefficient after adjustment for the block effect, CV_P phenotypic variation coefficient after adjustment for the block effect, CV_R residual coefficient of variation, CV_{BP} between-population coefficient of variation, Q_{ST} quantitative genetic variation between populations (Spitze 1993)

^a We provide P value for Q_{ST} and F_{ST} comparison based on Whitlock's method and our method based on Kosorok test

The significance of random effects is indicated after each variance estimator: ns $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

differentiation for quantitative traits could have been reached by genetic drift alone, and (iii) if $Q_{ST} < F_{ST}$, the observed degree of differentiation is lower than expected from genetic drift alone. Consistent with previous reports (Waldmann et al. 2005; O'Hara and Merilä 2005), we found that F_{ST}^* and Q_{ST}^* had skewed distributions (Fig. 2). Only ϕ and D_{mean} (together with D_{min} , D_{max} , data not shown) had a Q_{ST}^* distribution that differed from the F_{ST}^* distribution ($P < 0.0001$ and $P = 0.0008$, respectively). For, A_{Leaf} (and W_a , data not shown), the difference between Q_{ST}^* and F_{ST}^* values was centered on 0 (see Fig. 2c, right panel), and it was, therefore, not possible to distinguish between drift and selection ($P = 0.23$). Conversely, differences between the Q_{ST}^* and F_{ST}^* distributions for D_{mean} were centered on -0.13 , suggesting that the studied populations were less differentiated than would be expected in the presence of drift alone (Fig. 2a). Thus, natural selection favors the same phenotypic mean in different populations. For ϕ , the difference between Q_{ST}^* and F_{ST}^* distributions was centered on 0.34, suggesting that the studied populations displayed more differentiation than would be expected with drift alone (Fig. 2b). The population sampling protocol lead us to select population bearing different mitotypes and chlorotypes reflecting different evolutionary histories (Vendramin et al. 1998; Burban and Petit 2003). It is worth noticing that this procedure did not bias the population genetic differentiation upward because our F_{ST} estimation (0.10) was close to the lower bound of F_{ST} estimated for this species (0.10–0.14, Bucci et al. 2007).

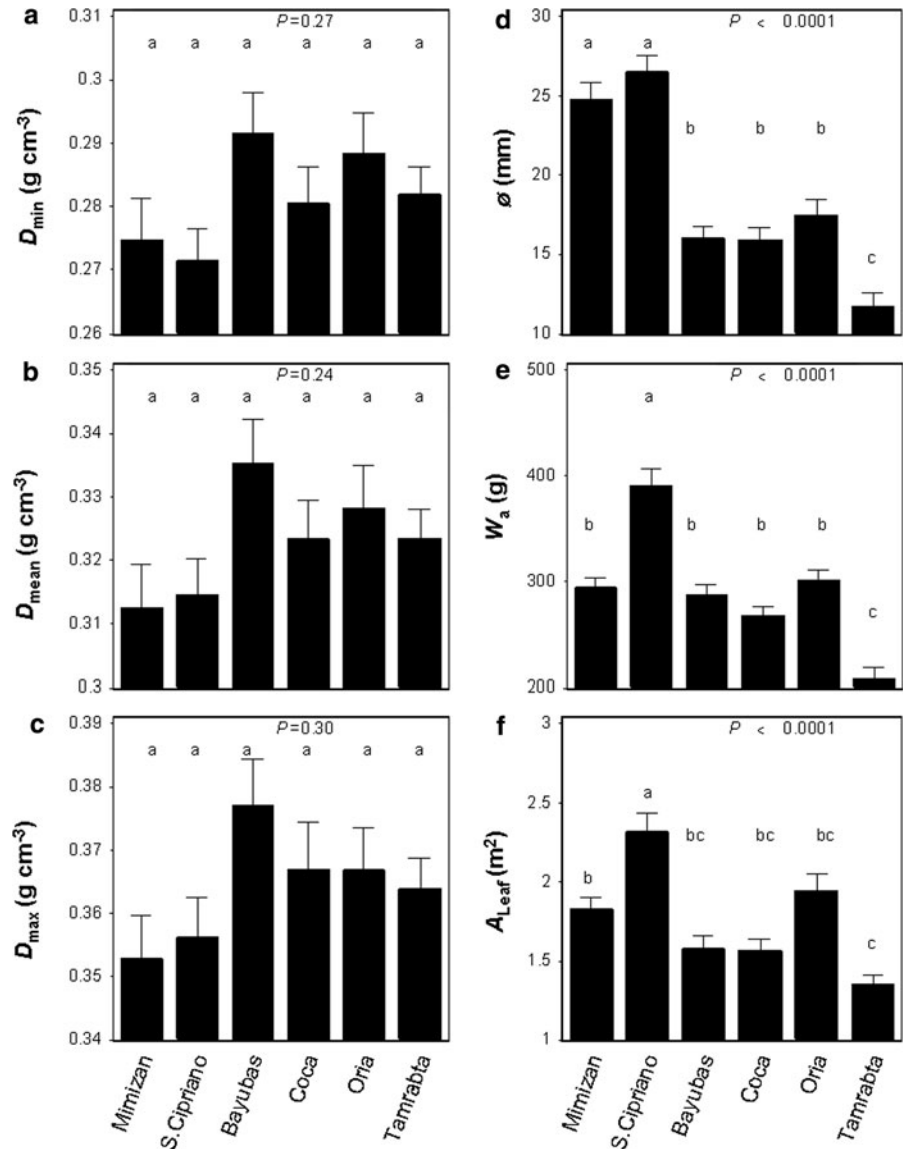
Correlation between traits and climate

We found a significant negative phenotypic correlation between D_{mean} and ϕ ($r = -0.33$, $P = 0.02$), indicating that individuals with denser wood tended to have a smaller stem diameter. However, the genetic correlation was not significant ($r = -0.20$, $P = 0.16$). We observed no significant phenotypic correlation between D_{mean} (or D_{max} and D_{min}) and cavitation resistance (P_{50}) (Fig. 3). No significant correlations were found between D_{mean} and W_a , A_{Leaf} . Soil water deficit and evapotranspiration are the main contributors to the first axis of the PCA of climatic variable at population origin (see “Plant material” section). Therefore, this axis represents an index of aridity for each provenance site. No significant relationships were found between population's traits and aridity, except for ϕ ($P = 0.02$, data not shown).

Discussion

We here evidenced that juvenile wood density (D_{min} , D_{mean} , and D_{max}) had a low additive variance and moderate heritability. Although the studied populations originated from contrasting climates and presented different evolutionary histories, no significant difference between populations was detected and the variability between populations was smaller than expected under genetic drift alone ($Q_{ST} < F_{ST}$). In other words, these populations from different climates

Fig. 1 Histogram showing, for each population, the 10th percentile of wood density distribution (D_{\min} **a**), mean values of mean wood density (D_{mean} **b**), 90th percentile of wood density distribution (D_{\max} **c**), stem diameter in 2007 (ϕ **d**), total above-ground tree dry biomass in 2005 (W_a **e**), total tree leaf (needle) area (A_{Leaf} **f**), for each studied population ($n = 40$ per population). Error bars standard errors. The P value is that for the mixed model and indicates whether the population effect is significant. Different letters indicate significant differences between populations at the $\alpha = 0.05$ level (from a fixed effect model)

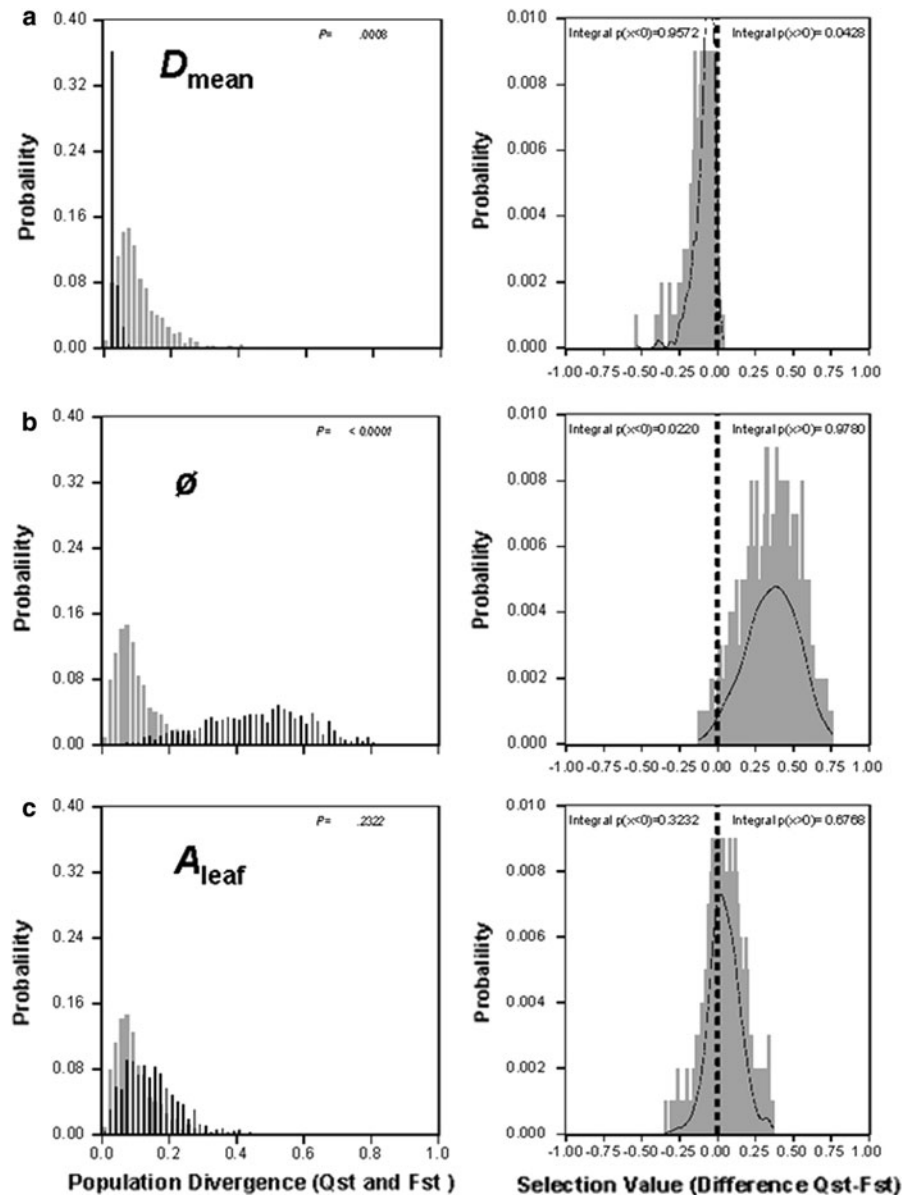


and with different evolutionary histories shared the same trait value, suggesting an evolutionary process had driven the trait variability. Conversely, growth (collar diameter) presented a higher additive genetic variance and differentiation between populations, consistent with the action of diversifying selection on the studied populations. Concerning other biomass estimates (W_a , A_{Leaf}), we were unable to disentangle the effects of genetic drift and natural selection. We discuss below the possible evolutionary mechanisms underlying the low degree of variability of juvenile wood density between populations.

Juvenile wood density variability

Our results showed that mean juvenile wood density did not change between *Pinus pinaster* ecotypes. Wood density variability has been thoroughly dissected in previous studies (Gapare et al. 2009), but generally in mature tree with a narrow genetic backgrounds (elite trees, crossing between local populations) or mature interspecific hybrids. Conversely, few studies have considered the between-population variability of wood density because this information is usually not required for classical

Fig. 2 Comparison between F_{ST} (histogram in gray) and Q_{ST} (histogram in black) distributions for mean wood density (D_{mean} a), stem diameter (ϕ b), total tree leaf (needle) area in 2006 (A_{leaf} c), in the left panel. The observed distribution (gray histogram) and kernel density (black curves) of the $Q_{ST} - F_{ST}$ difference are represented on the right panel for each trait. On the right panel, we also show the integral probability of the distribution (using the kernel density estimator) above (see “Integral $p(x > 0)$ ”) on the right panel) and below (see “Integral $p(x < 0)$ ”) on the right panel) zero (marked with the dashed and dotted lines)



breeding programs. In some instance, evolutionary biologists have compared between-population variability and expectations under genetic drift for wood density as for *Picea glauca*, *Eucalyptus globulus*, and *Pinus contorta* (Yang et al. 1996; Jaramillo-Correa et al. 2001; Steane et al. 2006). In some studies, between-population variation was detected, but comparisons with ours remain difficult because: (i) these previous studies were not designed to assess the level and structure of variation across a wide range of ecotypes. Population sampling was usually restricted

to a subset of the distribution area. The results were, therefore, biased toward low F_{ST} values (< 0.1). For instance, Jaramillo-Correa et al. (2001) obtained an F_{ST} of 0.02, much lower than that found by Tremblay and Simon (1989) ($F_{ST} = 0.11$) for broader sampling in *Picea Glauca*, (ii) the protocols for assessing wood density differ between studies: with the use of pilodyn (Steane et al. 2006) or the water displacement method to estimate wood-specific gravity (Yang et al. 1996), (iii) the age at which wood density was measured also differed considerably between studies. Apio

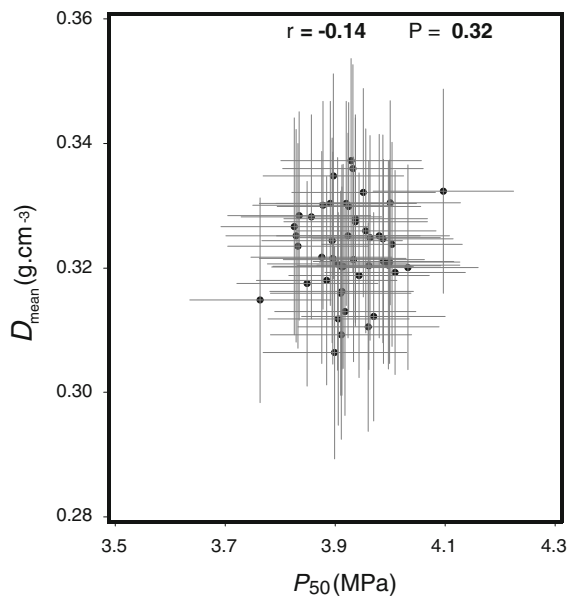


Fig. 3 Adjusted mean values of wood density (D_{mean}) versus cavitation resistance (P_{50} , xylem pressure inducing 50 % loss of hydraulic conductance). Dots correspond to the composite BLUP estimate and the error bar to the associated standard error ($n = 48$). The BLUP estimate for each family is calculated as the sum of population BLUP, family BLUP and the grand mean

(2011) and Zamudio et al. (2002) showed that genetic variability in *Pinus radiata* increases with age for wood density, and mature wood and juvenile wood are known to be the subject of different genetic controls (Plomion et al. 2001). The ring's age in our studies is much more younger compare to Jaramillo-Correa et al. (2001) and Yang et al. (1996), who worked on 22-year-old and 10-year-old trees, respectively. Our conclusions could, therefore, not be extrapolated to mature wood (and mature tree) even for *Pinus pinaster*. Low variability within population for juvenile wood density and the lack of genetic differentiation between populations may have different consequences; (i) breeding different ecotypes will not bring much more genetic variation for this trait; (ii) it may be more valuable to select for a better diameter growth and/or for a greater proportion of the latewood in the juvenile wood. It is important to keep in mind that all the above-mentioned studies, including our own, have sample size (<1,000 individuals) still far from the standard required by quantitative genetics theory ($\gg 1000$ individuals), more studies are needed to draw robust conclusions.

Impact of environment variation on wood density

Comparing CV_R between traits, it seems that wood density is less sensitive to environmental variance compare to growth-related traits. Wood density should exhibit some phenotypic plasticity but in a less extent compare to growth trait, and it may be possible that such phenotypic plasticity is stereotyped. To specifically study the reaction norm of mean wood density, multi-site approach would be valuable to draw robust conclusion about the environmental sensitivity of such trait (Vitasse et al. 2010). Another alternative to explore phenotypic plasticity is to study reaction norm of wood density along the whole profile (Martinez-Meier et al. 2011). It was not possible to investigate more deeply the reaction norm of wood density in this study because of the low number of wood rings (3-year-old branches). In addition, the extraction of phenotypic plasticity out of the total wood density signal is still a conceptual (no mechanistic model of wood density genesis) and statistical challenge (non-linearity with time). However, there are some pertinent approaches at the intra-annual level (Martinez-Meier et al. 2011).

Evolutionary significance of trait conservatism and underlying mechanisms

In our study, variance between populations (Q_{ST}) was lower than expected under a hypothesis of genetic drift (F_{ST} , from neutral markers), suggesting an evolutionary stasis for wood density. Such a pattern of variability was recently described for cavitation resistance-related traits in a study based on the same experiment (Lamy et al. 2011). Here, we demonstrated, using another complex trait (wood density), that functional traits are not always labile and prone to diversifying selection or homoplasy. Explaining trait conservatism (the extreme form of conservatism lead to stasis) is one of the most challenging questions in evolutionary biology (Bradshaw 1991). Uniform selection is often proposed as a classical explanation of narrow between-population trait variation resulting in evolutionary stasis. According to this model, independent stabilizing selection events act within each population, with the same selection optimum. However, this model provides no clues as to why the selection optimum should be the same. Genetic constraints (such as canalization) could also represent alternative explanations, particularly for

traits still displaying some degree of variability within populations (Gould and Lewontin 1979; Bradshaw 1991; Lamy et al. 2011). Simulations have also shown that the $Q_{ST} < F_{ST}$ pattern can be obtained for traits that are non additively genetically controlled (Goudet and Buchi 2006; Lopez-Fanjul et al. 2007). Deciphering uniform selection from canalization hypotheses is a difficult task. Across 21 *Pinus* species, Creese et al. (2011) have shown that wood density presented the lowest coefficient of variation among plant mass, hydraulic conductivity, and transpiring surface on the conductive surface ratio, and they found no relation with climatic variables despite a large range of mean annual precipitation (500–2,000 mm). If closely related species living in different climate have the same value of trait (i.e., evolutionary stasis), it is difficult to conceive that all the populations of each species share the same value of selective optima as it is assumed in the uniform selection hypothesis. Therefore, the alternative hypothesis that evolutionary stasis is due to a robust genetic architecture that narrows trait variability (i.e., canalization) should be considered with more attention.

Wood density and other adaptive traits

Juvenile wood density is negatively correlated with growth trait as diameter and/or height in mature tree (Bouffier et al. 2008). Between water use efficiency and wood density, the relationships are difficult to predict as a ring could be build up with the carbon from the previous years. Indeed, theoretically, the correlation is very loose between these two processes. For hydraulic safety traits (drought tolerance), at the interspecific level, Hacke and Sperry (2001) showed that cavitation resistance is correlated with wood density, species with higher wood density being more resistant to cavitation. In *Pseudotsuga menziesii* and *Picea abies* clones, a negative correlation was found between wood density and cavitation resistance (Rosner et al. 2008; Dalla-Salda et al. 2011). Even with considerable statistical power compared to these previous studies, we found no relationship between wood density and cavitation resistance within species (Fig. 3). In provenance tests, Corcuera et al. (2011) and Wortemann (2011) also found no relationship between wood density and cavitation resistance. In *Pinus sylvestris*, Martinez-Vilalta et al. (2009) did not detect any correlation between these traits in situ in

natural populations. We propose two hypotheses to account for these discrepancies: (i) despite the greater statistical power of our design, the relationship between cavitation resistance and wood density does not seem to be robust to environmental variation and genetic variation and (ii) from a physiological point of view, this lack of correlation is unsurprising, because there is no direct causal link between wood density and cavitation resistance (Delzon et al. 2010; Jansen et al. 2012) in *Pinus* genera. For instance, Delzon et al. (2010) showed that bordered pit dimensions are much more important for cavitation resistance than total tracheid lumen area. However, even if these two traits are not correlated, they have other features in common: they both have a low CV_A (<7 %) and display extremely low levels of variation between populations ($CV_{BP} \leq 1$ %), suggesting that this trend follows a general rule for wood anatomy-related traits. These findings indicate that additional, more detailed investigations of the naturally occurring variability of wood anatomy-related traits (e.g., cell wall thickness, aperture pit dimensions, torus diameter, margo pore diameter, etc.) are required to gain greater insight into canalization. However, it should be pointed out that our study focused on juvenile wood, and trait values at the start of ontogeny are known to be canalized (Milton et al. 2003; Sangster et al. 2008). Further studies are required to confirm that conservatism for mean wood density is also conserved in mature trees.

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