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Jose Manuel Torres Ruiz, Antoine Kremer, M. R. Carins-Murphy, T. J. Brodribb, Laurent Lamarque, Laura Truffaut, Fabrice Bonne, Alexis Ducouso, Sylvain Delzon

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1 **Genetic differentiation in functional traits among European sessile oak**  
2 **populations**

3 JM Torres-Ruiz<sup>1\*</sup>, A Kremer<sup>2</sup>, MR Carins-Murphy<sup>3</sup>, TJ Brodribb<sup>3</sup>, LJ Lamarque<sup>2</sup>, L  
4 Truffaut<sup>2</sup>, F Bonne<sup>4</sup>, A Ducouso<sup>2</sup> and S Delzon<sup>2</sup>.

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6 <sup>1</sup> Université Clermont-Auvergne, INRA, PIAF, 63000, Clermont-Ferrand, France.

7 <sup>2</sup>Unité Mixte de Recherche Biodiversité Genes & Communautés (UMR 1202 BIOGECO), Institut National  
8 de la Recherche Agronomique (INRA) – Université de Bordeaux, 69 route d’Arcachon, Cestas F-33610,  
9 France.

10 <sup>3</sup>School of Natural Sciences, University of Tasmania, Private Bag 55, Hobart, Tasmania 7001, Australia.

11 <sup>4</sup>UMR SILVA, INRA, Nancy, France.

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14 *\*author for correspondence:*

15 José M. Torres-Ruiz.

16 Université Clermont-Auvergne, INRA, PIAF, 63000, Clermont-Ferrand, France.

17 Email : [torresruizjm@gmail.com](mailto:torresruizjm@gmail.com)

18 Telephone: +33 4 43 76 14 06

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25 ecophysiology, plant functional traits.

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29 **Abstract**

30 The vulnerability of forest species and tree populations to climate change is related to the  
31 exposure of the ecosystem to extreme climatic conditions and to the adaptive capacity of  
32 the population to cope with those conditions. Adaptive capacity is a relatively under-  
33 researched topic within the forest science community and there is an urgent need to  
34 understand to what extent particular combinations of traits have been shaped by natural  
35 selection under climatic gradients, potentially resulting in adaptive multi-trait  
36 associations. Thus, our aim was to quantify genetic variation in several leaf and woody  
37 traits that may contribute to multi-trait associations in which intraspecific variation could  
38 represent a source for species adaptation to climate change. A multi-trait approach was  
39 performed using nine *Quercus petraea* provenances originating from different locations  
40 that cover most of the species' distribution range over Europe and that were grown in a  
41 common garden. Multiple adaptive differences were observed between oak provenances  
42 but also some evolutionary stasis. Also, our results revealed higher genetic differentiation  
43 in traits related to phenology and growth than in those related to xylem anatomy,  
44 physiology and hydraulics for which no genetic differentiation was observed. The  
45 multiple associations between those traits and climate variables resulting from  
46 multivariate and path analyses suggest a multi-trait association largely involving  
47 phenological and growth traits for *Quercus petraea*.

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## 54 INTRODUCTION

55 Climate change and the associated increase in mean temperature and reduction in  
56 precipitation are expected to induce significant shifts in species' distributions due to  
57 drought-induced population diebacks (Bertin 2008, Allen et al. 2010 201, Delzon et al.  
58 2013, Allen 2014). This has raised important concerns not only about our ability to predict  
59 population mortality and its impact on ecosystem function (McDowell et al. 2013,  
60 Cailleret et al. 2017) but also about the capacity of species to adapt in a timely manner to  
61 the expected warmer and drier climates (Corlett and Westcott 2013, Sáenz-Romero et al.  
62 2017, González-Muñoz et al. 2018). These concerns are especially relevant to forest  
63 species given, on the one hand, the rapid rate of environmental change and, on the other,  
64 the long life-span of most tree species (Aitken et al. 2008). Therefore, crucial questions  
65 for evaluating and predicting the consequences of ongoing climate change are i) which  
66 key traits can evolve within a few generations allowing the adaptation of trees to climate  
67 change?; and ii) how fast can such adaptation occur in response to a changing  
68 environment?

69 During the last decade, many studies have focused on addressing these questions by  
70 monitoring woody plants under controlled or induced environmental changes (Hoffmann  
71 and Sgrò 2011, Franks et al. 2014). As a result, different traits have been identified that  
72 respond phenotypically to temperature, thus making them potential targets for  
73 microevolution (e.g. Amano Tatsuya et al. 2010). Phenology is one of the most well-  
74 known sensitive indicators of climate change. In oaks, previous studies have shown  
75 genetic differentiation in bud phenology that has resulted in a shift of two to three days

76 in spring and 0.3 to 1.6 days in autumn per decade over the last 50 years, extending the  
77 growing season (Vitasse et al. 2009). Although the benefits can vary across species, it has  
78 been shown that individuals that flower early produce flowers throughout the entire  
79 growing season, thus maximizing fitness compared with late-flowering individuals  
80 (Anderson et al. 2012). However, much less is known about the genetic determinism of  
81 leaf functional traits in oaks such as stomatal density, leaf size and leaf thickness, that  
82 have significant influence on net carbon gain but also on plant water balance. The genetic  
83 determinism of xylem and hydraulic traits that are related to the hydraulic failure of the  
84 plant water transport system due to embolism formation is also largely unknown in oak  
85 despite embolism formation being considered to be one of the main mechanism leading  
86 drought-induced plant mortality (Brodribb and Cochard 2009, Urli et al. 2015, Salmon et  
87 al. 2015, Choat et al. 2018). Understanding intra-specific variation in these traits would  
88 therefore help us to evaluate the capacity of tree species to face and adapt to new  
89 environmental conditions induced by ongoing climate change.

90 In this study, we investigated intraspecific variation of numerous traits related to leaf  
91 phenology and physiology with the aim to better understand the potential for adaptation  
92 of oak populations. Indeed, genetic variation could help to ensure survival of at least some  
93 individuals or populations during extreme events, buffering the population or the species  
94 against extinction (Meireles et al. 2017).

95 Some of these traits have previously been assessed in common garden experiments and  
96 exhibited clinal genetic variation along geographic gradients as a result of diversifying  
97 selection (Vitasse et al. 2009, Alberto et al. 2011). Thus, divergent intraspecific profiles  
98 for “intrinsic” water-use efficiency (Farquhar and Richards 1984) have been linked to the  
99 distribution of genotypes across gradients in air humidity and soil water availability for  
100 different species (Pennington et al. 1999, Cregg and Zhang 2001, Aletà i Soler et al.

101 2009). Also, a previous study in oaks carried out in a common garden with populations  
102 from different locations along an elevation gradient showed how genetic differentiation  
103 accounted for up to 28% of total variation in traits such as leaf mass area and nitrogen  
104 content for European oak and beech (Bresson et al. 2011). However, some studies  
105 reported no evidence of genetic differentiation for growth traits between populations  
106 along an aridity gradient (Deacon and Cavender-Bares 2015, Ramírez-Valiente et al.  
107 2017). Environmental changes can also affect wood and water metabolism-related traits,  
108 such as wood density, vulnerability to embolism and water use efficiency (Mencuccini  
109 2003, Schume et al. 2004, Brienen et al. 2011). In fact, genetic differences in both wood  
110 density and vulnerability to embolism have been reported for some species (Arnold et al.  
111 2004, Sotelo Montes and Weber 2009, David-Schwartz et al. 2016) but not for others  
112 (Lamy et al. 2011).

113 In this contribution, we purposely implemented a multitrait approach by considering  
114 different functional traits related to either growth, phenology, structure (wood density),  
115 and physiology. Our aim was to investigate whether diversifying selection along climate  
116 gradients triggered specific associations of multiple traits whose genetic variation could  
117 represent a source for adaptation. Although previous studies have shown that some traits  
118 may follow clinal genetic variation along climatic gradients, their pattern of genetic  
119 variation has never been investigated collectively in a single and large common garden.  
120 We studied nine *Quercus petraea* L. provenances diverging from a common source  
121 population (from the last glacial period, 15000 years BP) and originating from different  
122 locations that cover most of the species' distribution range over Europe and grown in a  
123 common garden. We focused on a paneuropean oak species: *Quercus petraea* (sessile  
124 oak). This species has been extensively monitored at the molecular level to document  
125 spatial and temporal differences across Europe, but to a much lesser extent at the

126 phenotypic level, which was our main aim. Also, *Quercus petraea* is an ideal species for  
127 drawing adaptive inferences based on genetic divergence. Indeed, recent  
128 microevolutionary patterns of variation were shown to be less blurred by historical or  
129 demographic noise in oaks in comparison to other species, due to their very low level of  
130 subdivision on the basis of neutral markers (Firmat et al. 2017).

131

## 132 MATERIAL AND METHODS

### 133 Common-garden experiment

134 This study utilised a common garden experiment that was planted in 1989 and 1993 in  
135 the Forêt Domaniale de Sillégné (France) which contains 107 sessile oak provenances  
136 (Ducousso et al. 1996). From these, 9 provenances diverging from a common source  
137 population and representing different climatic regions within the distribution range of the  
138 species in Europe, from Northern Germany to Southern France, were selected for this  
139 study (Fig. 1 and Table 1, climate data source: Worldclim; period: 1960-1990). The initial  
140 density of the plantation was 1904 individuals per hectare (spacing 3 m × 1.75 m) with  
141 each provenance replicated from ten to fifteen plots with 24 trees per plot. At the time of  
142 the study, most of the trees were 25 year-old and 10 m tall on average (see details about  
143 plantation years in Table 1). Although sample size differed between the different traits  
144 studied because of varying complexity of measurements, all traits were evaluated in the  
145 exact same set of trees per provenance.

### 146 *Phenology and tree height*

147 We monitored spring and fall phenology in the nine selected provenances in 2014. Leaf  
148 unfolding (LU) in spring was monitored every ten days in 25 individuals per provenance

149 distributed in 7 to 12 replicated plots. Leaf senescence (LS) was monitored two times in  
150 late September and mid-October 2014 in the same individuals monitored for LU.  
151 Phenological observations were made using binoculars (magnifying power: 109) at a  
152 distance of approximately 10 m from each tree, by the same observer. In spring, we  
153 recorded the development stages from bud dormancy to leaf unfolding, using a scale with  
154 five intermediate stages according to Vitasse *et al.* (2009). We considered that a bud had  
155 reached leaf unfolding (LU) stage when at least one of its leaves was fully unfolded. At  
156 the tree level, LU date was determined when 50% of the buds had reached this threshold.  
157 In fall, due to the low number of field campaigns, we were not able to estimate a date of  
158 LS and therefore used the score of LS obtained during the October campaign (the  
159 September campaign was not discriminative enough). The senescence score corresponds  
160 in percentage to the amount of non-functional leaves, either coloured or fallen according  
161 to Vitasse *et al.* (2009). Height of all individuals was measured in January 2015.

162 *Mean leaf area, specific leaf area and wood density*

163 Mean leaf area (MLA, mm<sup>2</sup>) and specific leaf area (SLA, m<sup>2</sup> kg<sup>-1</sup>) was determined for 22  
164 to 28 trees per provenance randomly selected in 7 to 12 blocks per provenance and with  
165 at least 1 tree per block. Between 10 to 15 fully expanded and non-damaged leaves were  
166 collected per tree. To avoid any possible effects of different light exposure within the  
167 crown on leaf traits, all samples were collected from the upper and outer part of the crown  
168 to ensure a similar light exposure among them. All leaves were collected within 48 hours  
169 starting on June 14<sup>th</sup>, 2014. They were collected from a single branch per tree using a pole  
170 pruner or by shooting them down. Immediately after being collected, leaves were placed  
171 in sealed plastic bags to avoid desiccation, stored in cooling boxes and transported to the  
172 lab. Once in the lab, 6 to 8 leaves per tree were scanned to measure the area of each  
173 individual leaf using a desktop scanner (Expression 10000 XL, Epson, Japan) and



174 WinFolia software (Regent Instruments Inc., Quebec, Canada). For determining the SLA,  
175 the dry mass of leaves was measured after drying them in an oven at 65°C until a constant  
176 mass was reached. The SLA was assessed as the ratio of the leaf area to its mass.  
177 Individual leaf data were later averaged over all leaves per tree.

178 Wood density was estimated using X-ray imagery (Polge 1966) on a section of  
179 dry branch. One ca. 10-cm long branch segment per individual was collected from 4 to  
180 16 individuals per provenance. As for leaves, to avoid any possible effects of different  
181 light exposure within the crown on wood density, all branches were collected from the  
182 upper and outer part of the crown to ensure similar light exposure conditions among them.  
183 All branches had the same age (two years-old) within and between provenances. For each  
184 sample, we used a double-bladed saw to cut a transverse section with a constant thickness  
185 of 2 mm. Wood density was measured on the transverse section by using an x-ray image  
186 calibration procedure. Sections were exposed to X rays and were then scanned with a  
187 microdensitometer. Images were analysed using Windendro (Guay et al. 1992) to obtain  
188 two radial density profiles per section. Ring limits were determined automatically,  
189 checked manually, and then corrected with this software. We then calculated mean wood  
190 density ( $D$ , g cm<sup>-3</sup>).

#### 191 *Stable C and N isotopes analysis*

192 After MLA and SLA measurements, the same leaves were used for determining the  
193 carbon and nitrogen content (C and N g/kg, respectively) and isotopic discrimination  
194 ( $\Delta^{13}\text{C}$  and  $\Delta^{15}\text{N}$  for C and N, respectively). The dry samples were ground to powder  
195 using a wood grinding sample system (Labman, Stokesley, North Yorkshire, UK) and put  
196 into a tin capsule for mass spectrometry. The C and N isotope ratios as well as C and N  
197 contents were measured on 3 mg samples at the Microbiology & Agronomics Platform at  
198 INRA Reims (France) by using an isotope ratio mass spectrometer (Delta Advantage,

199 Thermo Scientific, Bremen Germany). The carbon isotopic composition expressed as  
200  $\delta^{13}\text{C}$  in ‰ (Craig 1957) was then converted into carbon discrimination  $\Delta^{13}\text{C}$  in ‰  
201 (Farquhar and Richards 1984). The  $\Delta^{13}\text{C}$  values were corrected for the Suess Effect  
202 (decrease in  $\delta^{13}\text{C}$  of atmospheric  $\text{CO}_2$  since the beginning of industrialization) resulting  
203 from the emission of fossil carbon dioxide, which is depleted in  $^{13}\text{C}$  (Francey et al. 1999,  
204 McCarroll and Loader 2004) even if the sampling has been done at the same date.  $\Delta^{13}\text{C}$   
205 can be related to the ratio of  $\text{CO}_2$  assimilation ( $A$ ) to stomatal conductance ( $g_s$ ), also  
206 named the intrinsic water-use efficiency.

#### 207 *Leaf vein and stomatal density*

208 Vein density (total vein length per  $\text{mm}^2$  of leaf area) was determined from paradermal  
209 sections of five fresh leaves (one leaf per tree, five trees per provenance) similar to those  
210 used for MLA and SLA. Sections were prepared and measured following the protocols  
211 described by Carins Murphy et al. (2012). In brief, this involved removing the adaxial  
212 epidermis and palisade tissue, clearing all pigment with bleach, and measuring VD from  
213 slide mounts of the sections using image analysis of digital photomicrographs (5 fields of  
214 view per section). Stomatal density (total stomata per  $\text{mm}^2$  of leaf area) was also  
215 determined from cuticles (1 per leaf and 5 fields of view per cuticle) prepared and  
216 measured following the protocols of Carins Murphy et al. (2012).

#### 217 *Vulnerability to embolism*

218 Due to the complexity of these measurements, vulnerability to xylem embolism was  
219 determined in three out of the 9 selected provenances and one extra provenance  
220 (originally from Ireland) for which the aridity of the provenance origin was much lower  
221 (Table 1). For each provenance, 15 individuals randomly selected in 7 to 9 blocks per  
222 provenance and with at least 1 tree per block were evaluated in June/July 2015. These

223 four provenances were selected according to their aridity index (AI) which was calculated  
224 as:

$$225 \quad \text{AI} = \text{MAP} / \text{MAE}$$

226 where MAP and MAE represent the mean annual precipitation and mean annual potential  
227 evapotranspiration, respectively. The four provenances selected were Grésigne  
228 (Southeastern France), Killarney (Southern Ireland), Vachères (Southwestern France) and  
229 Göhrde (Northern Germany), and represent different climatic regions, ranging from a dry  
230 Mediterranean region in France to a continental temperate climate in Germany (plus the  
231 additional provenance from a cool and humid oceanic climate in Ireland).

232 Xylem vulnerability to embolism was measured using the Cavitron technique  
233 (Cochard, 2002; Cochard, 2005) at the Caviplace laboratory (GENOBOIS platform,  
234 INRA-University of Bordeaux, France). To prevent artefactual losses in hydraulic  
235 conductance due to the induction of embolism during the sample preparation (Torres-  
236 Ruiz et al. 2015) or the presence of open vessels in the samples (Torres-Ruiz, Cochard,  
237 Choat, et al. 2017), >2 m-long branches were collected from the trees (one branch per  
238 tree), wrapped in moist paper and plastic bags to kill transpiration and transported to the  
239 laboratory. Once in the lab, branches were progressively recut under water to release the  
240 xylem tension according to Torres-Ruiz et al. (2015) and to adjust them to a 1 m-long  
241 length. Branches were debarked at both ends and installed in a large cavitron equipped  
242 with a 1 m-diameter custom-built honeycomb rotor (DGMeca, Gradignan, France; (Lobo  
243 et al. 2018). Several branches were used to test the presence of open vessels by air  
244 injection at 2 bars and none of them presented open vessels in 1 m long branches. Samples  
245 were spun for three minutes at a given speed to decrease the xylem pressure progressively  
246 at its centre from -0.8 MPa to -10.5 MPa (those pressures correspond to centrifugation  
247 rotation from 764 rpm to 2768 rpm respectively). Vulnerability curves to embolism were

248 generated by plotting the percentage loss of hydraulic conductivity (PLC) at the different  
249 target pressures applied and fitting a sigmoidal equation (Pammenter and Van der  
250 Willigen 1998). Mean  $P_{50}$  values, i.e. the xylem pressure inducing 50% of PLC, were  
251 obtained by averaging the values of 13 to 15 samples per provenance. The  $P_{50}$  value is  
252 commonly used as a proxy for tree drought resistance: the lower the  $P_{50}$  value, the more  
253 drought tolerant the species (Delzon 2015, Torres-Ruiz, Cochard, Fonseca, et al. 2017).

#### 254 *Statistical analyses*

255 Differences in phenological, physiological, anatomical and hydraulic traits among the  
256 nine provenances of *Q. petraea* were tested with a generalized linear mixed model  
257 (MIXED procedure, restricted maximum likelihood (REML) method in SAS, version 9.4,  
258 SAS Institute, NC, USA) where plots and provenances were respectively treated as fixed  
259 and random factors. The provenance effect was further assessed using a log likelihood  
260 ratio test from the full and reduced models (Littell et al. 2007). The data were checked to  
261 satisfy the assumptions of normality and homogeneous variance prior to analyses. The  
262 ratio of the provenance variance component to total variance was estimated according to  
263 Vitasse et al. (2009) using the VARCOMP procedure with the restricted maximum  
264 likelihood (REML). These analyses used the following statistical model:  $Y_{ijk} = \mu + P_i +$   
265  $b_j + (Pb)_{ij} + \varepsilon_{ijk}$ , with  $Y_{ijk}$  being the observed trait of the seedling  $k$  from the provenance  
266  $i$  and block  $j$ ,  $\mu$  the overall mean of the analysed characters,  $P_i$  the random effect of  
267 provenance  $i$ ,  $b_j$  the fixed effect of block (here replicated plot)  $j$ ,  $(Pb)_{ij}$  the interaction  
268 between provenance  $i$  and block  $j$ , and  $\varepsilon_{ijk}$  the residual variation including the effect of  
269 tree  $k$  belonging to combination  $ijk$ . Variances of random effects (provenance  $\sigma_P^2$ ,  
270 interaction  $\sigma_{bP}^2$ , residual  $\sigma_\varepsilon^2$ ) were also computed. The overall differentiation among  
271 provenances ( $D$ ) was calculated as the ratio (%) of the variance component of provenance  
272 to total variance estimated by analysis of variance, i.e as  $\sigma_P^2 / (\sigma_P^2 + \sigma_{bP}^2 + \sigma_\varepsilon^2)$ , and

273 varied from 0 to 100.  $D$  is an analog of  $Q_{st}$  (Spitze 1993), which is the genetic  
274 differentiation of quantitative traits ( $Q_{st} = \sigma_P^2 / (\sigma_P^2 + 2\sigma_A^2)$ , where  $\sigma_A^2$  is the within  
275 provenance additive variance. In our study based on provenance and not descendant test,  
276  $\sigma_A^2$  could not be estimated and we thus used the overall within population phenotypic  
277 variance (i.e.  $D$ ) rather than the overall genetic variance as it is for  $Q_{st}$  (Vitasse et al. 2009,  
278 Bresson et al. 2011).

279 The variability of each trait was evaluated by estimating both the intra- and inter-  
280 provenance coefficient of variation. The  $CV_{intra}$  was calculated for each provenance and  
281 then averaged at the species level, while the  $CV_{inter}$  was obtained from the between-  
282 provenance standard deviation and the overall mean value.

283 Weighted linear regression analyses were used to assess (i) relationships between  
284 the functional traits that showed significant differences among provenances, and (ii)  
285 whether genetic variation was explained by the local environmental conditions (i.e.  
286 latitude, temperature and precipitation) of the provenances' origin. Also, relationships  
287 between phenological and functional traits with climate variables were investigated using  
288 principal component analysis (PCA) in order to identify patterns of interrelationships.  
289 Finally, a path analysis was carried out to test different conceptual models that could  
290 explain how the climate at the original location of each provenance determines the  
291 variance of the different functional traits. Those models were based on different  
292 hypotheses considering only the traits that were the most influenced by temperature and  
293 precipitation according to the PCA analyses (**Supplementary information** Fig. S3). The  
294 best model based on the Akaike information criterion (AIC) would therefore provide  
295 valuable information about the associations of climate and multitrait values.

296

## 297 **RESULTS**

298 *Genetic differentiation*

299 Significant genetic differentiation between provenances was observed in four out of the  
300 13 studied traits (Table 2). The provenances differ in both the timing of leaf spring and  
301 autumn phenology (leaf unfolding and senescence, respectively). Tree height (H) and  
302 SLA were also significantly different between provenances, whereas a lack of genetic  
303 differentiation was found for MLA,  $\Delta^{13}\text{C}$ ,  $\Delta^{15}\text{N}$ , leaf C and N content, stomatal density,  
304  $P_{50}$ , vein density and wood density. Overall genetic differentiation for the different traits  
305 evaluated ranged from 0 up to 79. Thus, SLA and leaf unfolding amounted the highest  
306 values with 79 and 62, respectively, whereas it was weaker for, leaf senescence (32) and  
307 MLA (5). A null (0.0) overall differentiation was observed for all the other traits (Table  
308 2). Most traits showed, to a greater or a lesser extent, higher coefficient of variation across  
309 provenances than within provenances (Table 2). Only leaf vein density showed a slightly  
310 higher variation within (9) than across provenances (8).

312 *Genetic clines*

313 Correlations between traits and the climate and latitude of provenance origin reveal some  
314 relevant trends (Fig 2). Thus, positive and negative trends were observed between leaf  
315 unfolding and both latitude and temperature of the provenance origin, respectively. Thus,  
316 the earliest provenance to begin leaf flushing was the one originating from the southern  
317 margin (Latitude 43.98°) whereas the latest was one from northern Germany (Latitude  
318 53.18°). Both the mean annual maximum and minimum temperatures correlate with the  
319 date of leaf unfolding, with earlier budburst in provenances from warmer areas. Also, late  
320 leaf unfolding was observed in areas with lower precipitation. Thus, for the Grésigne  
321 provenance with a mean annual precipitation of 806 mm leaf unfolding occurred in DOY  
322 (day of year) 93.7, whereas for Göhrde with a precipitation of 629 mm/year it occurred

323 in DOY 104.2, i.e 10.5 days later than for Grésigne. Concerning leaf shedding, while  
324 significant genetic differentiation was observed between provenances (Table 2) no  
325 significant cline with the climate of provenances was detected (**Supplementary**  
326 **information** Table S1). A similar pattern as in LU was observed for SLA, with higher  
327 values in provenances originating from higher latitudes and colder and drier areas. Thus,  
328 mean SLA ranged from 9.95 to 12.39 m<sup>2</sup> kg<sup>-1</sup> within a range in temperature of 17.14 to  
329 4.42°C and in total annual precipitation of 838 to 598mm. Tree height, however, was not  
330 significantly affected by latitude or maximum temperature, but significantly increased  
331 with increasing minimum temperature and precipitation. Thus, trees are taller (up to 10.74  
332 m) in provenances with higher minimum temperatures (6.8°C) and annual precipitation  
333 (838 mm) (Fig. 2). Interestingly, xylem resistance to embolism showed similar  $P_{50}$  values  
334 across the four provenances evaluated (i.e. no genetic differentiation, Fig. 3A), but a  
335 significant correlation with mean annual temperature (MAT), showing increased  
336 resistance to embolism with higher MAT (Fig. 3B). Surprisingly, aridity index did not  
337 correlate with any of the evaluated traits (**Supplementary information** Table S1).

338

339 *Relationships between phenological and functional traits, and climate variables.*

340 Only a few significant correlations were observed between those traits exhibiting a  
341 provenance effect (Table 3). Thus, tight correlations were observed between LS and H  
342 (Fig. 4A) and between SLA and leaf unfolding (Fig. 4B). Thus, lower SLA was observed  
343 in those provenances with an earlier flushing, with Grésigne having the lowest SLA value  
344 (9.9 m<sup>2</sup> kg<sup>-1</sup>) and flushing in DOY= 94. On the contrary, the highest SLA values was  
345 observed for Lappwald (12.4 m<sup>2</sup> kg<sup>-1</sup>) that flushed in DOY= 103. The correlation between  
346 LS and H was observed (Fig.4A) reporting a lower percentage of coloured leaves on in  
347 taller trees. The PCA showed how the first two principal components together explained



348 59.2% of the variance (Fig. 5A). Thus, Axis 1 (variance 37.5%) was strongly associated  
349 with the main climate variables, i.e. maximum and minimum temperature and  
350 precipitation, as well as with functional leaf traits leaf unfolding,  $P_{50}$ , SLA and N content  
351 (Fig. 5B). Axis 2 (variance 21.7%), on the contrary, was more defined by the variance in  
352 leaf senescence, MLA,  $\Delta 15N$  and tree height than by climate variables (**Supplementary**  
353 **information** Fig. S2). When running the path analyses to detect associations between the  
354 main climate variables and the functional traits we found that the minimum temperature  
355 at the provenance origin seemed to be the most relevant trait determining their leaf  
356 unfolding, SLA and N content values (Fig. 6).

357

## 358 **DISCUSSION**

359 Nine provenances of *Q. petraea* originating from an extensive portion of the species'  
360 overall distribution in Europe showed significant genetic differentiation in traits related  
361 to leaf phenology, morphology and growth but not in traits related to xylem anatomy and  
362 hydraulics when grown in a common-garden. Phenology and some functional traits such  
363 as  $P_{50}$  also showed significant clines with the latitude and climate of provenance origin.  
364 Results from PCA and path analyses reported multiple associations between climate  
365 variables and both phenology and functional traits that would be driven by a diversifying  
366 selection along the studied climatic gradient.

### 367 *Multi-trait associations*

368 The effect of climate change on phenological traits has been extensively studied (Peñuelas  
369 et al. 2002, Gordo and Sanz 2005), especially the effect of temperature on the timing of  
370 leaf unfolding and flowering, which both occur earlier as temperature rises (Doi and  
371 Katano 2008, Chung et al. 2013). However, unlike this study, many previous studies have



372 been carried out ‘in situ’, and thus do not allow for any attribution of trait variation to  
373 either plasticity or genetic differentiation. Our results from trees grown in a common  
374 garden showed a significant cline between leaf unfolding and the climate of provenance  
375 origin, with earlier bud burst in provenances from the warm margin. Therefore, local  
376 adaptation of phenology to the environmental conditions is observed for *Q. petraea*.  
377 Similar results have been already reported for different tree species including oaks  
378 (Menzel and Fabian 1999, Matsumoto et al. 2003, Vitasse et al. 2009), showing the  
379 important role of local temperature in driving adaptation of the growing season length.  
380 This may partially explain the significant differences in growth (i.e. tree height) observed  
381 between provenances although a more complete understanding of the role of the  
382 vegetative growth period length and assimilation rates on the final growth of the  
383 populations would require a more focused study. In fact, our results also agree with a  
384 previous study by Kuster et al. (2014) in which three different oak species were grown  
385 on two different soils and exposed to air warming and drought. Their results showed  
386 earlier leaf unfolding for trees exposed to higher air temperatures, leading to an earlier  
387 start of shoot growth, in that they estimated an advance in leaf unfolding of 1-3 days °C<sup>-1</sup>.  
388 Our results also agree with those reported by (Vitasse et al. 2009) who observed negative  
389 genetic clines in leaf unfolding with increasing temperature at the origin of the  
390 provenance for *Quercus petraea*. More recent studies (Alberto et al. 2011, Firmat et al.  
391 2017) reported a similar genetic cline for both germination and bud burst timing with  
392 provenance elevation in *Q. petraea*, showing that spring leaf phenological traits are  
393 critical for local adaptation in oaks. Those genetic variations in leaf phenology observed  
394 in oaks are probably due to differences in heat requirement for bud burst rather than  
395 differences in chilling-associated with leaf shedding (Dantec et al. 2015).

396 In contrast with other studies (Vitasse et al. 2009), a clinal trend with temperature  
397 was not observed for leaf senescence in our study despite the significant variation in this  
398 trait among provenances and despite showing the highest between-provenance variation  
399 value. This is confirmed by the results from the PCA showing that, contrary to leaf  
400 unfolding, the variation in leaf senescence is barely determined by the temperature and  
401 precipitation regimes of the provenances' origin.

402 Significant genetic differentiation in SLA, a trait directly related to light  
403 conditions and nutrient availability (Milla et al. 2008), was observed between  
404 provenances, indicating genetic variation among provenances induced by the climate at  
405 their original location. Similar to “in situ” observations from Bresson et al. (2011) for  
406 *Quercus petraea*, we observed a negative correlation between SLA and both temperature  
407 and precipitation. In fact, results from the PCA show exactly this: a high influence of the  
408 minimum and maximum temperatures and precipitation not only on SLA, but also on  
409 other traits such as  $P_{50}$  and leaf unfolding. Intraspecific variation in SLA with air  
410 temperature and precipitation have been already reported for other species including  
411 maize and wheat for which, under N fertilization and irrigation controlled conditions,  
412 responses to both climate variables explained 43.7 % of the variation in SLA (Martin et  
413 al. 2018). Earlier leaf unfolding would increase leaf lifespan which has been reported to  
414 be correlated with SLA. Thus, across species, Reich et al. (1991) showed that species  
415 with short leaf lifespan generally have thinner leaves (high SLA) than those with longer  
416 leaf lifespan, which agrees with our results within species. As the authors suggested, the  
417 correlation between leaf lifespan, and therefore leaf unfolding, and SLA would result  
418 from different allocation trade-offs between species to enhance productivity, nutrient  
419 conservation or defence. This could also be the case at the intraspecific level and may  
420 explain observed differences between provenances. The relevance of the adaptation of

421 SLA to climate resides in the fact that it may contribute to a species' ability to adjust to  
422 different air temperatures and precipitation regimes (Albert et al. 2010, Long et al. 2011),  
423 because SLA is a key plant functional trait reflecting the trade-off between resource  
424 capture and conservation (Wright et al. 2004). Interestingly, no genetic differences were  
425 found in leaf size between provenances. This finding links with a recent study evaluating  
426 the bivariate leaf size-climate relationships for 7670 plant species from 682 sites  
427 worldwide that shows how day and night time leaf-to-air temperature differences are key  
428 to explaining the latitudinal gradient in leaf size (Wright et al. 2017). So, considering the  
429 results from Wright et al. (2017) and our results, latitudinal changes in leaf size would be  
430 likely due to phenotypic plasticity rather than to genetic variation.

#### 431 *Lack of evidence for local adaptation*

432 Leaf vein and stomatal density are directly linked with plant transpiration  
433 (Brodribb et al. 2007, Franks and Beerling 2009) and also highly influenced by  
434 environmental factors (Woodward and Bazzaz 1988, Uhl and Mosbrugger 1999), with  
435 higher stomatal and venation densities in drier areas (Herbig and Kull 1992, Carlson et  
436 al. 2016). Furthermore, both traits, tend to remain proportional during leaf acclimation to  
437 light intensity and VPD in woody angiosperm species (Brodribb and Jordan 2011, Carins  
438 Murphy et al. 2012, 2014, 2016). However, the determinism of these traits has received  
439 limited attention so far (Zhu et al. 2012). Assuming these traits varied between  
440 provenances in situ, which is likely given the capacity for acclimation in vein density to  
441 variation in evaporative demand among the upper and lower canopy in the closely related  
442 *Quercus rubra* (Zwieniecki et al. 2004), our results suggest that leaf capacity to acclimate  
443 to different conditions via changes to these anatomical traits is not translated into genetic  
444 differences. In fact, there is no evidence for genetically driven effects on stomatal density  
445 in *Quercus petraea* L. and *Fagus sylvatica*, although the phenotypic effect of temperature

446 is large (Bresson et al. 2011). However, contrary to this, vein density was found to be  
447 genotypically fixed in *Quercus variabilis* (Zhu et al. 2012). A possible explanation for  
448 our observation that these traits that tend to show large phenotypic variation appear to  
449 have not responded to divergent selection here is that the populations sampled could have  
450 not spanned all the variation within the distribution area of this species. However, an  
451 efficient phenotypic plasticity response could also have had an important role in the lack  
452 of variation observed for these traits since it would lead to convergence among  
453 populations in trait expression when grown in a common garden. An alternative  
454 explanation is that other traits not measured in this study but that also contribute to leaf  
455 water supply and demand varied among provenances. For example, stomatal size, along  
456 with stomatal density, determines stomatal conductance to water vapour. Likewise, the  
457 distance between vein tips and stomata, along with the horizontal spacing of veins,  
458 determines the length of the post-vein pathway from the end of the vascular system to the  
459 sites of evaporation in the leaf which itself is correlated with leaf hydraulic conductance  
460 (Brodribb et al. 2007). In this study differences were found in SLA but not in leaf size  
461 implying that leaf thickness varied among provenances. This may have altered the post-  
462 vein pathlength for water. However, the distance between veins tends to equal the distance  
463 between veins and the evaporative surface in derived angiosperms (Zwieniecki and Boyce  
464 2014). More detailed study is therefore required to determine whether the lack of variation  
465 in vein and stomatal density among provenances is due to strong plasticity or genetic  
466 changes in other leaf traits.

467 In many cases, traits exhibiting plasticity also show genetic differentiation  
468 (Kremer et al. 2013), unless their heritability is extremely low. Therefore, an interesting  
469 follow up of this study would be to explore whether there is enough genetic diversity

470 within populations on which diversifying selection may act since it may be very important  
471 for the species potential to adapt to future changes in climate.

472         Increases in shoot growth and leaf area allocation due to higher temperatures result  
473 in decreases in plant N concentration and leaf N content (Weih and Karlsson 2002). This  
474 explains the negative correlation reported by the PCA between leaf N content and  
475 temperature and precipitation for the different provenances. In fact, from all the models  
476 tested to identify which climate variable determines the variance in the functional traits  
477 most influenced by climate, the one that fits best with our results suggests that minimum  
478 temperature is the main climate variable determining leaf N content, SLA and leaf  
479 unfolding for each provenance.

480         Water Use Efficiency (WUE), which corresponds to the ratio of biomass  
481 produced to the rate of transpiration can be estimated by measuring the carbon isotope  
482 discrimination. While previous studies in oak showed intraspecific genetic variations in  
483  $\Delta^{13}\text{C}$  in *Quercus* (*Q. robur* and *Q. petraea*, Ponton et al. 2002) (*Q. robur* and *Q.*  
484 *pyrenaica*, Granda et al. 2018) in *Pinus pinaster* (Lamy et al. 2014) and in *Populus nigra*  
485 (Guet et al. 2015), our results showed no significant genetic differentiation between  
486 provenances in *Quercus petraea*. The lack of genetic differentiation between populations  
487 might be explained by the advantage of increased efficiency of water use in conserving  
488 soil moisture, which could be more predominant in Mediterranean and semiarid climates.  
489 Our results however agree with those reported by Chamaillard et al. (2011) showing no  
490 differences in leaf C content among three populations of *Populus nigra* grown in a  
491 common garden, even when they were subjected to different water treatments. Whether  
492 our target species, *Quercus petraea*, is less prone to the occurrence of genetic differences  
493 for given physiological traits than other species is an emerging question that needs to be  
494 addressed in future studies.

495 Resistance to embolism varies across plants species and helps to explain species  
496 distributions across climatic gradients (i.e. water scarcity; Choat et al. 2012). However,  
497 low or no variability in resistance to embolism are usually reported within species (Lamy  
498 et al. 2014, González-Muñoz et al. 2018). Lamy et al. (2011) showed a strong uniform  
499 selection or canalisation for this trait shaped the lack of genetic variation. There are some  
500 studies showing a significant correlation between  $P_{50}$  and climate variables (Brodribb et  
501 al. 2014, Larter et al. 2017), especially temperature (Kavanagh et al. 1999), although this  
502 is not a common pattern in either 'in situ' studies or common garden experiments  
503 (Martínez-Vilalta et al. 2009, Lamy et al. 2014). Recently, Stojnić et al. (2018) attributed  
504 lack of variability to an ascertainment bias, as most populations in reported studies  
505 originate from the core of the species distribution. Hence, significant differences are  
506 observed when marginal populations growing in areas subject to unsuitable conditions  
507 for the species are considered. In our study, despite the contrasting climate at the original  
508 location of the four provenances that would induce differences in potential  
509 evapotranspiration (PET, no data available), xylem resistance to embolism showed  
510 similar  $P_{50}$  values. Therefore, contrary to Cavender-Bares (2018) our results do not  
511 evidence a role of evolvability for hydraulic traits that could have allowed adaptation to  
512 changing environments. The minor differences observed however, correlate with mean  
513 temperature, with more resistant individuals originating from the warm margin. This  
514 could affect the performance of individuals from the different populations although only  
515 slightly. In fact, as for SLA, leaf unfolding and leaf N content,  $P_{50}$  seems to be altered by  
516 the main climate variables evaluated (i.e. precipitation and maximum and minimum  
517 temperatures) as the PCA shows. This result is similar to what has been observed for  
518 *Quercus oleoides* in which variation among populations associated with climates of origin  
519 have been found despite a lack of local adaptation (Cavender-Bares 2018). Blackman et

520 al. (2017) also showed how  $P_{50}$  was related to mean annual temperature across eight  
521 populations of *Corymbia calophylla* grown in a common garden experiment. Wood  
522 density has been theoretically linked with resistance to embolism based on the avoidance  
523 of implosion/collapse events in the xylem vessels (Hacke et al. 2001), because it is closely  
524 related to climate, and in particular precipitation and aridity, across species (Martínez-  
525 Cabrera et al. 2009). The lack of significant correlation between wood density and the  
526 climate variables analysed and the low contribution to the principal component 1 of the  
527 PCA (i.e. the one more influenced by climate), suggests that this anatomical trait is  
528 differently affected by climate than resistance to embolism and that, therefore, it could  
529 not work as an accurate proxy for  $P_{50}$  at an intra-specific level. Despite this, more studies  
530 including more provenances from the marginal distribution area of the species and more  
531 species with different xylem anatomical characteristics are required to definitely reject  
532 wood density as proxy for embolism resistance within species.

533

## 534 **CONCLUSIONS**

535 Our results evidenced that different *Quercus petraea* provenances originating from an  
536 extensive portion of the species' overall distribution in Europe showed important genetic  
537 variation for traits related to phenology and growth. In fact, genetic differentiation is  
538 much higher for leaf phenological traits than for other functional traits directly related  
539 with plant tolerance to drought such as hydraulic traits. Most anatomical, physiological  
540 and hydraulics traits evaluated, except SLA, did not show any significant variation  
541 between provenances, or very minor genetic differentiation, although large between trees  
542 (within populations) variations were observed. This raises the question of whether the  
543 multiple-trait associations are more driven by very integrated traits, i.e. by traits that can  
544 give synthetic information about interactions between the plant and their environment

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545 such as growth, phenology and SLA. Despite this, there are some less integrated traits,  
546 e.g. leaf N content, highly influenced by the climate at the origin of the provenances that,  
547 although they did not show genetic divergence, they could be a good target of selection  
548 for individuals from marginal populations, i.e. with highly contrasted climate conditions.  
549 Whether the lack of variation in certain traits in this species is due to phenotypic plasticity  
550 remains to be investigated. In any case, we suspect that there is enough genetic variation  
551 within populations to trigger genetic divergence among populations. While experimental  
552 evolution cannot be implemented to check our hypothesis, future investigations will  
553 explore whether anatomical, physiological and hydraulics traits do exhibit significant  
554 selection gradients.

555

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## 566 **AUTHORS’ CONTRIBUTIONS**



567 AK and SD conceived the ideas and, together with JMT-R, designed methodology. JMT-  
568 R, LT, FB, AD and SD collected the samples and carried out most of the measurements  
569 related with phenology and anatomy. JMT-R, MRC-M and TB collected and analysed the  
570 vein and stomatal density data. LJL and JMT-R ran the statistical analyses. All authors  
571 contributed critically to the drafts and gave final approval for publication.

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Torres Ruiz, J., Kremer, A., Carins-Murphy, M. R., Brodrigg, T. J., Lamarque, L., Truffaut, L.,  
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873 **FIGURE LEGENDS**

874

875 **Fig. 1:** Distribution of *Quercus petraea* in Europe. Red dots indicate the origin of the  
876 provenances grown in the common garden located in Sillegny (yellow star) and used in  
877 this study (See Table 1 for climate information and abbreviation of each site).

878

879 **Fig. 2:** Specific leaf area (SLA), mean tree height (H) and leaf unfolding of the nine  
880 provenances studied in the common-garden at Sillegny versus latitude, mean annual  
881 maximum and minimum temperature (Tmax and Tmin, respectively) and precipitation.  $r$   
882 indicates Pearson product-moment correlation;  $p$  indicates significance levels of the  $p$   
883 values of the slope.

884

885 **Fig. 3: A)** Box plot of  $P_{50}$  (i.e. pressure at which 50% loss of stem conductivity occurs)  
886 for each of the four provenances in which resistance to embolism was evaluated. From  
887 13 to 15 different branches from different trees were used for each provenance. No  
888 significant differences between provenances were observed in  $P_{50}$  ( $p = 0.21$ ). **B)**  
889 Significant correlation between  $P_{50}$  and mean temperature. A linear regression was fitted  
890 to the whole dataset ( $r$ , Pearson product-moment correlation;  $p$ , significance levels of the  
891  $p$  values of the slope). The error bars represent  $\pm$  the standard error.

892

893 **Fig. 4:** Significant correlations between pairs of phenological and ecophysiological traits.  
894 Each point represents the mean value of 22 to 28 trees per provenance. Linear regressions  
895 were fitted for the whole dataset ( $r$ , Pearson product-moment correlation;  $p$ , significance  
896 levels of the  $p$  values of the slope).

897

898 **Fig. 5: A)** Percentage of variances explained by the different axes (components) resulting  
899 from the principal component analysis (PCA). **B)** Variables factor map on the first two  
900 axes of the PCA performed on the different climate variables and functional traits. For  
901 facilitating its evaluation, only those traits with a higher contribution to component 1 and  
902 2 ( $\cos^2 > 0.25$ ) are shown. The variables factor map including all traits is included in the  
903 supplementary information of the article (Fig. S1).

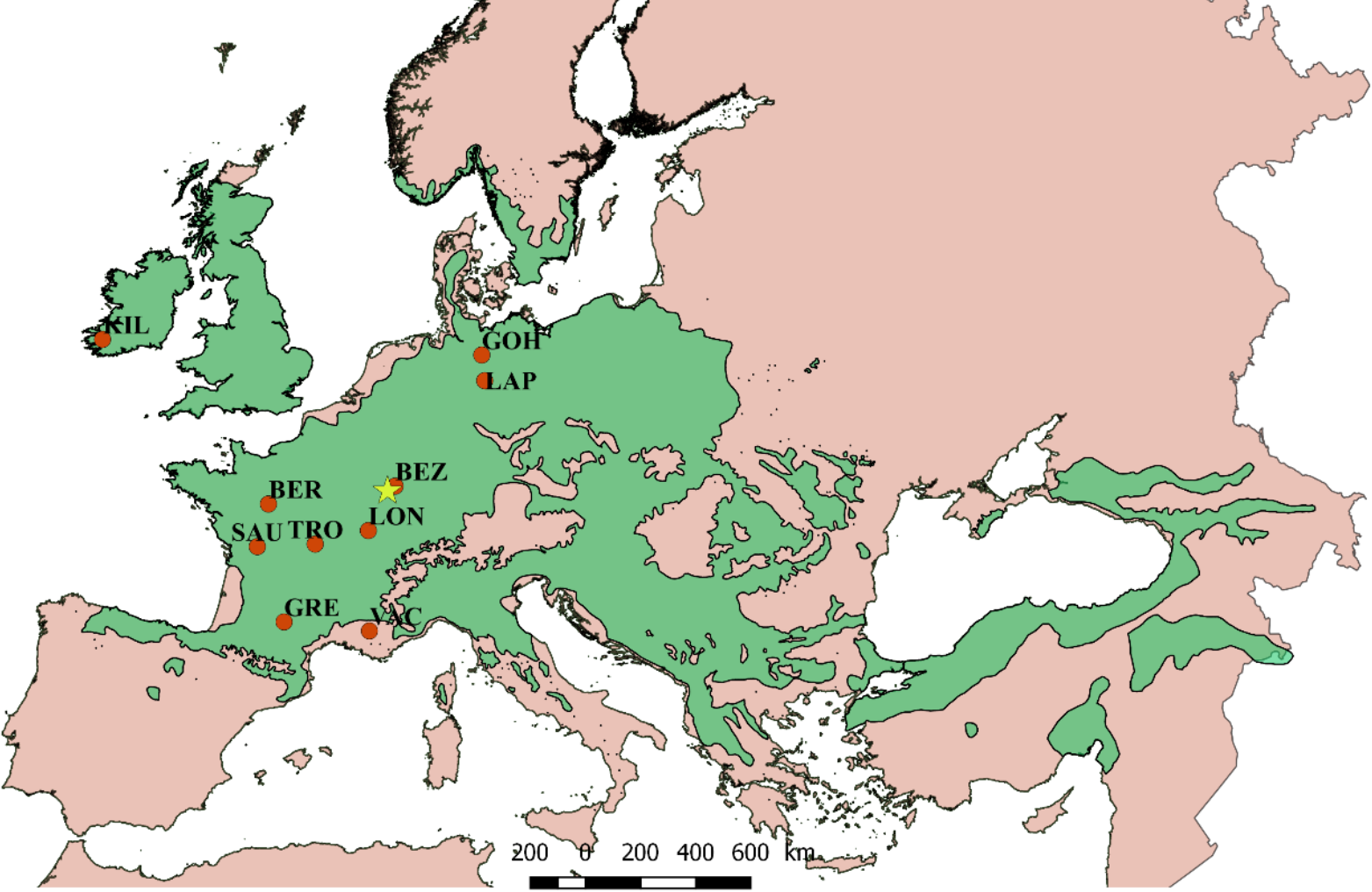
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905 **Fig. 6:** Path diagram of the model that best describes (lower Akaike information criterion  
906 (AIC)) the relationship between climate variables and functional traits. Solid and dashed  
907 lines indicate direct or indirect effects, respectively, between variables and traits. Values  
908 in the arrows indicate the standardized coefficients (std.all), and refer to standardized  
909 estimates of the variances of both continuous observed and latent variables. LU: Leaf  
910 unfolding; SLA: Specific Leaf Area; N: Leaf Nitrogen content; Tmin: minimum  
911 temperature.

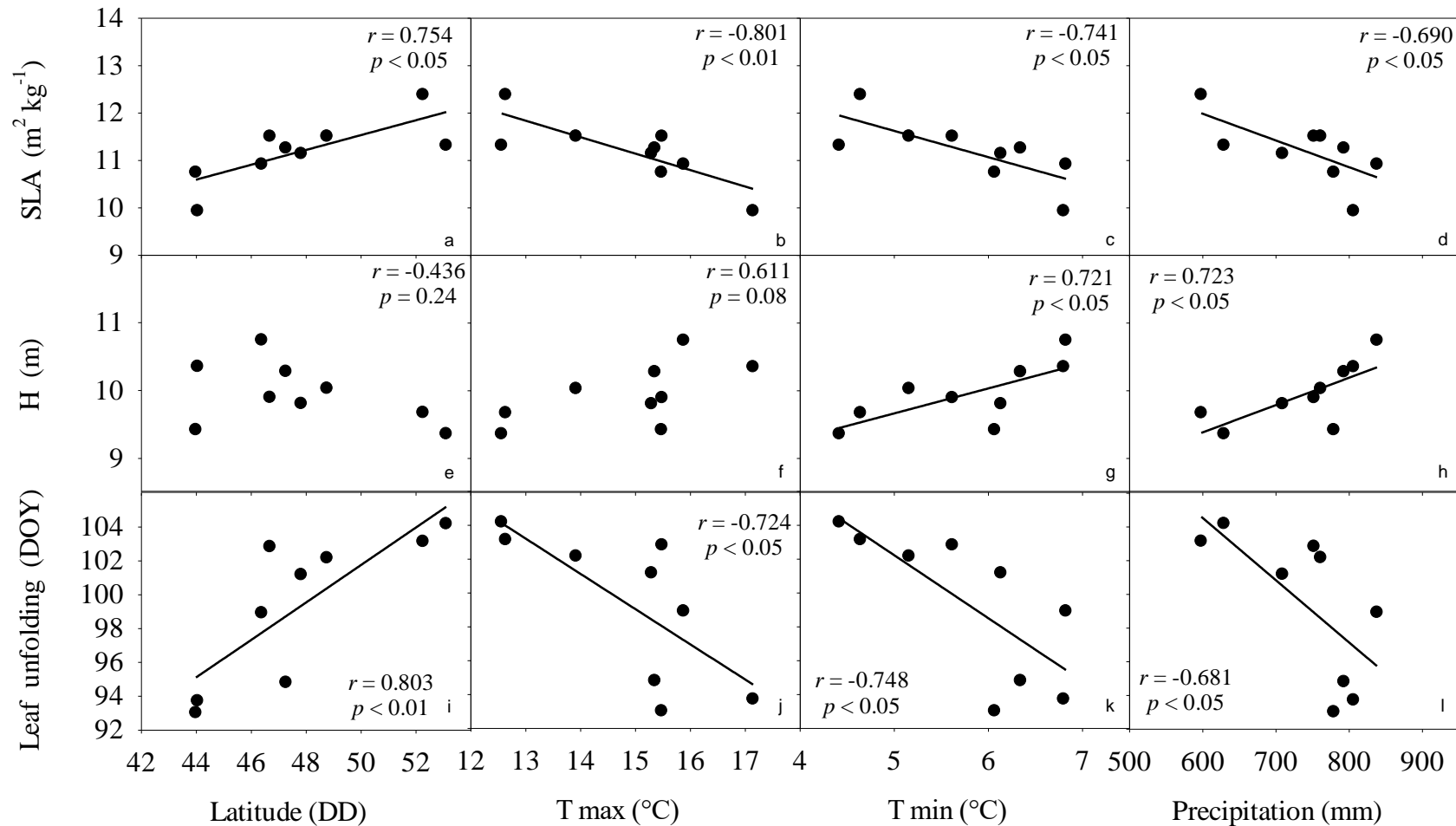
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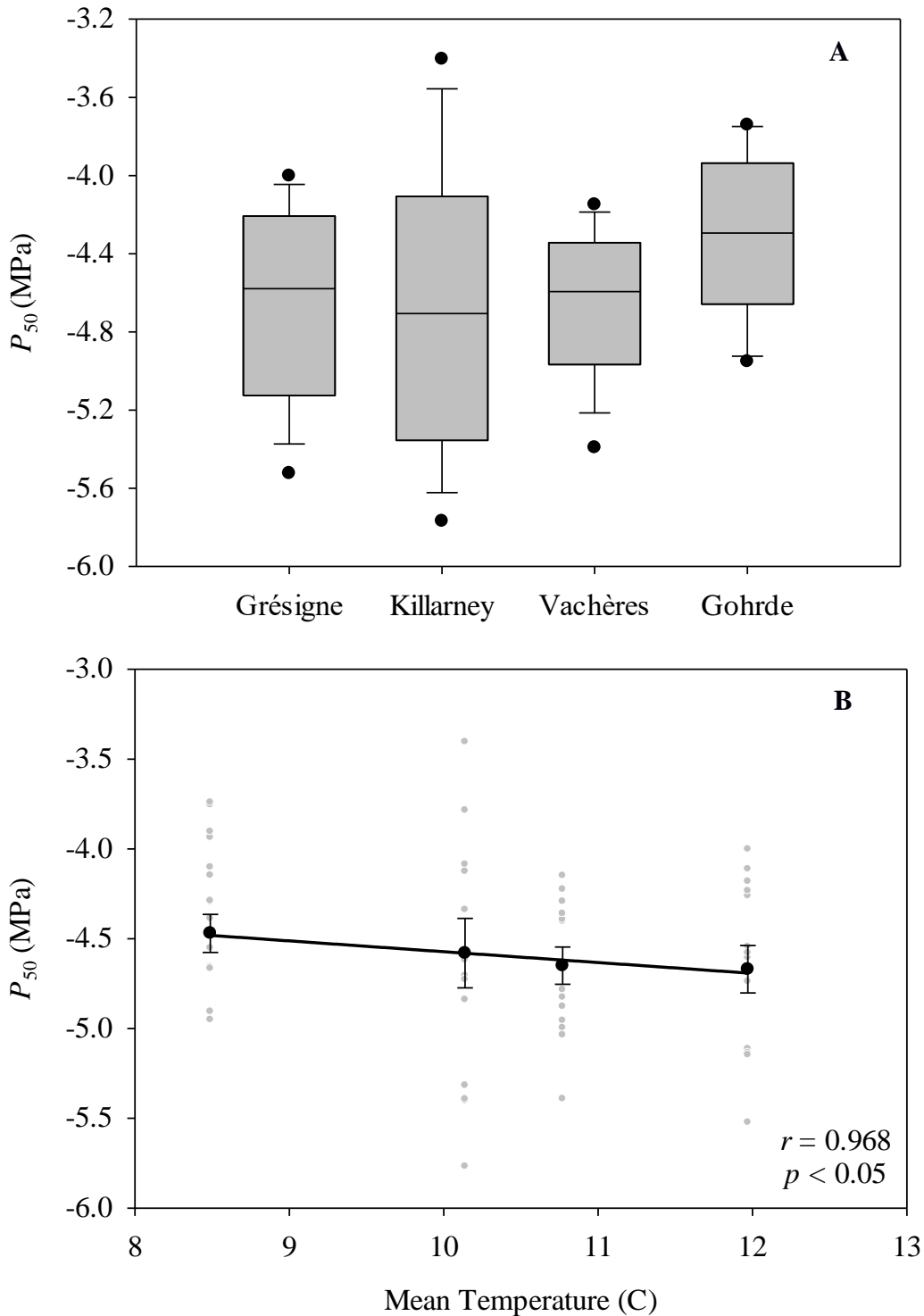
**Fig. 1:** Distribution of *Quercus petraea* in Europe. Red dots indicate the origin of the provenances grown in the common garden located in Sillegny (yellow star) and used in this study (See Table 1 for climate information and abbreviation of each site).



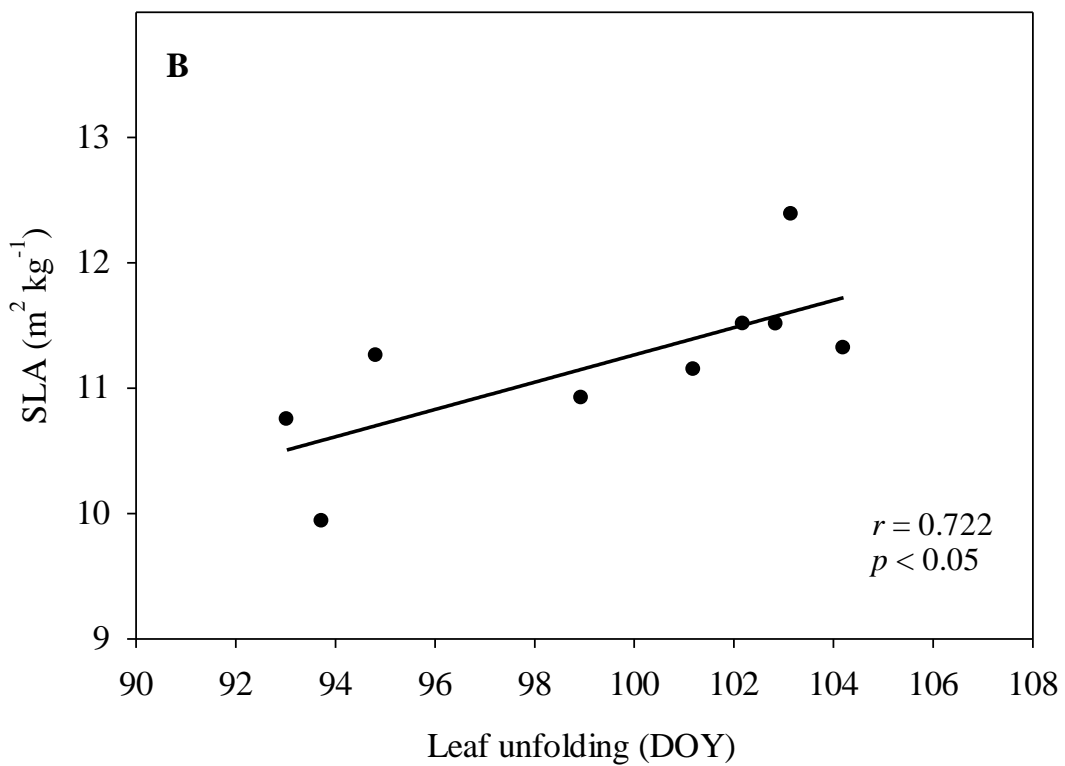
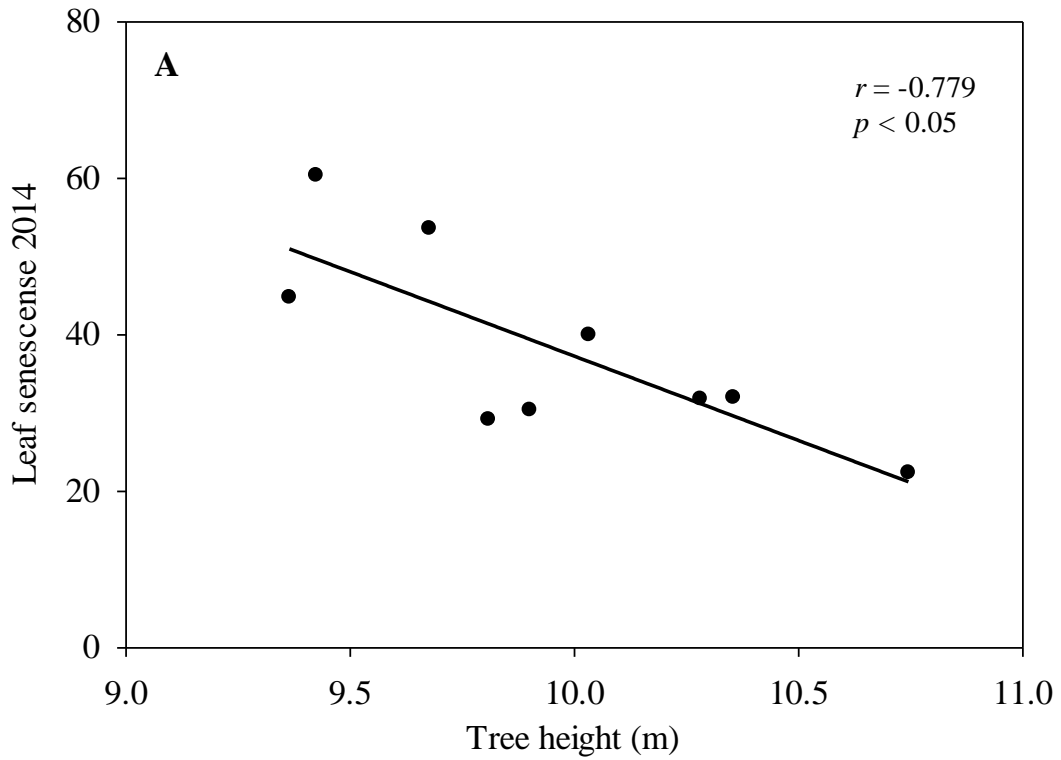
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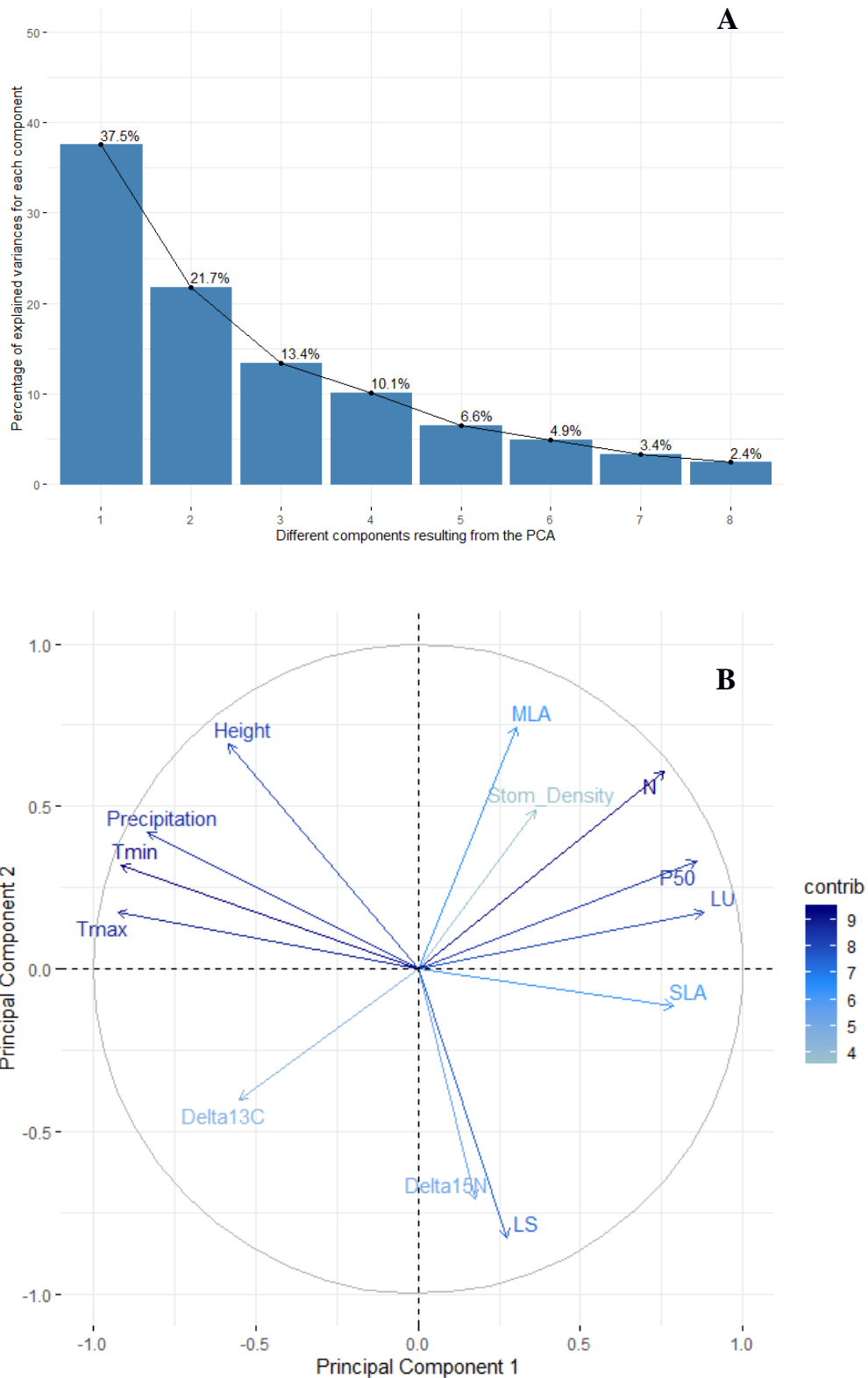
**Fig. 3: A)** Box plot of  $P_{50}$  (i.e. pressure at which 50% loss of stem conductivity occurs) for each of the four provenances in which resistance to embolism was evaluated. From 13 to 15 different branches from different trees were used for each provenance. No significant differences between provenances were observed in  $P_{50}$  ( $p = 0.21$ ). **B)** Significant correlations between  $P_{50}$  and mean temperature. A linear regression was fitted to the whole dataset ( $r$ , Pearson product-moment correlation;  $p$ , significance levels of the  $p$  values of the slope). The error bars represent  $\pm$  the standard error.



**Fig. 4:** Significant correlations between pairs of phenological and ecophysiological traits. Each point represents the mean value of 22 to 28 trees per provenance. Linear regressions were fitted for the whole dataset ( $r$ , Pearson product-moment correlation;  $p$ , significance levels of the  $p$  values of the slope).



**Fig. 5: A)** Percentage of variances explained by the different axes (components) resulting from the principal component analysis (PCA). **B)** Variables factor map on the first two axes of the PCA performed on the different climate variables and functional traits. For facilitating its evaluation, only those traits with a higher contribution to component 1 and 2 ( $\cos^2 > 0.25$ ) are shown. The variables factor map including all traits is included in the supplementary information of the article (Fig. S1).



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**Fig. 6:** Path diagram of the model that best describes (lower Akaike information criterion (AIC)) the relationship between climate variables and those functional traits with a higher contribution to the component 1 of the PCA analyses, i.e. the one in which climate variables have a higher contribution. Solid and dashed lines indicate direct or indirect effects, respectively, between variables and traits. Values in the arrows indicate the standardized coefficients (std.all), and refer to standardized estimates of the variances of both continuous observed and latent variables. LU: Leaf unfolding; SLA: Specific Leaf Area; N: Leaf Nitrogen content; Tmin: minimum temperature; Tmax: maximum temperature.

