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

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JM Torres-Ruiz[1]*, A Kremer[2], MR Carins-Murphy[3], TJ Brodribb[3], LJ Lamarque[2], L Truffaut[2], F Bonne[4], A Ducousso[2] and S Delzon[2].

[1] Universite Clermont-Auvergne, INRA, PIAF, 63000, Clermont-Ferrand, France.

[2] Unité Mixte de Recherche Biodiversité Genes & Communautés (UMR 1202 BIOGECO), Institut National de la Recherche Agronomique (INRA) – Université de Bordeaux, 69 route d'Arcachon, Cestas F-33610, France.

[3] School of Natural Sciences, University of Tasmania, Private Bag 55, Hobart, Tasmania 7001, Australia.

[4] UMR SILVA, INRA, Nancy, France.

*author for correspondence:

José M. Torres-Ruiz.

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

Email : *torresruizjm@gmail.com*

Telephone: +33 4 43 76 14 06

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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.

4

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

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 Sillégny (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^2) and specific leaf area (SLA, $m^2\ kg^{-1}$) 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 14th, 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-3).

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   (Δ13C and Δ15N 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    $\delta^{13}C$ in ‰ (Craig 1957) was then converted into carbon discrimination $\Delta^{13}C$ in ‰

201   (Farquhar and Richards 1984). The $\Delta^{13}C$ values were corrected for the Suess Effect

202   (decrease in $\delta^{13}C$ of atmospheric CO_2 since the beginning of industrialization) resulting

203   from the emission of fossil carbon dioxide, which is depleted in ^{13}C (Francey et al. 1999,

204   McCarroll and Loader 2004) even if the sampling has been done at the same date. $\Delta^{13}C$

205   can be related to the ratio of 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 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 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

9

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

224   as:

225   AI = MAP / 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

10

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 $Yijk$ being the observed trait of the seedling *k* from the provenance

266   *i* and block *j*, μ 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 ε_{ijk} the residual variation including the effect of

269   tree *k* belonging to combination *ijk*. Variances of random effects (provenance σ_P^2,

270   interaction σ_{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 σ_A^2 is the within

275   provenance additive variance. In our study based on provenance and not descendant test,

276   σ_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, $\Delta\,^{13}$C, $\Delta\,^{15}$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).

311

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^2 kg^{-1} 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^2 kg^{-1}) and flushing in DOY= 94. On the contrary, the highest SLA values was

345   observed for Lappwald (12.4 m^2 kg^{-1}) 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, Δ15N 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

15

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 $°C^{-1}$.

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   Δ13C 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

CONCLUSIONS

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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

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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873   **FIGURE LEGENDS**

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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 ± 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 (cos2>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).
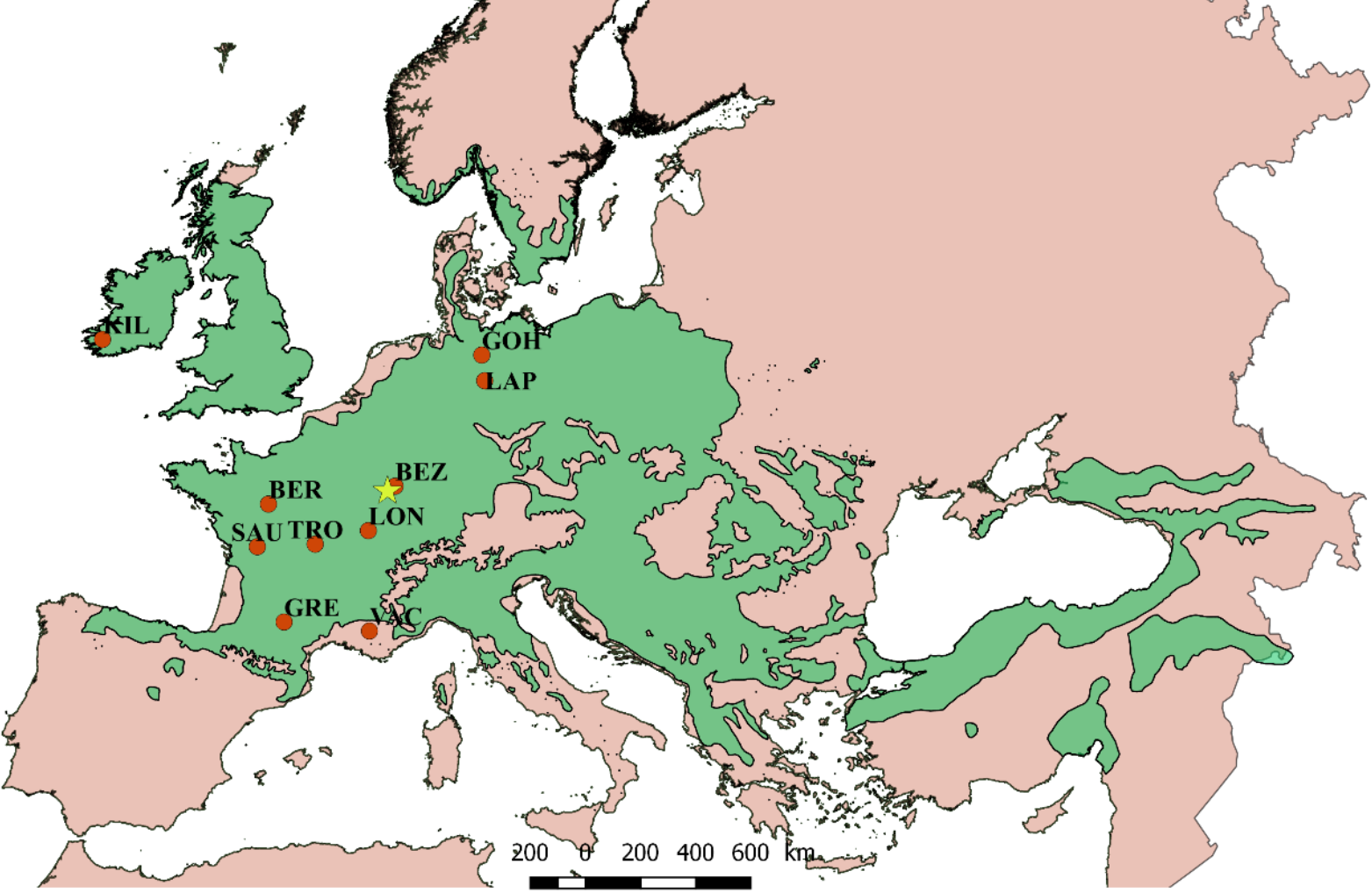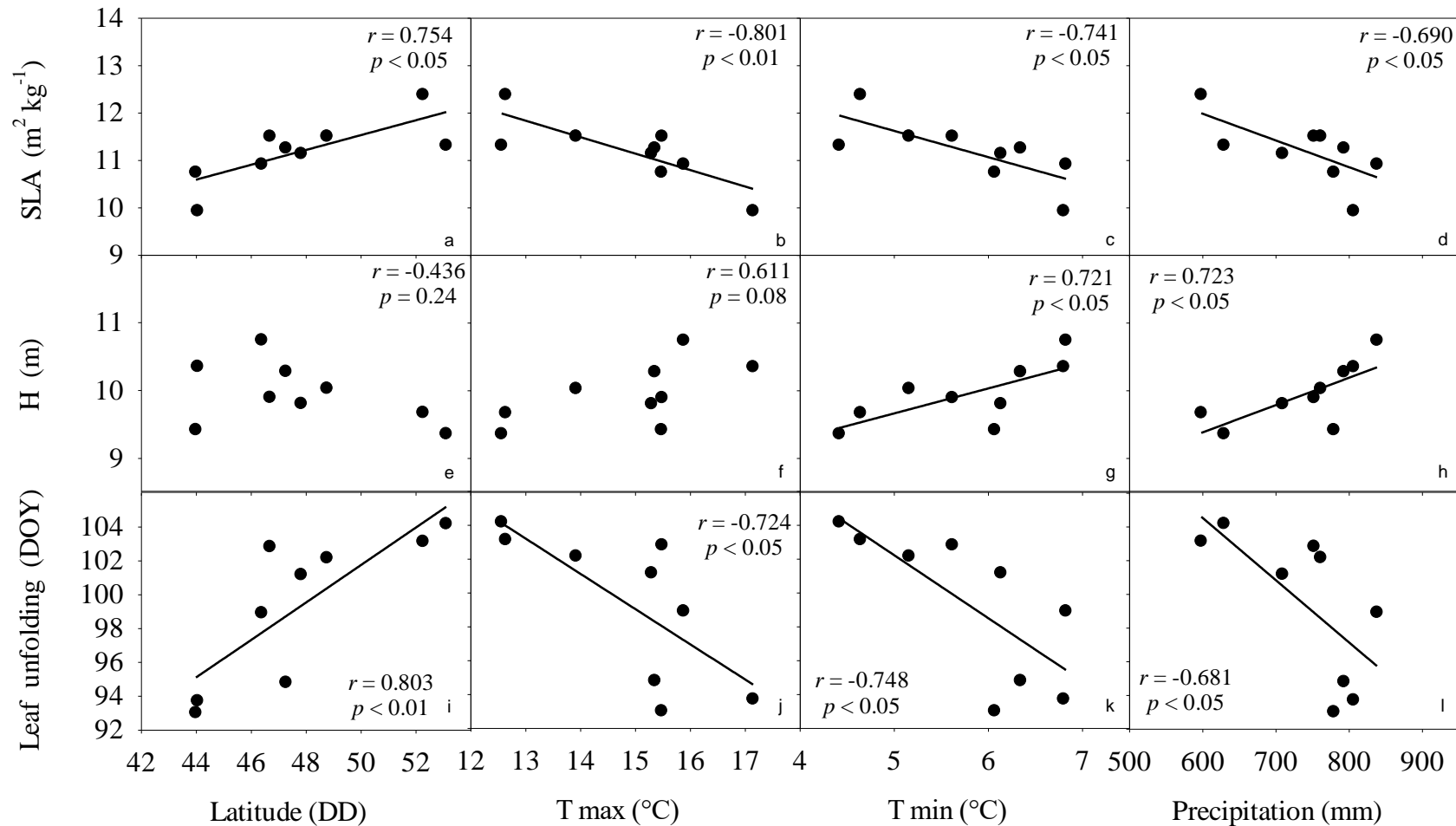
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Fig. 3: A) Box plot of P_{50} (i.e. pressure at which 50% loss of stem conductivity occurs) for each of the four provenances in which resistance to embolism was evaluated. From 13 to 15 different branches from different trees were used for each provenance. 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 ± the standard error.
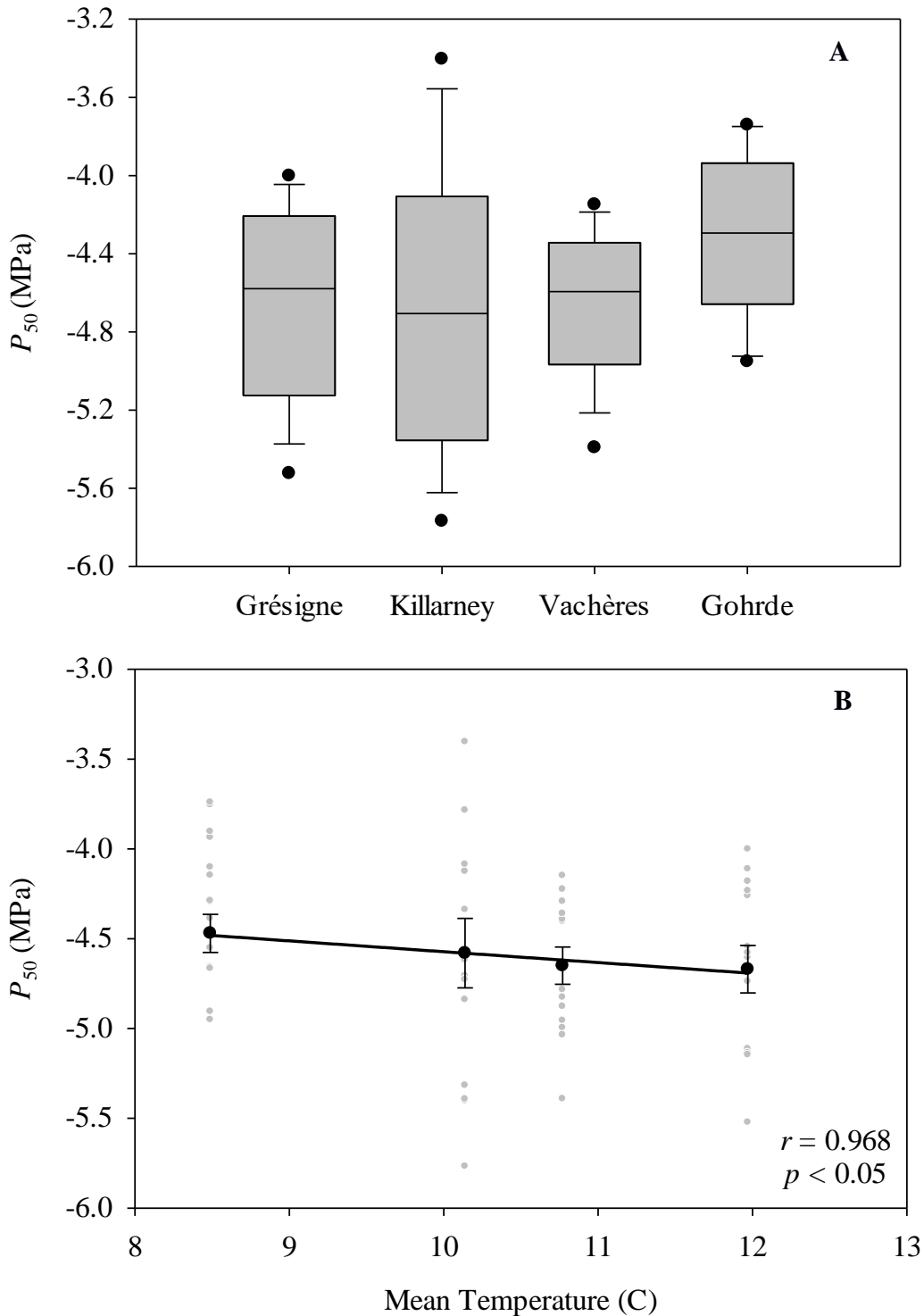
Fig. 4: Significant correlations between pairs of phenological and ecophysiological traits. Each point represents the mean value of 22 to 28 trees per provenance. Linear regressions were fitted for the whole dataset (*r*, Pearson product-moment correlation; *p*, significance levels of the *p* values of the slope).
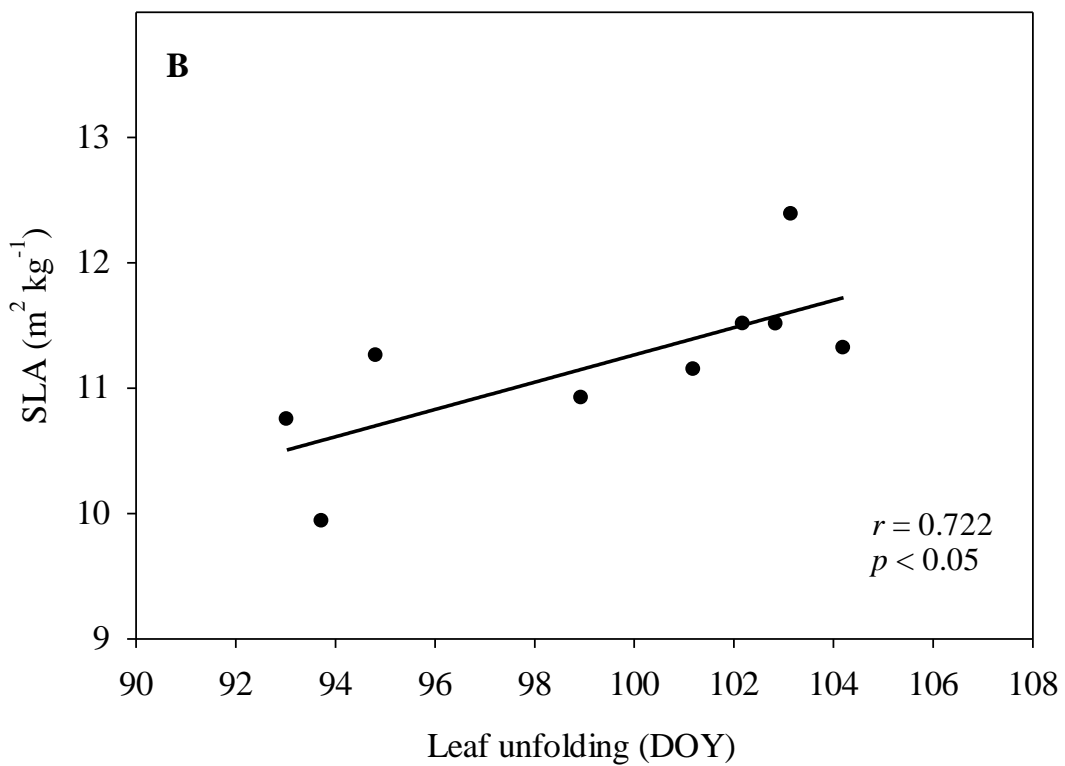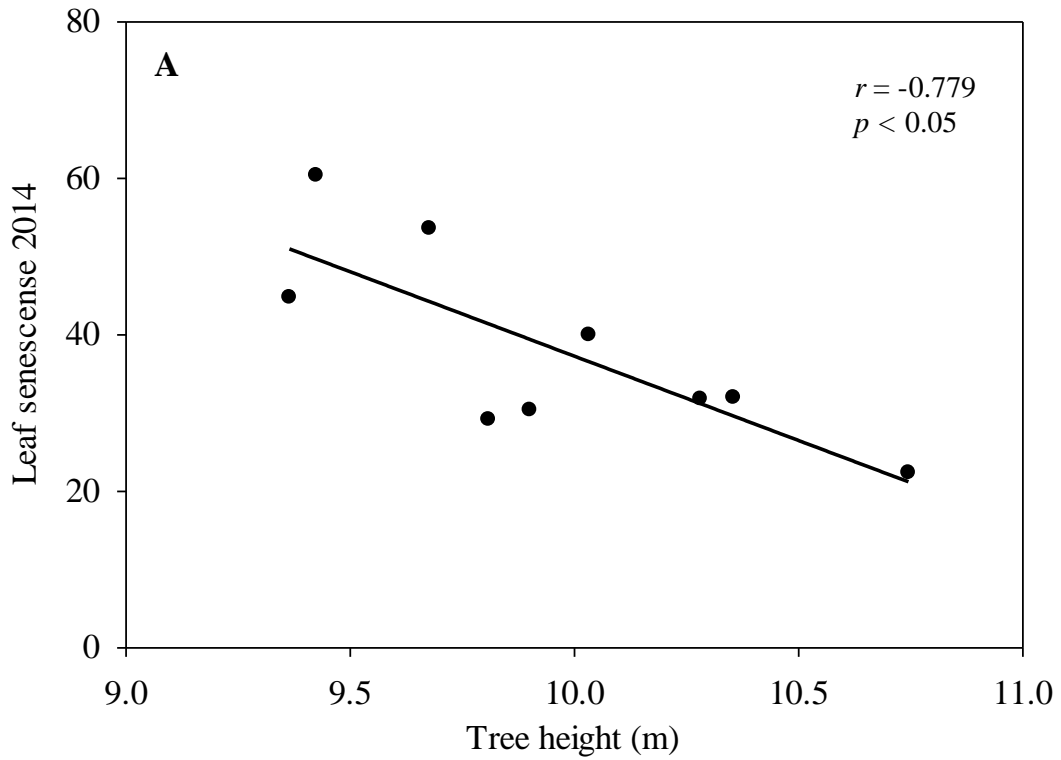
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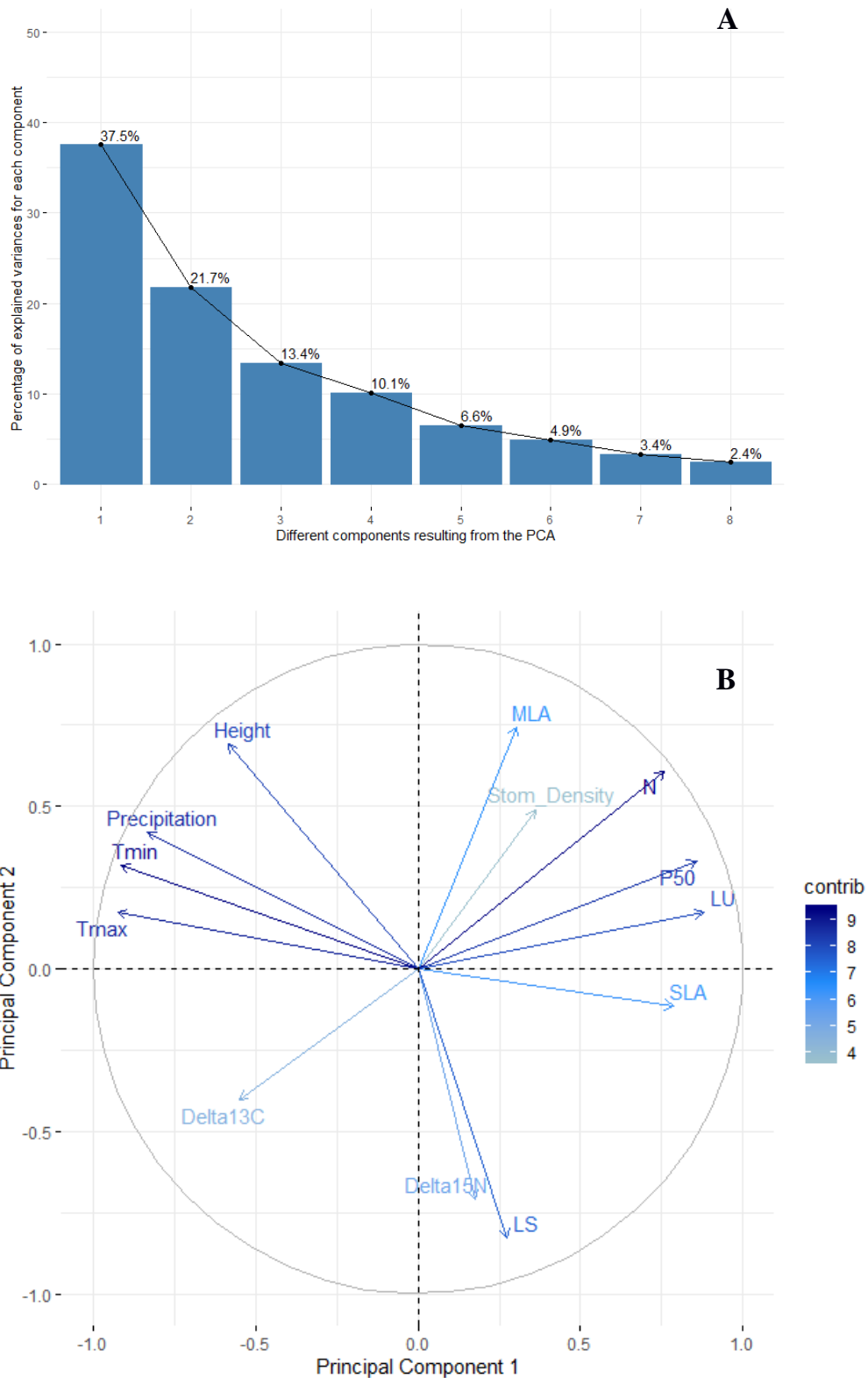
Fig. 6: Path diagram of the model that best describes (lower Akaike information criterion (AIC)) the relationship between climate variables and those functional traits with a higher contribution to the component 1 of the PCA analyses, i.e. the one in which climate variables have a higher contribution. 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.