

# Genetic differentiation in functional traits among European sessile oak populations

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1 2	Genetic differentiation in functional traits among European sessile oak populations
3 4	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 Truffaut <sup>2</sup> , F Bonne <sup>4</sup> , A Ducousso <sup>2</sup> and S Delzon <sup>2</sup> .
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6	<sup>1</sup> Universite Clermont-Auvergne, INRA, PIAF, 63000, Clermont-Ferrand, France.
7 8 9	<sup>2</sup> 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.
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.
12	
13	
14	*author for correspondence:
15	José M. Torres-Ruiz.
16	Université Clermont-Auvergne, INRA, PIAF, 63000, Clermont-Ferrand, France.
17	Email : <u>torresruizjm@gmail.com</u>
18	Telephone: +33 4 43 76 14 06
19	
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#### 29 Abstract

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

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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 population mortality and its impact on ecosystem function (McDowell et al. 2013, 59 60 Cailleret et al. 2017) but also about the capacity of species to adapt in a timely manner to the expected warmer and drier climates (Corlett and Westcott 2013, Sáenz-Romero et al. 61 2017, González-Muñoz et al. 2018). These concerns are especially relevant to forest 62 species given, on the one hand, the rapid rate of environmental change and, on the other, 63 the long life-span of most tree species (Aitken et al. 2008). Therefore, crucial questions 64 65 for evaluating and predicting the consequences of ongoing climate change are i) which key traits can evolve within a few generations allowing the adaptation of trees to climate 66 change?; and ii) how fast can such adaptation occur in response to a changing 67 68 environment?

During the last decade, many studies have focused on addressing these questions by monitoring woody plants under controlled or induced environmental changes (Hoffmann and Sgrò 2011, Franks et al. 2014). As a result, different traits have been identified that respond phenotypically to temperature, thus making them potential targets for microevolution (e.g. Amano Tatsuya et al. 2010). Phenology is one of the most well– known sensitive indicators of climate change. In oaks, previous studies have shown 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 leaf functional traits in oaks such as stomatal density, leaf size and leaf thickness, that 81 82 have significant influence on net carbon gain but also on plant water balance. The genetic determinism of xylem and hydraulic traits that are related to the hydraulic failure of the 83 plant water transport system due to embolism formation is also largely unknown in oak 84 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 al. 2015, Choat et al. 2018). Understanding intra-specific variation in these traits would 87 88 therefore help us to evaluate the capacity of tree species to face and adapt to new environmental conditions induced by ongoing climate change. 89

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

Some of these traits have previously been assessed in common garden experiments and exhibited clinal genetic variation along geographic gradients as a result of diversifying selection (Vitasse et al. 2009, Alberto et al. 2011). Thus, divergent intraspecific profiles for "intrinsic" water-use efficiency (Farquhar and Richards 1984) have been linked to the distribution of genotypes across gradients in air humidity and soil water availability for 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, such as wood density, vulnerability to embolism and water use efficiency (Mencuccini 108 109 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 (Lamy et al. 2011). 112

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

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

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#### 132 MATERIAL AND METHODS

133 Common-garden experiment

134 This study utilised a common garden experiment that was planted in 1989 and 1993 in the Forêt Domaniale de Sillégny (France) which contains 107 sessile oak provenances 135 136 (Ducousso et al. 1996). From these, 9 provenances diverging from a common source population and representing different climatic regions within the distribution range of the 137 species in Europe, from Northern Germany to Southern France, were selected for this 138 study (Fig. 1 and Table 1, climate data source: Worldclim; period: 1960-1990). The initial 139 density of the plantation was 1904 individuals per hectare (spacing  $3 \text{ m} \times 1.75 \text{ m}$ ) with 140 141 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 exact same set of trees per provenance. 145

146 *Phenology and tree height* 

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

distributed in 7 to 12 replicated plots. Leaf senescence (LS) was monitored two times in 149 150 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 the tree level, LU date was determined when 50% of the buds had reached this threshold. 156 In fall, due to the low number of field campaigns, we were not able to estimate a date of 157 158 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

Mean leaf area (MLA, mm<sup>2</sup>) and specific leaf area (SLA, m<sup>2</sup> kg<sup>-1</sup>) was determined for 22 163 to 28 trees per provenance randomly selected in 7 to 12 blocks per provenance and with 164 at least 1 tree per block. Between 10 to 15 fully expanded and non-damaged leaves were 165 166 collected per tree. To avoid any possible effects of different light exposure within the crown on leaf traits, all samples were collected from the upper and outer part of the crown 167 to ensure a similar light exposure among them. All leaves were collected within 48 hours 168 starting on June 14<sup>th</sup>, 2014. They were collected from a single branch per tree using a pole 169 170 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

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WinFolia software (Regent Instruments Inc., Quebec, Canada). For determining the SLA,
the dry mass of leaves was measured after drying them in an oven at 65°C until a constant
mass was reached. The SLA was assessed as the ratio of the leaf area to its mass.
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 dry branch. One ca. 10-cm long branch segment per individual was collected from 4 to 179 180 16 individuals per provenance. As for leaves, to avoid any possible effects of different light exposure within the crown on wood density, all branches were collected from the 181 upper and outer part of the crown to ensure similar light exposure conditions among them. 182 183 All branches had the same age (two years-old) within and between provenances. For each sample, we used a double-bladed saw to cut a transverse section with a constant thickness 184 of 2 mm. Wood density was measured on the transverse section by using an x-ray image 185 calibration procedure. Sections were exposed to X rays and were then scanned with a 186 microdensitometer. Images were analysed using Windendro (Guay et al. 1992) to obtain 187 188 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 density (D, g cm-3). 190

# 191 Stable C and N isotopes analysis

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

Thermo Scientific, Bremen Germany). The carbon isotopic composition expressed as 199  $\delta^{13}$ C in ‰ (Craig 1957) was then converted into carbon discrimination  $\Delta^{13}$ C in ‰ 200 (Farguhar and Richards 1984). The  $\Delta^{13}$ C values were corrected for the Suess Effect 201 (decrease in  $\delta^{13}$ C of atmospheric CO<sub>2</sub> since the beginning of industrialization) resulting 202 from the emission of fossil carbon dioxide, which is depleted in  ${}^{13}C$  (Francey et al. 1999, 203 McCarroll and Loader 2004) even if the sampling has been done at the same date.  $\Delta^{13}$ C 204 can be related to the ratio of  $CO_2$  assimilation (A) to stomatal conductance ( $g_s$ ), also 205 named the intrinsic water-use efficiency. 206

# 207 *Leaf vein and stomatal density*

Vein density (total vein length per  $mm^2$  of leaf area) was determined from paradermal 208 sections of five fresh leaves (one leaf per tree, five trees per provenance) similar to those 209 210 used for MLA and SLA. Sections were prepared and measured following the protocols described by Carins Murphy et al. (2012). In brief, this involved removing the adaxial 211 212 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 view per section). Stomatal density (total stomata per mm<sup>2</sup> of leaf area) was also 214 determined from cuticles (1 per leaf and 5 fields of view per cuticle) prepared and 215 216 measured following the protocols of Carins Murphy et al. (2012).

# 217 Vulnerability to embolism

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

225

## AI = MAP / MAE

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

Xylem vulnerability to embolism was measured using the Cavitron technique 232 233 (Cochard, 2002; Cochard, 2005) at the Caviplace laboratory (GENOBOIS platform, 234 INRA-University of Bordeaux, France). To prevent artefactual losses in hydraulic conductance due to the induction of embolism during the sample preparation (Torres-235 236 Ruiz et al. 2015) or the presence of open vessels in the samples (Torres-Ruiz, Cochard, Choat, et al. 2017), >2 m-long branches were collected from the trees (one branch per 237 tree), wrapped in moist paper and plastic bags to kill transpiration and transported to the 238 239 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 length. Branches were debarked at both ends and installed in a large cavitron equipped 241 242 with a 1 m-diameter custom-built honeycomb rotor (DGMeca, Gradignan, France; (Lobo et al. 2018). Several branches were used to test the presence of open vessels by air 243 244 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 at its centre from -0.8 MPa to -10.5 MPa (those pressures correspond to centrifugation 246 rotation from 764 rpm to 2768 rpm respectively). Vulnerability curves to embolism were 247

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generated by plotting the percentage loss of hydraulic conductivity (PLC) at the different target pressures applied and fitting a sigmoidal equation (Pammenter and Van der Willigen 1998). Mean  $P_{50}$  values, i.e. the xylem pressure inducing 50% of PLC, were obtained by averaging the values of 13 to 15 samples per provenance. The  $P_{50}$  value is commonly used as a proxy for tree drought resistance: the lower the  $P_{50}$  value, the more 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 nine provenances of *Q. petraea* were tested with a generalized linear mixed model 256 (MIXED procedure, restricted maximum likelihood (REML) method in SAS, version 9.4, 257 SAS Institute, NC, USA) where plots and provenances were respectively treated as fixed 258 259 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 Vitasse et al. (2009) using the VARCOMP procedure with the restricted maximum 263 likelihood (REML). These analyses used the following statistical model:  $Y_{ijk} = \mu + P_i + P_i$ 264  $b_i + (Pb)_{ii} + \varepsilon_{iik}$ , with Yijk being the observed trait of the seedling k from the provenance 265 i and block j,  $\mu$  the overall mean of the analysed characters,  $P_i$  the random effect of 266 provenance *i*,  $b_i$  the fixed effect of block (here replicated plot) *j*,  $(Pb)_{ii}$  the interaction 267 268 between provenance *i* and block *j*, and  $\varepsilon_{ijk}$  the residual variation including the effect of tree k belonging to combination *ijk*. Variances of random effects (provenance  $\sigma_{\rm P}^2$ , 269 interaction  $\sigma_{bP}^2$ , residual  $\sigma \epsilon^2$ ) were also computed. The overall differentiation among 270 271 provenances (D) was calculated as the ratio (%) of the variance component of provenance to total variance estimated by analysis of variance, i.e as  $\sigma_P^2 / (\sigma_P^2 + \sigma_{bP}^2 + \sigma_{\epsilon}^2)$ , and 272

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

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

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

296

#### 297 **RESULTS**

299 Significant genetic differentiation between provenances was observed in four out of the 13 studied traits (Table 2). The provenances differ in both the timing of leaf spring and 300 301 autumn phenology (leaf unfolding and senescence, respectively). Tree height (H) and SLA were also significantly different between provenances, whereas a lack of genetic 302 differentiation was found for MLA,  $\Delta^{13}$ C,  $\Delta^{15}$ N, leaf C and N content, stomatal density, 303  $P_{50}$ , vein density and wood density. Overall genetic differentiation for the different traits 304 evaluated ranged from 0 up to 79. Thus, SLA and leaf unfolding amounted the highest 305 values with 79 and 62, respectively, whereas it was weaker for, leaf senescence (32) and 306 307 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*

Correlations between traits and the climate and latitude of provenance origin reveal some 313 314 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, the earliest provenance to begin leaf flushing was the one originating from the southern 316 margin (Latitude 43.98°) whereas the latest was one from northern Germany (Latitude 317 318 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 provenance with a mean annual precipitation of 806 mm leaf unfolding occurred in DOY 321 (day of year) 93.7, whereas for Göhrde with a precipitation of 629 mm/year it occurred 322

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

338

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

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

59.2% of the variance (Fig. 5A). Thus, Axis 1 (variance 37.5%) was strongly associated 348 349 with the main climate variables, i.e. maximum and minimum temperature and precipitation, as well as with functional leaf traits leaf unfolding, P<sub>50</sub>, SLA and N content 350 (Fig. 5B). Axis 2 (variance 21.7%), on the contrary, was more defined by the variance in 351 leaf senescence, MLA,  $\Delta 15N$  and tree height than by climate variables (Supplementary 352 information Fig. S2). When running the path analyses to detect associations between the 353 354 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**

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

367 Multi-trait associations

The effect of climate change on phenological traits has been extensively studied (Peñuelas et al. 2002, Gordo and Sanz 2005), especially the effect of temperature on the timing of leaf unfolding and flowering, which both occur earlier as temperature rises (Doi and 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. Similar results have been already reported for different tree species including oaks 377 378 (Menzel and Fabian 1999, Matsumoto et al. 2003, Vitasse et al. 2009), showing the important role of local temperature in driving adaptation of the growing season length. 379 This may partially explain the significant differences in growth (i.e. tree height) observed 380 381 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 populations would require a more focused study. In fact, our results also agree with a 383 384 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 earlier leaf unfolding for trees exposed to higher air temperatures, leading to an earlier 386 start of shoot growth, in that they estimated an advance in leaf unfolding of 1-3 days  $^{\circ}C^{-1}$ . 387 Our results also agree with those reported by (Vitasse et al. 2009) who observed negative 388 genetic clines in leaf unfolding with increasing temperature at the origin of the 389 provenance for Quercus petraea. More recent studies (Alberto et al. 2011, Firmat et al. 2017) reported a similar genetic cline for both germination and bud burst timing with provenance elevation in Q. petraea, showing that spring leaf phenological traits are 392 393 critical for local adaptation in oaks. Those genetic variations in leaf phenology observed in oaks are probably due to differences in heat requirement for bud burst rather than 394 differences in chilling-associated with leaf shedding (Dantec et al. 2015). 395

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Comment citer ce document Torres Ruiz, J., Kremer, A., Carins-Murphy, M. R., Brodribb, T. J., Lamarque, L., Truffaut, L., Bonne, F., Ducousso, A., Delzon, S. (2019). Genetic differenciation in functional traits among European sessile oak populations. Tree Physiology (Oxford Academic), 39 (10), 1736-1749., DOI In contrast with other studies (Vitasse et al. 2009), a clinal trend with temperature was not observed for leaf senescence in our study despite the significant variation in this trait among provenances and despite showing the highest between-provenance variation value. This is confirmed by the results from the PCA showing that, contrary to leaf unfolding, the variation in leaf senescence is barely determined by the temperature and precipitation regimes of the provenances' origin.

402 Significant genetic differentiation in SLA, a trait directly related to light conditions and nutrient availability (Milla et al. 2008), was observed between 403 provenances, indicating genetic variation among provenances induced by the climate at 404 405 their original location. Similar to "in situ" observations from Bresson et al. (2011) for *Quercus petraea*, we observed a negative correlation between SLA and both temperature 406 and precipitation. In fact, results from the PCA show exactly this: a high influence of the 407 minimum and maximum temperatures and precipitation not only on SLA, but also on 408 409 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, responses to both climate variables explained 43.7 % of the variation in SLA (Martin et 412 413 al. 2018). Earlier leaf unfolding would increase leaf lifespan which has been reported to be correlated with SLA. Thus, across species, Reich et al. (1991) showed that species 414 415 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 explain observed differences between provenances. The relevance of the adaptation of 420

SLA to climate resides in the fact that it may contribute to a species' ability to adjust to 421 422 different air temperatures and precipitation regimes (Albert et al. 2010, Long et al. 2011), because SLA is a key plant functional trait reflecting the trade-off between resource 423 424 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 the bivariate leaf size-climate relationships for 7670 plant species from 682 sites 426 427 worldwide that shows how day and night time leaf-to-air temperature differences are key to explaining the latitudinal gradient in leaf size (Wright et al. 2017). So, considering the 428 429 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 (Brodribb et al. 2007, Franks and Beerling 2009) and also highly influenced by 433 434 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 al. 2016). Furthermore, both traits, tend to remain proportional during leaf acclimation to 436 light intensity and VPD in woody angiosperm species (Brodribb and Jordan 2011, Carins 437 Murphy et al. 2012, 2014, 2016). However, the determinism of these traits has received 438 limited attention so far (Zhu et al. 2012). Assuming these traits varied between 439 provenances in situ, which is likely given the capacity for acclimation in vein density to 440 441 variation in evaporative demand among the upper and lower canopy in the closely related Quercus rubra (Zwieniecki et al. 2004), our results suggest that leaf capacity to acclimate 442 443 to different conditions via changes to these anatomical traits is not translated into genetic differences. In fact, there is no evidence for genetically driven effects on stomatal density 444 in Quercus petraea L. and Fagus sylvatica, although the phenotypic effect of temperature 445

is large (Bresson et al. 2011). However, contrary to this, vein density was found to be 446 447 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 populations in trait expression when grown in a common garden. An alternative 453 454 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 sites of evaporation in the leaf which itself is correlated with leaf hydraulic conductance 459 460 (Brodribb et al. 2007). In this study differences were found in SLA but not in leaf size implying that leaf thickness varied among provenances. This may have altered the post-461 462 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.

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

within populations on which diversifying selection may act since it may be very importantfor 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 temperature and precipitation for the different provenances. In fact, from all the models 475 476 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 temperature is the main climate variable determining leaf N content, SLA and leaf 478 479 unfolding for each provenance.

Water Use Efficiency (WUE), which corresponds to the ratio of biomass 480 481 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  $\Delta 13C$  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 (Guet et al. 2015), our results showed no significant genetic differentiation between 485 provenances in Quercus petraea. The lack of genetic differentiation between populations 486 might be explained by the advantage of increased efficiency of water use in conserving 487 soil moisture, which could be more predominant in Mediterranean and semiarid climates. 488 Our results however agree with those reported by Chamaillard et al. (2011) showing no 489 490 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 for given physiological traits than other species is an emerging question that needs to be 493 494 addressed in future studies.

Resistance to embolism varies across plants species and helps to explain species 495 496 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 et al. 2014, González-Muñoz et al. 2018). Lamy et al. (2011) showed a strong uniform 498 499 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 is not a common pattern in either 'in situ' studies or common garden experiments 502 503 (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 location of the four provenances that would induce differences in potential 508 509 evapotranspiration (PET, no data available), xylem resistance to embolism showed similar  $P_{50}$  values. Therefore, contrary to Cavender-Bares (2018) our results do not 510 511 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 could affect the performance of individuals from the different populations although only 514 515 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 Quercus oleoides in which variation among populations associated with climates of origin 518 have been found despite a lack of local adaptation (Cavender-Bares 2018). Blackman et 519

al. (2017) also showed how  $P_{50}$  was related to mean annual temperature across eight 520 521 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-Cabrera et al. 2009). The lack of significant correlation between wood density and the 525 526 climate variables analysed and the low contribution to the principal component 1 of the PCA (i.e. the one more influenced by climate), suggests that this anatomical trait is 527 528 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 species with different xylem anatomical characteristics are required to definitely reject 531 532 wood density as proxy for embolism resistance within species.

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# 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 variation for traits related to phenology and growth. In fact, genetic differentiation is 537 much higher for leaf phenological traits than for other functional traits directly related 538 539 with plant tolerance to drought such as hydraulic traits. Most anatomical, physiological and hydraulics traits evaluated, except SLA, did not show any significant variation 540 541 between provenances, or very minor genetic differentiation, although large between trees 542 (within populations) variations were observed. This raises the question of whether the multiple-trait associations are more driven by very integrated traits, i.e. by traits that can 543 give synthetic information about interactions between the plant and their environment 544

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, although they did not show genetic divergence, they could be a good target of selection 547 548 for individuals from marginal populations, i.e. with highly contrasted climate conditions. Whether the lack of variation in certain traits in this species is due to phenotypic plasticity 549 550 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 evolution cannot be implemented to check our hypothesis, future investigations will 552 explore whether anatomical, physiological and hydraulics traits do exhibit significant 553 554 selection gradients.

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# 566 AUTHORS' CONTRIBUTIONS

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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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# 73 **FIGURE LEGENDS**

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

**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 correlation 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 (cos2>0.25) are shown. The variables factor map including all traits is included in the supplementary information of the article (Fig. S1).

**Fig. 6:** Path diagram of the model that best describes (lower Akaike information criterion (AIC)) the relationship between climate variables and functional traits. 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.

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Mean Temperature (C)

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