

# Genomic data and common garden experiments reveal climate-driven selection on ecophysiological traits in two Mediterranean oaks

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## Abstract

Improving our knowledge of how past climate-driven selection has acted on present-day trait population divergence is essential to understand local adaptation processes and improve our predictions of evolutionary trajectories in the face of altered selection pressures resulting from climate change. In this study, we investigated signals of selection on traits related to drought tolerance and growth rates in two Mediterranean oak species (*Quercus faginea* and *Q. lusitanica*) with contrasting distribution ranges and climatic niches. We genotyped 182 individuals from 24 natural populations of the two species using restriction-site-associated DNA sequencing and conducted a thorough functional characterization in 1602 seedlings from 21 populations cultivated in common garden experiments under contrasting watering treatments. Our genomic data revealed that both *Q. faginea* and *Q. lusitanica* have very weak population genetic structure, probably as a result of high rates of pollen-mediated gene flow among populations and large effective population sizes. In contrast, common garden experiments showed evidence of climate-driven divergent selection among populations on traits related to leaf morphology, physiology and growth in both species. Overall, our study suggests that climate is an important selective factor for Mediterranean oaks and that ecophysiological traits have evolved in drought-prone environments even in a context of very high rates of gene flow among populations.

## KEY WORDS

adaptive evolution, divergent selection, drought tolerance, genetic drift, leaf economics spectrum, local adaptation

## 1 | INTRODUCTION

Understanding environmental factors that promote and maintain adaptive genetic diversity within species is a fundamental question

in evolutionary ecology. A possible approach to this question is to investigate genetic variation among populations along environmental gradients, which may provide indirect evidence of environmental selection driving adaptive population divergence. However, extant

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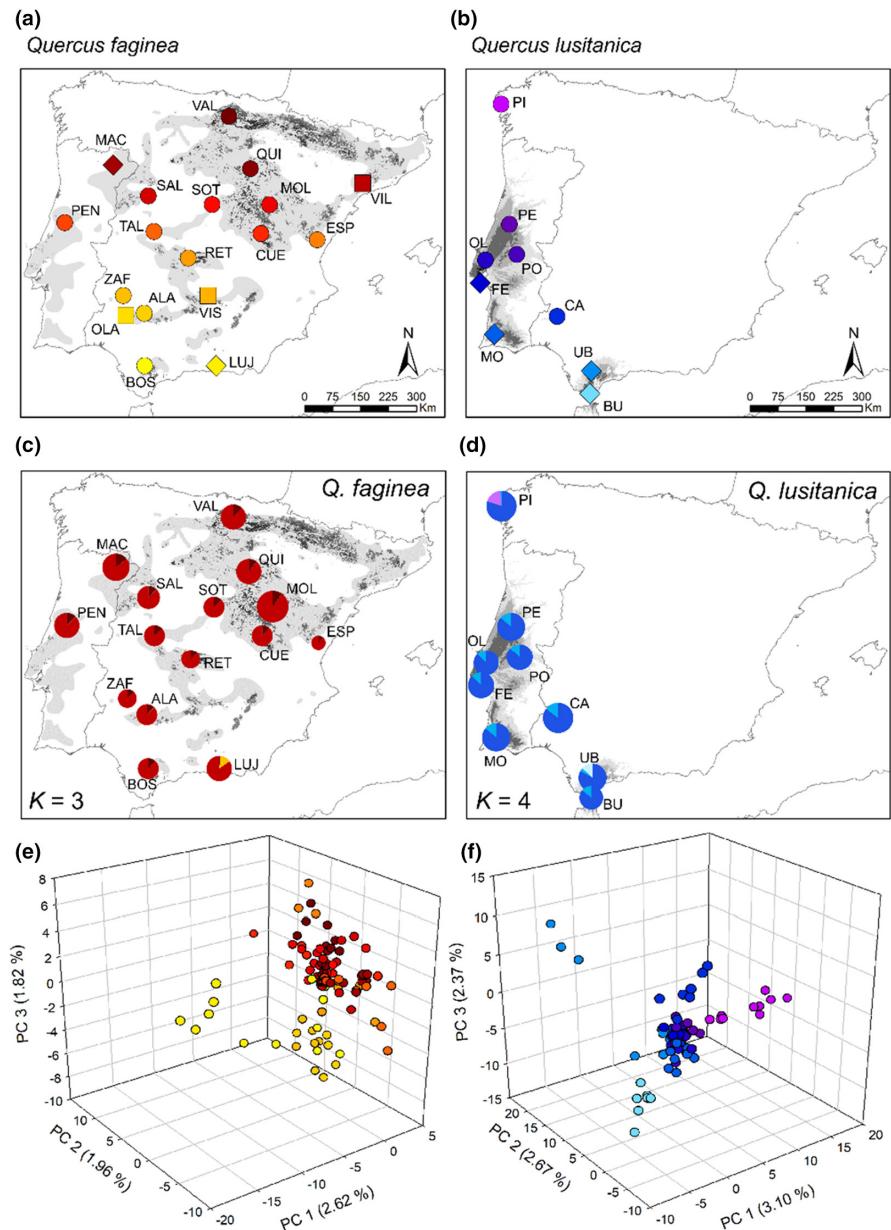
patterns of genetic divergence among populations are also largely impacted by neutral demographic processes, such as range shifts, gene flow and genetic drift, thus confounding inference of selection (Leinonen et al., 2013; Villemereuil et al., 2022). In forest tree species, palynological and ancient DNA studies have provided strong evidence of range shifts triggered by Holocene postglacial warming, particularly in northern temperate regions (Davis & Shaw, 2001; Saltré et al., 2013; Giesecke & Brewer, 2018; Wagner et al., 2018). The rate and scale of species migrations have differed across continents, mainly due to factors such as climate and the geographical configuration of corridors and barriers to dispersal. Specifically, the dynamics of temperate species were governed largely by long-distance expansions in central Europe, Asia and most areas from North America (Brewer et al., 2002; Petit, Brewer, et al., 2002; de Lafontaine et al., 2010; Cao et al., 2015). In contrast, many Mediterranean species from Europe and North America expanded at lower scales from multiple refugia (Grivet et al., 2006; López de Heredia et al., 2007). Migration routes are generally reflected in the geographical distribution of maternally inherited organelle haplotypes, as a result of demographic expansions and founding events via seed dispersal (Petit, Csaikl, et al., 2002; Pham et al., 2017; Sork, Fitz-Gibbon, et al., 2016; Sork, Gugger, et al., 2016). Subsequent among-population gene flow, especially via long-distance pollen dispersal, tends to erode nuclear genetic differentiation produced by ancient migration (Savolainen et al., 2007; Kremer & Le Corre, 2012), interacting with divergent selection and drift to produce present-day patterns of population genetic divergence.

Despite extensive long-distance gene flow among forest tree populations, there is strong evidence that suggests the emergence and maintenance of adaptive population divergence after postglacial colonization (Kremer & Hipp, 2020, and references therein). For example, widely distributed European oaks exhibit strong population differences in traits related to growth rates, vegetative phenology and drought tolerance (Alberto et al., 2013; Torres-Ruiz et al., 2019; Sáenz-Romero et al., 2017) that have presumably occurred as a result of divergent selection during the last 8000 years despite high gene flow (Alberto et al., 2013; Kremer & Hipp, 2020). Climate has been considered the main selective agent driving population genetic differentiation in forest tree species (Alberto et al., 2013; Aitken & Bemmels, 2016; Baughman et al., 2019; Ramírez-Valiente et al., 2022). In particular, temperature and precipitation regimes have been related to the evolution of traits important for fitness (i.e., functional traits) across species' ranges (Alberto et al., 2013; Ramírez-Valiente et al., 2022). Conversely, there are also well-documented examples of functional trait population divergence in animal and plant species, including forest trees, of a magnitude that is consistent with neutral demographic processes alone. For example, López-Goldar et al. (2019) showed that demographic processes could explain by themselves population differentiation in constitutive secondary metabolites in the Mediterranean pine *Pinus pinaster*, a species showing a marked genetic structure. Similarly, Vázquez-González et al. (2019) in a study on the same species found that genetic differentiation in stem anatomical traits could be largely explained by genetic drift.

Other studies have reported coincident geographical patterns in neutral and adaptive population genetic variation for species with weaker spatial genetic structure as well. This is the case, among others, of the Mediterranean oak *Quercus suber*, for which a longitudinal pattern of population variation in fitness-related traits, phenology and growth architecture (e.g., Gandour et al., 2007; Ramírez-Valiente et al., 2014; Sampaio et al., 2016) mirrors the neutral genetic structure in both nuclear and organelle genomes (Magri et al., 2007; Pina-Martins et al., 2019).

Mediterranean regions are characterized by summers with low precipitation, which creates a period of water stress for plants (Larcher, 2000). Differences in the length and severity of the dry season have been suggested to exert differential selective pressures driving genetic divergence in traits related to drought tolerance and resource-use strategies (e.g., Gratani et al., 2003; Ramírez-Valiente et al., 2009; Ramírez-Valiente, Lorenzo, et al., 2009; Ramírez-Valiente 2010; Andivia et al., 2012; Ghoul et al., 2020; Solé-Medina et al., 2022). However, for most Mediterranean species the extent to which nuclear genomic divergence and functional trait divergence may have been influenced by population demographic dynamics is still not well understood. In this study, we integrate genomic analyses and a thorough functional characterization of seedlings cultivated in common garden experiments to investigate the role of provenance climate in the divergent evolution of traits related to resource-use strategies among populations of two Mediterranean oaks: *Quercus faginea* and *Q. lusitanica*. *Q. faginea* and *Q. lusitanica* are two closely related oak species that belong to the section *Quercus* (white oaks). They are mainly distributed in the Iberian Peninsula and show contrasting growth forms, leaf habits and climatic niches (Figure 1). *Q. faginea* is the most abundant white oak in Iberian ecosystems (Pérez-Ramos & Maraño, 2009); it spans a wide ecological and climatic range, with an altitudinal gradient of ~1700 m (Alía et al., 2009), and exhibits a range of leaf habits—from evergreen to deciduous—and growth forms—from shrub-like to large trees—observable across natural populations. *Q. lusitanica* is a shrub or small tree (seldom taller than 1 m), with evergreen or brevideciduous leaf habit and a distribution range restricted to the Atlantic coast of the Iberian Peninsula. It inhabits areas with oceanic influence and dry summers, except a northern isolated population that grows under a rainy temperate climate (Figure 1). By studying both species in the same common-garden experiment, potential interspecific differences in observed patterns of trait population divergence and in selection signals may be ascribed to species-specific genetic and selective factors, rather than to contemporary growing-environment effects. Both *Q. faginea* and *Q. lusitanica* are closely related phylogenetically to widespread temperate European white oaks such as *Q. robur* and *Q. petraea* (Hipp et al., 2020). Analyses of chloroplast DNA (cpDNA) of white oaks have revealed four geographically structured haplotypic groups within the Iberian Peninsula shared across all species, including *Q. faginea*, which have been related to the existence of different glacial refugia followed by subsequent postglacial expansions and intra- and interspecific gene flow (Olalde et al., 2002; Petit et al., 2002).

**FIGURE 1** Top panels show the location of the studied populations of *Quercus faginea* (a) and *Q. lusitanica* (b) in the Iberian Peninsula. Light and dark grey shaded areas in (a) indicate the Iberian distribution range of *Q. faginea* according to Caudullo et al. (2017) and De Rigo et al. (2016), respectively. The shaded area in (b) shows the distribution range of *Q. lusitanica* estimated using species distribution modelling, where a higher probability of occurrence is represented by darker grey colours. Circles indicate populations with genomic and phenotypic data, squares indicate populations with only phenotypic data, and diamonds indicate populations with only genomic data. Middle panels represent the genetic structure of populations of (c) *Q. faginea* and (d) *Q. lusitanica* based on the Bayesian clustering method implemented in STRUCTURE for the  $K$  values with the highest  $\ln \Pr(X|K)$ . Admixture proportions are represented using pie charts, with each colour indicating a different genetic cluster. Pie chart size is proportional to the number of genotyped individuals at each location. Population codes are described in Table 1. Bottom panels display the first three axes of the DAPCs of genomic variation for (e) *Q. faginea* and (f) *Q. lusitanica*. Dots indicate individual PC scores. The colour scale used for populations and individuals of *Q. faginea* and *Q. lusitanica* reflects their latitudinal ranking to help visualization of geographical patterns in the DAPCs.



Here, we test the hypothesis that climate has driven the divergent evolution of functional traits related to drought tolerance and growth among populations of both *Q. faginea* and *Q. lusitanica*, even in a probable scenario of high gene flow among conspecific populations inhabiting contrasting environmental conditions (Savolainen et al., 2007; Kremer & Hipp, 2020). As observed in other oak species, we expect patterns of divergent selection to be particularly evident on morphological traits and growth rates (Ramírez-Valiente et al., 2009; Ramírez-Valiente et al., 2018). Specifically, we expect populations from drier areas to exhibit conservative strategies, with more sclerophyllous and smaller leaves, providing increased desiccation tolerance under low water potentials (Ramírez-Valiente et al., 2010, 2014). We also test the effect of experimental environment on inferred signals of selection. Despite genotype-by-environment interactions being ubiquitous for many traits and species (Matesanz & Ramírez-Valiente, 2019), only a few

studies, mainly on animals, have tested the influence of experimental growing environments on selection inference (Palo et al., 2003; Hangartner et al., 2012; Goodrich et al., 2016). In our study, we expect that experimentally increasing water availability should help to reveal an acquisitive strategy for seedlings from mesic populations, enhancing the expression of faster growth relative to seedlings from xeric populations, which would suggest an adaptive increase in competitive ability under favourable conditions. Finally, we expect patterns of population divergence and trait selection to be stronger in *Q. faginea* than in *Q. lusitanica*, as a result of the more restricted climatic niche and lower climatic variability across the distributional range of the latter. Such potential differences between two closely related species with contrasting climatic niches and ecological strategies might improve our understanding of the role of environmental heterogeneity in driving local adaptation. We address the following specific questions: Do *Q. faginea* and *Q. lusitanica* exhibit population

genetic structure in nuclear genomic markers? Do their respective populations differ genetically in leaf morphology, physiology and growth rates in response to water availability? Is population genetic divergence in functional traits the result of divergent selection? Are there associations between population genetic divergence and provenance climate?

## 2 | MATERIALS AND METHODS

### 2.1 | Population genomic analyses

Between 2016 and 2018, we collected leaves from adult trees from 15 natural populations of *Quercus faginea* ( $n = 104$  individuals) and nine populations of *Quercus lusitanica* ( $n = 78$  individuals) covering most of the distribution range of the two species (Table 1, Figure 1). Leaf samples were stored at  $-20^{\circ}\text{C}$  until needed. Frozen leaf tissue was ground using a mixer mill and tungsten beads and DNA extracted with NucleoSpin Plant II kits (Machery-Nagel). Then, DNA was processed into genomic libraries following the double-digestion restriction-fragment-based procedure (ddRAD-seq) described in Peterson et al. (2012). Briefly, DNA was double-digested using EcoRI and *Mse*I restriction enzymes (New England Biolabs), followed by the ligation of Illumina adaptors and unique 7-bp barcodes. Ligation products were pooled, size-selected between 350 and 450 bp using a Pippin Prep (Sage Science) machine, and amplified by iProof<sup>TM</sup> High-Fidelity DNA Polymerase (Bio-Rad) with 12 cycles. Single-read 151-bp sequencing was performed on an Illumina HiSeq2500 platform at the Centre for Applied Genomics (Toronto, ON, Canada).

We used STACKS version 1.35 (Hohenlohe et al., 2010; Catchen et al., 2011, 2013) to assemble our sequences into de novo loci and call genotypes, a pipeline that produces data sets consistent with those obtained using reference genome-based mapping approaches (Shafer et al., 2017; see also Fitz-Gibbon et al., 2017). Briefly, we demultiplexed and filtered reads for overall quality using the program *process\_radtags*, retaining reads with a Phred score  $> 10$  (using a sliding window of 15%), no adaptor contamination, and having an unambiguous barcode and restriction cut site. We screened raw reads for quality with FASTQC version 0.11.5 (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) and trimmed all sequences to 129 bp using SEQTk (Heng Li, <https://github.com/lh3/seqtk>) in order to remove low-quality reads near the 3' ends. We assembled filtered reads de novo into putative loci with the program *ustacks*. We set the minimum stack depth ( $m$ ) to three and allowed a maximum distance ( $M$ ) of two nucleotide mismatches to group reads into a "stack." We used the "removal" ( $r$ ) and "deleveraging" ( $d$ ) algorithms to eliminate highly repetitive stacks and resolve over-merged loci, respectively. We identified single nucleotide polymorphisms (SNPs) at each locus and called genotypes using a multinomial-based likelihood model that accounts for sequencing errors, with the upper bound of the error rate ( $\epsilon$ ) set to 0.2 (Hohenlohe et al., 2010; Catchen et al., 2011, 2013). Then, we built a catalogue of loci using the *cstacks* program, with loci recognized as homologous across individuals if the number

of nucleotide mismatches between consensus sequences ( $n$ ) was  $\leq 2$ . Finally, we matched each individual data against this catalogue using the program *sstacks* and exported output files in different formats for subsequent analyses using the program *populations*. For all subsequent analyses, we exported only the first SNP per RAD locus and retained loci with a minimum stack depth  $\geq 5$  ( $m = 5$ ), a minimum minor allele frequency (MAF)  $\geq 0.01$  (min\_maf = 0.01) and represented in at least 80% of the populations ( $p = 12$  for *Q. faginea* and  $p = 7$  for *Q. lusitanica*) and 50% of the individuals within each population ( $r = .5$ ).

### 2.2 | Common garden experiments

For the ecophysiological characterization, we used a common garden experiment established at ICIFOR-INIA-CSIC (Madrid, Spain) greenhouse facilities in 2018. This common garden experiment has been used in a previous analysis of *Q. faginea* multivariate phenotypic variation and plasticity (Solé-Medina et al., 2022). In the present study, we conducted a functional characterization for both *Q. faginea* and *Q. lusitanica* using all plants in the experiment, and integrated this information with new genomic data to explore patterns of selection in both species. To establish this common garden, we collected acorns in autumn 2017 from 106 open-pollinated mother trees from 10 climatically contrasting populations of *Q. faginea* and from 35 open-pollinated mother trees from five populations of *Q. lusitanica* (Table 1), covering the distribution range of both species. Seeds were stored at  $4^{\circ}\text{C}$ . In spring 2018, we sowed the seeds in 2.3-L pots (CC-1028R; Sansan) filled with fine sand and placed them randomly within the greenhouse. Seedlings were maintained under ambient light and  $10\text{--}25^{\circ}\text{C}$  of temperature for 2 months after emergence. Then, seedlings were transplanted into double pots (i.e., two 2.3-L CC-1028R Sansan pots stacked on top of each other, after removing the bottom of the upper pot) with a total volume of 4.5 L and a length of 55 cm. We used a soil mixture of 1:1 (v/v) peat and fine sand. The experiment followed a complete block design with six blocks. Between one and five seedlings per family of each species were placed within each block, depending on seedling availability. In total, the experiment included 1350 seedlings, 966 of *Q. faginea* and 384 of *Q. lusitanica*. After watering all seedlings every other day for 2 weeks, watering was withdrawn in three blocks to induce gradual water stress (dry treatment), whereas seedlings in the other three blocks were watered to field capacity every 2–3 days (well-watered treatment). Volumetric water content (VWC) was measured once a week in seven randomly selected pots per block using time domain reflectometry (ProCheck, Decagon Devices, Inc.). In the well-watered treatment, VWC was maintained between 28.3% and 34.3% throughout the experiment, while it was gradually reduced down to an average low of 6.7% in the dry treatment at the end of the experiment. Additionally, for *Q. faginea*, we established a common garden experiment under field conditions (outdoor common garden experiment) to contrast inferred patterns of divergent selection based on phenotypes expressed in the greenhouse vs. in more natural environmental conditions (Methods S1).

TABLE 1 Geographical location and elevation of the studied populations of *Quercus faginea* and *Q. lusitanica*

Population	Code	Species	Latitude	Longitude	Elevation	Garden	n Fam	n Gen
Valderejo	VAL	<i>Q. faginea</i>	42.89713	-3.23803	1066	GH	15	8
Quintana R.	QUI	<i>Q. faginea</i>	41.62061	-2.65374	1024	GH	5	8
Macedo de C.	MAC	<i>Q. faginea</i>	41.51642	-6.95816	541			9
Vilaplana	VIL	<i>Q. faginea</i>	41.24001	1.05709	658	GH	9	
Salamanca	SAL	<i>Q. faginea</i>	40.87523	-5.82989	864	OE, GH	6,7	7
Molina	MOL	<i>Q. faginea</i>	40.79333	-1.82689	1139	OE, GH	6,15	11
Soto del Real	SOT	<i>Q. faginea</i>	40.74432	-3.74622	909	OE, GH	6,12	6
Cuenca	CUE	<i>Q. faginea</i>	40.06702	-2.20115	917	OE	5	6
Penela	PEN	<i>Q. faginea</i>	40.03422	-8.37145	152	GH	11	8
Talayuela	TAL	<i>Q. faginea</i>	39.94701	-5.58068	270	OE	4	6
Espadán	ESP	<i>Q. faginea</i>	39.91521	-0.36951	548	OE	2	3
Retuerta del B.	RET	<i>Q. faginea</i>	39.43268	-4.34608	694	OE	4	5
Viso del Marqués	VIS	<i>Q. faginea</i>	38.48316	-3.73880	879	GH	13	
Zafra	ZAF	<i>Q. faginea</i>	38.38436	-6.28528	561	OE	6	5
Alanis	ALA	<i>Q. faginea</i>	38.04015	-5.67355	677	OE, GH	6,9	6
Olalla	OLA	<i>Q. faginea</i>	37.93880	-6.17445	451	OE	5	
Lújar	LUJ	<i>Q. faginea</i>	36.82401	-3.40702	1750			8
El Bosque	BOS	<i>Q. faginea</i>	36.77460	-5.53451	463	OE, GH	6,10	6
Monte Pindo	PI	<i>Q. lusitanica</i>	42.87456	-9.10553	60	GH	13	10
Penela	PE	<i>Q. lusitanica</i>	40.04035	-8.36233	217	GH	7	9
Ponte de Sor	PO	<i>Q. lusitanica</i>	39.31391	-8.02107	179	GH	2	8
Olhalvo	OL	<i>Q. lusitanica</i>	39.11740	-9.04872	125	GH	4	8
Fernão Ferro	FE	<i>Q. lusitanica</i>	38.54203	-9.08829	79			8
Castaño Robledo	CA	<i>Q. lusitanica</i>	37.89580	-6.70790	735	GH	6	10
Monchique	MO	<i>Q. lusitanica</i>	37.32890	-8.50259	484			9
Ubrique	UB	<i>Q. lusitanica</i>	36.58720	-5.51819	609			9
El Bujeo	BU	<i>Q. lusitanica</i>	36.07097	-5.52980	275			7

Note: Garden indicates the common garden experiment in which populations were assayed (GH = greenhouse experiment, OE = outdoor experiment). Elevation is given in metres a.s.l. n Fam is the number of open-pollinated maternal families in the common garden, and n Gen indicates the number of genotyped individuals.

### 2.3 | Ecophysiological measurements

We measured stem height and basal diameter of all seedlings at the time of the start of the watering treatments (1 month after transplantation) and the end of the experiment (5 months after transplantation). Absolute growth rate (AGR) and relative growth rate (RGR) were calculated following Ramírez-Valiente & Cavender-Bares (2017), as  $AGR = (M_{final} - M_{initial})/(T_{final} - T_{initial})$ , where  $M_{final}$  and  $M_{initial}$  are the values measured at the end and the beginning of the experiment, respectively, and  $T_{final}$  and  $T_{initial}$  are the dates of the final measurement and of the start of the watering treatment, respectively. Relative growth rate was calculated as  $RGR = [\log_e(M_{final}) - \log_e(M_{initial})]/(T_{final} - T_{initial})$ . AGR and RGR for height and diameter were highly correlated ( $r = .75, p < .001$  and  $r = .65, p < .001$ , respectively), so only height growth rates were used for further analyses.

One month after the start of the watering treatments, we measured a set of physiological parameters related to leaf photochemical

efficiency, carbon assimilation rates, photoprotection and transpiration on sun leaves of two to five seedlings per maternal family per treatment. For this, we measured chlorophyll *a* fluorescence using a portable pulse-modulated fluorimeter (FMS2, Hansatech Instruments). Minimal ( $F_0$ ) and maximal ( $F_m$ ) fluorescence were measured at predawn ( $n = 1088$ ). Around solar noon (12–14 p.m.), we measured maximal ( $F_m'$ ) and steady-state ( $F_s$ ) fluorescence and applied a far-red pulse (740 nm) for 5 s to obtain minimum light-adapted fluorescence ( $F_0'$ ) ( $n = 1149$ ). For further analyses, we calculated Stern–Volmer nonphotochemical quenching, NPQ [ $(F_m - F_m')/F_m'$ ], which represents the excess energy dissipation via heat; the effective quantum yield of Photosystem II,  $\phi_{PSII}$  [ $(F_m' - F_s)/F_m'$ ], which represents the proportion of absorbed energy used in photochemistry; and maximum quantum yield of Photosystem II in light [ $(F_m' - F_0')/F_m' = F_v'/F_m'$ ], which represents the photochemical efficiency if all PSII centres were open under light conditions (Cavender-Bares & Bazzaz, 2004). We measured stomatal conductance around

solar noon (12–14 p.m.) using a leaf porometer (SC-1; Decagon Devices) ( $n = 1300$ ). Stomatal conductance was calculated on both area ( $g_{s,area}$ ) and mass ( $g_{s,mass}$ ) basis multiplying  $g_{s,area}$  by the specific leaf area (see paragraph below).

Leaves used for measuring physiological parameters were subsequently collected for analyses of leaf morphology. Leaf thickness was measured using a micrometre when leaves were still fresh. Then, we scanned them to determine leaf lamina area and perimeter using WinFOLIA software (Regent Instruments Inc.). We calculated the perimeter-to-area ratio, which combines information on leaf area and the degree of lobulation of the leaf. Finally, we oven-dried the leaves at 60°C for 48 h and weighed them to calculate specific leaf area (SLA), a trait related to carbon, nitrogen and phosphorous economics, by dividing fresh leaf area by leaf dry mass.

## 2.4 | Population genetic structure

We analysed patterns of SNP genetic structure for each species separately using the Bayesian Markov chain Monte Carlo (MCMC) clustering method implemented in the program STRUCTURE version 2.3.3 (Pritchard et al., 2000; Falush et al., 2003; Hubisz et al., 2009) and discriminant analysis of principal components (DAPC; Jombart et al., 2010). Neither of the two clustering methods requires a priori population delimitation, but they differ in their analytical approaches and assumptions. STRUCTURE builds clusters by minimizing Hardy-Weinberg and gametic disequilibrium within clusters and typically fails to detect isolation-by-distance (IBD). By contrast, the multivariate DAPC does not rely on the assumptions of STRUCTURE and could be more efficient to detect complex patterns of genetic differentiation (Jombart et al., 2010).

For the Bayesian clustering analyses in STRUCTURE, we considered correlated allele frequencies and an admixture model without prior information on population origin (Hubisz et al., 2009). We performed 15 independent runs for each value of  $K$  ( $K = 1\text{--}8$  for each species) with a burn-in period of 200,000 steps and a run length of 1,000,000 MCMC cycles. We retained the 10 runs having the highest likelihood for each value of  $K$  and estimated the best-supported number of genetic clusters with the log probability of the data [ $\ln \Pr(X|K)$ ] (Pritchard et al., 2000) and the  $\Delta K$  method (Evanno et al., 2005) as implemented in STRUCTURE HARVESTER (Earl & VonHoldt, 2012). For visualization, we used the “full search” algorithm in the program CLUMPP version 1.1.2 to align replicated runs and average individual assignment probabilities for the same  $K$  value (Jakobsson & Rosenberg, 2007). DAPCs were implemented using the package “adegenet” (Jombart & Ahmed 2011) in R version 3.6.1 (R Core Team, 2022).

We additionally estimated the magnitude of neutral genetic structure of each species by calculating overall and pairwise  $F_{ST}$  values, using the estimator of Weir & Cockerham (1984) as implemented in the R package “hierfstat” (Goudet 2005).  $F_{ST}$  significance ( $p$  value) was estimated via 1000 random permutations of genotypes

among populations. The distribution of  $F_{ST}$  values and confidence intervals around point estimates were obtained via bootstrapping over loci with 1000 independent replicates.

## 2.5 | Population divergence in ecophysiological traits and signals of selection

We used the approach developed by Ovaskainen et al. (2011) and Karhunen et al. (2013) with modifications by Csilléry, Buchmann, et al. (2020) to investigate if traits were potentially under among-population divergent selection. This method appears to be preferable to the traditional comparison  $F_{ST}\text{-}Q_{ST}$  mainly due to the high evolutionary stochasticity associated with  $Q_{ST}$ , which may inflate type I error, especially for complex population structures and low population numbers (for reviews see Leinonen et al., 2013; Villemereuil et al., 2022). Ovaskainen et al. (2011) use a more flexible neutral demographic model, allowing for drift and migration differences among populations, which results in a null expectation of trait population divergence potentially enabling more powerful tests of selection (Villemereuil et al., 2022).

For the analyses, we first estimated a co-ancestry matrix (i.e., genetic distances based on genomic markers) between all pairs of populations using SNP allele frequencies assuming an admixture F-model (AFM) and using a Metropolis-Hastings algorithm implemented in the R package “RAFM” (Karhunen & Ovaskainen 2012). This model assumes that populations diverged from a common ancestral genetic pool and that genetic differences among populations have emerged from neutral drift. We conducted these analyses for each species and common garden, independently. We ran 30 independent Markov chains with a burn-in of 20,000 iterations followed by 10,000 iterations and a thinning interval of 10. To test for chain convergence, we estimated the mean of the off-diagonal values of  $F_{ST}$  of each matrix, ran Heidelberger's test (Heidelberger & Welch, 1981) and calculated Geweke's z-scores (Geweke, 1991) using the “coda” R package. Chains that did not converge were excluded from further analyses.

Second, we evaluated the overall evidence of selection across all populations by comparing mean additive trait values for the study populations with those estimated for the assumed ancestral population. For this purpose, we used the MH and S-test functions of the “driftsel” R package (Ovaskainen et al., 2011; Karhunen et al., 2013). MH conducts a Bayesian mixed-effects animal model that takes into account the co-ancestry matrix obtained from AFM (i.e., the genetic structure from SNP markers), the family structure among and within populations (i.e., pedigree) and the experimental design (i.e., covariates, fixed-effect factors and random-effect factors). Then, the S-test estimates to what extent population trait means differ between the study populations and the ancestral population using the S-statistic (“signal of selection” sensu Karhunen et al., 2014). An S-statistic equal to 0.5 would indicate a perfect match with neutral expectations; that is, the overall pattern of trait population differentiation is exactly as expected from neutral drift alone under the

assumed model of divergence;  $S = 0$  would indicate a perfect match with uniform selection expectations, with overall genetic differences among populations lower than expected under neutral drift; and  $S = 1$  would indicate a perfect match with divergent selection expectations, with overall trait genetic divergence higher than expected under drift. Values of  $S$  of 0–0.5 and of 0.5–1 would support uniform and divergent selection, respectively, at credibility levels depending on the precise  $S$  value. For example,  $S = 0.05$  would indicate uniform selection at a credibility level of 95%, while  $S = 0.95$  would indicate divergent selection at a credibility level of 95% (Karhunen et al., 2014). We conducted these analyses by species, common garden and watering treatment independently. Seedling height was included as a covariate, and block, row and column of the common gardens were included as fixed-effect factors in the models. Following Csilléry, Buchmann, et al. (2020), we also estimated the additive genetic trait values of single populations and their values expected under neutral drift. We assessed pairwise differences in trait values among populations based on the posterior distribution of their difference.

For the sake of comparison with the method developed by Ovaskainen et al. (2011) and Karhunen et al. (2013), we also conducted classic  $Q_{ST}$ – $F_{ST}$  analyses for each combination of species, trait and treatment. Variance components for the calculation of  $Q_{ST}$  were estimated using Bayesian mixed models, running 10 independent MCMC chains, with the “MCMCglmm” package in R (Hadfield, 2010). MCMC chains were run for 5.5 million iterations, sampling every 5000 and with a burn-in period of 500,000 iterations, yielding 1000 random samples used to infer the posterior distribution of  $Q_{ST}$ . For these analyses, seedlings within each sampled maternal family were considered as half-siblings.  $Q_{ST}$  was estimated as  $V_p/(V_p + 2 \times 4 \times V_f)$ , where  $V_p$  and  $V_f$  are the variance components of the population and maternal family, respectively. For  $Q_{ST}$ – $F_{ST}$  comparison, we followed the method outlined in Whitlock (2008). First, the previously obtained  $F_{ST}$  bootstrap distribution (see section 2.4) was multiplied by the Lewontin–Krakauer chi-square distribution (Lewontin & Krakauer, 1973), which accounts for deviations in  $F_{ST}$  due to demographic factors (Whitlock, 2008; Whitlock & Guillaume, 2009). Second, we tested for each trait whether  $Q_{ST} > F_{ST}$  by assessing whether the 0.05 quantile of the distribution of the difference ( $Q_{ST} - F_{ST}$ ) was larger than zero, with the distribution of the difference being obtained by pairing the 1000 values of the posterior  $Q_{ST}$  distribution with the 1000 values of the bootstrap  $F_{ST}$  distribution.

To explore selection from a multivariate phenotypic perspective, we conducted a principal component analysis on individual phenotypic values measured in the greenhouse experiment (PCA-phenotype). We conducted this analysis only for *Q. faginea* populations due to the low number of individuals of *Q. lusitanica* for which all traits were measured ( $n = 120$  in the dry treatment and 143 in the well-watered treatment). The phenotypic trait values were centred and scaled before performing the PCA using the `prcomp` function in R. We used the individual PC scores to test for multivariate signal of selection ( $S$ ).

## 2.6 | Environmental drivers of population genetic divergence in ecophysiological traits

For traits showing at least weak evidence of being under divergent selection (estimated  $S > 0.5$ ), we also implemented an  $H$ -test (sensu Karhunen et al., 2014) to identify the potential environmental drivers of population divergence, using the modification of this test developed by Csilléry, Buchmann, et al. (2020). Briefly, this test estimates a statistic ( $H^*$  sensu Csilléry, Buchmann, et al., 2020) that measures the association between an environmental variable and the degree of deviation from neutrality of a given trait. The  $H^*$ -statistic varies between 0 and 1, with values close to 1 indicating that trait differences are more strongly associated with climatic differences among populations than expected at random. To further explore potential drivers of population divergence in quantitative traits, we also implemented Pearson correlations between population environment and the additive population trait means (i.e., population means that account for genetic drift) obtained from the Bayesian mixed-effect animal model.

To test for associations between trait and environmental divergence via  $H^*$ -tests and correlations, we first performed two principal component analyses (PCA-climate) of environmental variables, one per species. These PCAs included 19 bioclimatic variables of the populations for the period 1970–2000 extracted from <http://www.worldclim.org/> (Fick & Hijmans, 2017), soil pH, extracted from Trabucco & Zomer (2010), and an annual and summer index of moisture ( $I_{m,annual}$  and  $I_{m,summer}$ , respectively). The annual index of moisture was calculated as  $I_{m,annual} = \sum(P_i - PET_i)$ , where  $P_i$  is the monthly precipitation and  $PET_i$  monthly potential evapotranspiration (see Ramírez-Valiente et al., 2018 for a similar procedure). Potential evapotranspiration was estimated using the method of Hargreaves & Samani (1985).  $I_{m,summer}$  was estimated as the difference between precipitation and potential evapotranspiration for July–September. The environmental variables were centred and scaled before performing the PCA using the `prcomp` function in R.

We used the individual PC scores from the PCA-phenotype of *Q. faginea* to test for associations between multivariate phenotypes and climate using  $H^*$ -tests and Pearson correlations.

## 3 | RESULTS

### 3.1 | Genomic data

A total of 192,377,504 (mean  $\pm$  SD = 1,849,784  $\pm$  987,363 reads per individual) and 189,338,285 (mean  $\pm$  SD = 2,427,414  $\pm$  1,699,312 reads per individual) reads were obtained for 104 and 78 individuals of *Quercus faginea* and *Q. lusitanica*, respectively. The data sets obtained with STACkS with parameters  $P = 12$  and  $P = 7$  contained 9244 and 11,463 SNPs, for *Q. faginea* and *Q. lusitanica*, respectively. Genetic diversity parameters for *Q. faginea* and *Q. lusitanica* populations are shown in Tables S1 and S2, respectively.

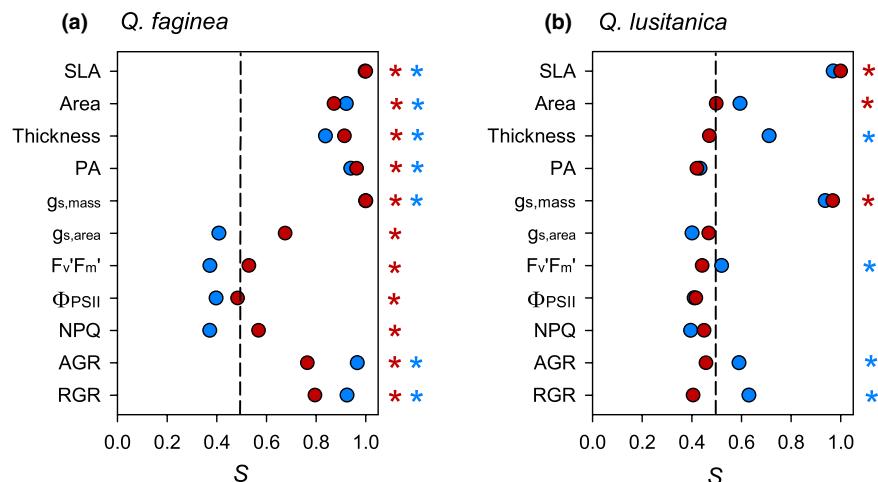
### 3.2 | Population genetic structure

STRUCTURE analyses revealed a weak geographical pattern of genetic structure within both *Q. faginea* and *Q. lusitanica*, with different populations across their respective ranges showing rather similar admixture proportions (Figure 1). For *Q. faginea*, the highest  $\ln \Pr(X|K)$  was reached for  $K = 3$  and the highest  $\Delta K$  for  $K = 2$  (Figure S1). For this species,  $K = 2$  did not show an evident clustering,  $K = 3$  slightly differentiated the Lújar population (LUJ), and  $K = 4$  partially grouped north-western populations (MAC, PEN and TAL) (Figure 1 and Figure S2). For *Q. lusitanica*, the highest  $\ln \Pr(X|K)$  was found for  $K = 4$  and the highest  $\Delta K$  for  $K = 2$  (Figure S1).  $K = 2$  slightly separated the northernmost population of *Q. lusitanica* (Monte Pindo, PI) from the rest,  $K = 3$  did not produce any additional clustering and  $K = 4$  weakly differentiated the southern population of Ubrique (UB) from the remaining populations (Figure 1 and Figure S2). Consistent with STRUCTURE results, DAPCs showed weak population segregation within both *Q. faginea* and *Q. lusitanica* (Figure 1), with the minimum Bayesian information criterion (BIC) obtained for  $K = 1$  in both species, indicating a limited population genetic structure. Global  $F_{ST}$  values for both *Q. faginea* (0.027; 95% confidence interval [CIs]: 0.024–0.029) and *Q. lusitanica* (0.044; 95% CI: 0.042–0.047) were low but significantly different from zero ( $p < .001$  in both species). Accordingly, several pairwise  $F_{ST}$  estimates were significantly different from zero but consistently low in both *Q. faginea* (range = 0.002–0.073; Table S3) and *Q. lusitanica* (range = 0.006–0.089; Table S4). Analyses based on data sets excluding outlier loci (i.e., putatively under selection; see details in Methods S2) produced virtually identical results in STRUCTURE (Figures S1 and S3) and DAPC (Figure S4) and yielded similar  $F_{ST}$  estimates (Tables S3 and S4).

### 3.3 | Signals of divergent selection on ecophysiological traits

Our results provided evidence of divergent selection in different leaf and growth traits. All traits for *Q. faginea* and seven out of 11 traits for *Q. lusitanica* exhibited differences among populations in at least one environment (Figure 2; Figure S5). Overall, more inland *Q. faginea* populations (MOL, QUI, SOT, SAL) exhibited lower specific leaf area and higher stomatal conductance ( $g_{s,mass}$ ;  $g_{s,area}$ ) in the greenhouse experiment, particularly under dry conditions, and lower absolute growth rates under well-watered conditions (Figure S6). In the outdoor common garden experiment, CUE, SOT and SAL had the lowest specific leaf area (Figure S7). For *Q. lusitanica*, there was not such a clear geographical pattern of trait variation across populations, although the northernmost population (PI) showed the highest leaf area,  $g_{s,mass}$  under dry conditions and absolute growth rate under well-watered conditions (Figure S8).

The signal of selection ( $S$ ) was highly variable across traits, species and common gardens.  $S$  was consistently high (supporting divergent selection) for specific leaf area, with  $S$  values ranging between 0.93 and 1 in both species, in both the outdoor and greenhouse experiments, and for both watering treatments in the greenhouse (Figure 2; Figure S5). Leaf area, perimeter-to-area ratio and thickness exhibited  $S$  values around 0.9 for *Q. faginea* in both greenhouse watering treatments, which contrasted with the  $S$  values close to 0.5 obtained for *Q. lusitanica* (Figure 2).  $S$ -tests provided strong evidence of divergent selection in mass-based stomatal conductance ( $g_{s,mass}$ ) for both *Q. faginea* and *Q. lusitanica* under dry and well-watered conditions, but not for other physiological traits (Figure 2). The signal of divergent selection was high also



**FIGURE 2** Signal of selection ( $S$ ) estimated following Ovaskainen et al. (2011) for quantitative traits measured in a greenhouse common-garden experiment of (a) *Quercus faginea* and (b) *Q. lusitanica*. The greenhouse experiment was established under well-watered (blue dots) and dry (red dots) conditions. The 95% credible intervals were small and are hidden behind the dots. Mass-based stomatal conductance ( $g_{s,mass}$ ) in the well-watered and dry conditions had the same signal of selection ( $S = 1$ ). Asterisks (\*) indicate at least one population pair with  $>95\%$  posterior probability of having different additive population means, as estimated in well-watered conditions (blue) and dry conditions (red) in the greenhouse common garden. Traits include specific leaf area (SLA), leaf lamina area (area), leaf thickness (thickness), leaf perimeter-to-area ratio (PA), mass-based stomatal conductance ( $g_{s,mass}$ ), area-based stomatal conductance ( $g_{s,area}$ ), maximum quantum yield of photosystem II in light ( $F_v'/F_m'$ ), effective quantum yield of photosystem II ( $\Phi_{PSII}$ ), nonphotochemical quenching (NPQ), and absolute and relative height growth rate (AGR and RGR).

in growth traits (AGR and RGR) in the well-watered treatment, but only for *Q. faginea* (Figure 2).

$Q_{ST}$ – $F_{ST}$  comparisons produced qualitatively similar results to those obtained from the method developed by Ovaskainen et al. (2011). Specifically, for *Q. faginea*, all traits exhibited  $Q_{ST}$  estimates significantly higher than  $F_{ST}$  in the dry treatment, while only three physiological traits did not have  $Q_{ST}$  estimates significantly higher than  $F_{ST}$  in the well-watered treatment (Table S5). For *Q. lusitanica*,  $Q_{ST}$  was significantly higher than  $F_{ST}$  for all morphological traits, for stomatal conductance (both mass-based and area-based) and relative growth rates in the dry treatment and for most physiological traits in the well-watered treatment.

### 3.4 | Potential role of climate on divergent selection

The first two axes of the PCA on environmental variables explained 76.7% and 72.7% of the variance of *Q. faginea* and *Q. lusitanica* populations, respectively (Figure 3). For *Q. faginea*, the first axis explained 45% of the variance and was negatively related (negative loadings) to mean temperature of the warmest quarter and annual mean temperature, and positively related (positive loadings) to summer precipitation, among others (Figure 3). Thus, higher values in PC-climate1 represented milder summers, with less severe drought and milder temperatures. The second axis (PC-climate2) explained 31.7% of the variance and was positively related to annual precipitation and winter temperatures, and negatively related to temperature seasonality and temperature annual range, among others (Figure 3). Thus, higher values in PC-climate2 represented wetter climates with milder winters and more constant temperatures over the year. For *Q. lusitanica*, the first axis explained 49.8% of the variance and was positively related to annual and summer precipitation and negatively related to summer temperature and temperature seasonality (Figure 3). Thus, higher values in PC-climate1 represented milder summers, with less severe drought and milder temperatures. The second axis (PC-climate2) explained 22.9% of the variance and was positively related to annual and winter temperature of *Q. lusitanica* populations (Figure 3).

For *Q. faginea*,  $H^*$ -tests and Pearson correlations showed strong associations between PC-climate2 and most traits that showed some evidence of divergent selection (Figure 3). Specifically, Pearson correlations showed that populations with higher values of PC-climate2 (i.e., from milder climatic provenances characterized by more mesic conditions and higher winter temperatures) exhibited higher SLA, larger and thinner leaves with higher growth rates in the well-watered treatment, and lower stomatal conductance ( $g_{s,area}$ ,  $g_{s,mass}$ ) and maximum quantum yield of PSII in light ( $F_v'/F_m'$ ) in the dry treatment (Figure 3). Most of these traits exhibited  $H^*$  values close to 1 in the greenhouse experiment, providing evidence of climate-driven divergent selection. For *Q. lusitanica*,  $H^*$ -tests and Pearson correlations showed weak associations between climatic PC axes and most traits (Figure 3). Leaf area and mass-based stomatal conductance ( $g_{s,mass}$ ) were positively associated with PC-climate1 and RGR was negatively associated with

PC-climate2 in the well-watered treatment (Figure 3). These results indicated that populations from more mesic provenances had larger leaves with higher  $g_{s,mass}$  under mesic conditions, whereas populations from warmer provenances had lower RGR (Figure 3).

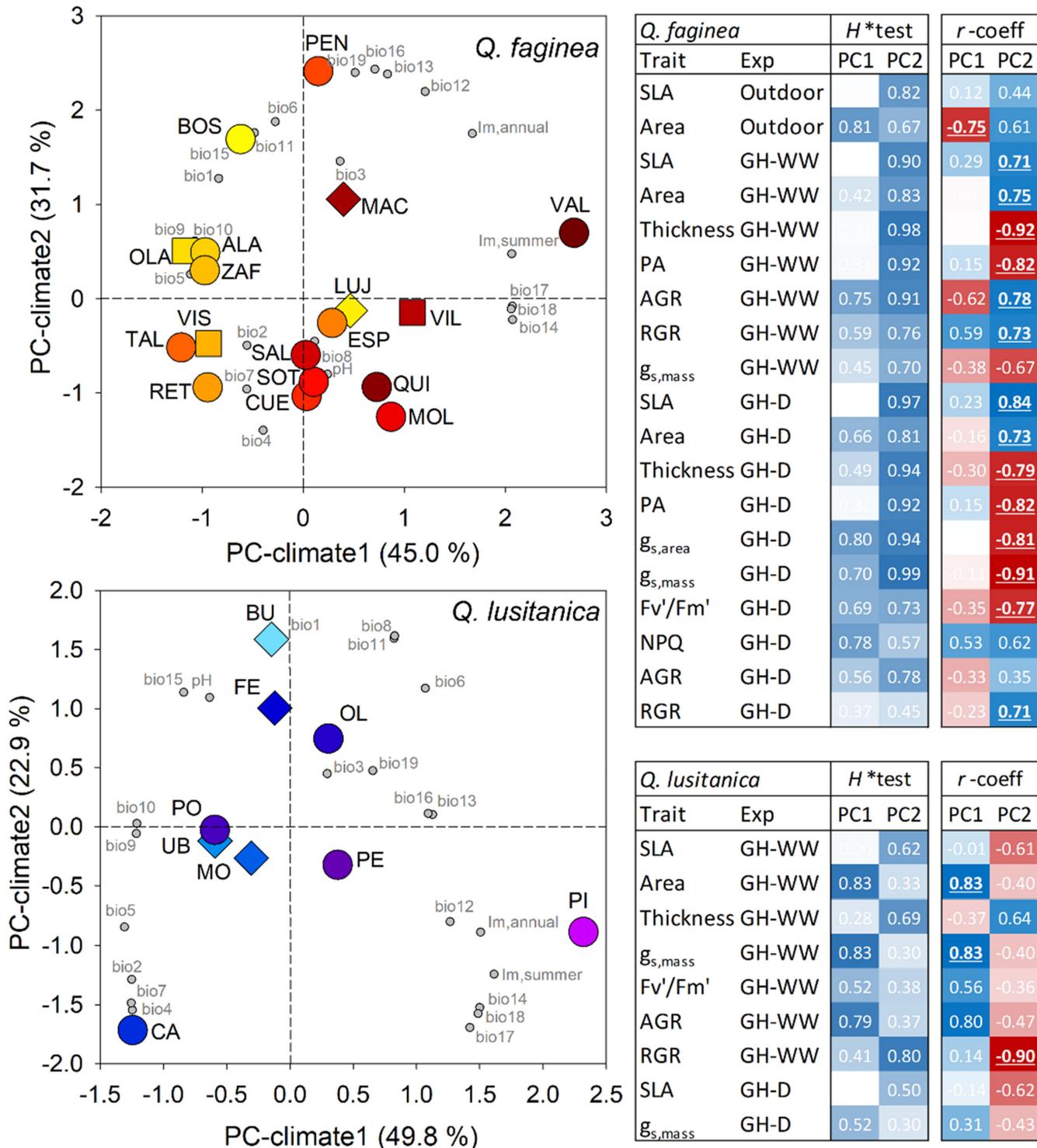
The first two PC axes of *Q. faginea* individual phenotypic values explained 52.2% of the variance (Figure 4). The first axis (PC-phenotype1) explained 30.3% of the variance and was negatively related (negative loadings) to physiological traits, mass-based stomatal conductance ( $g_{s,mass}$ ), area-based stomatal conductance ( $g_{s,area}$ ), maximum quantum yield of PSII in light ( $F_v'/F_m'$ ), effective quantum yield of PSII ( $\Phi_{PSII}$ ) and growth. The second axis (PC-phenotype2) explained 21.9% of the variance and was positively related (positive loadings) to leaf thickness and perimeter-to-area ratio and negatively related to SLA, leaf lamina area and growth (Figure 4). The signal of selection ( $S$ ) was 0.59 and 0.58 for the first axis (PC-phenotype1) and 0.99 and 0.98 for the second axis (PC-phenotype2) in the dry and well-watered treatment, respectively (Figure S9). The second axis of the phenotypic PCA (PC-phenotype2) was strongly negatively associated with the second axis of the environmental variables (PC-climate2) both in the dry and well-watered conditions (Figure 4). These results indicated that populations from more continental climates, with high temperature oscillation and drier conditions presented smaller (lower leaf area and perimeter-to-area ratio [PA]) and more sclerophyllous leaves (higher thickness and lower SLA). The  $H^*$  statistic provided strong evidence of climatic divergent selection driving multivariate phenotypic divergence among *Q. faginea* populations ( $H^* = 0.99$  for PC-phenotype2 in both watering treatments; Figure 4).

## 4 | DISCUSSION

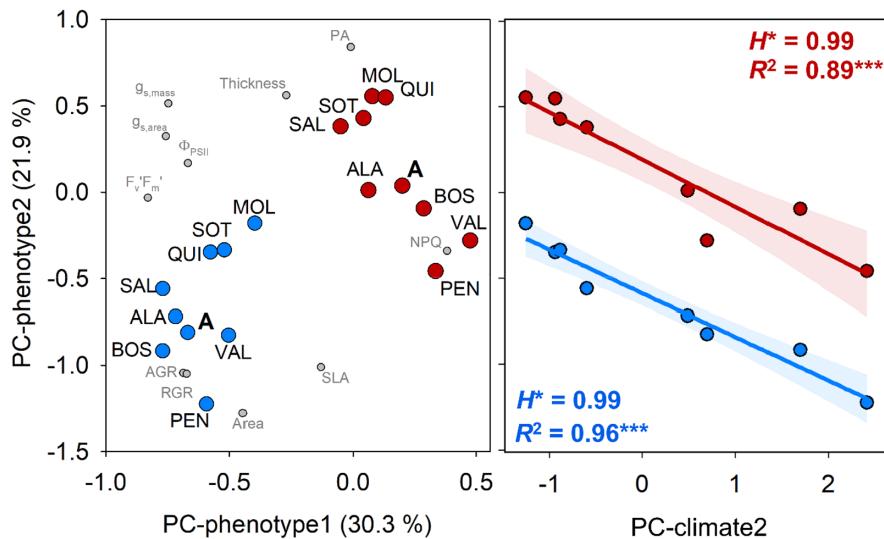
Our genotyping analysis revealed that *Quercus faginea* and *Q. lusitanica* exhibit weak population genetic structure, probably as a result of high historical rates of pollen-mediated gene flow among populations and large long-term effective population sizes. In contrast, our common garden experiments revealed substantial trait genetic variation and showed evidence of climate-driven divergent selection among populations of both species in traits related to leaf morphology, physiology and growth. These results suggest that climatic differences within species ranges have driven population genetic divergence in traits important for plant adaptation to heterogeneous Mediterranean ecosystems. Although dating the origin of such divergence would be complicated, given the continuous presence of oak species in the Iberian Peninsula throughout glacial periods, our results also suggest that it has not been precluded or eliminated by high levels of historical gene flow.

### 4.1 | Weak genetic structure in *Q. faginea* and *Q. lusitanica*

Previous studies have shown strong genetic structure of European white oaks in maternally inherited cpDNA (Petit, Csaikl, et al., 2002).



**FIGURE 3** Principal component analyses (PCAs) of 19 bioclimatic variables (data from [www.worldclim.org](http://www.worldclim.org)), soil pH (data from Trabucco & Zomer, 2010), and annual and summer index of moisture ( $I_{\text{m,annual}}$  and  $I_{\text{m,summer}}$ , respectively) for *Quercus faginea* and *Q. lusitanica* populations (left panels). Small grey circles and large coloured symbols indicate variable loadings and population scores for the first two PC axes (PC-climate1, PC-climate2), respectively. Population codes are described in Table 1. The colour scale used for populations reflects their latitudinal ranking following Figure 1. Circles indicate populations with genomic and phenotypic data, squares indicate populations only with phenotypic data and diamonds indicate populations only with genomic data. Right panels show the results of the  $H^*$ -test (Csilléry, Ovaskainen, et al., 2020; Karhunen et al., 2014) and  $r$ -coefficients from Pearson correlations between traits and the first two climatic PC-axes for each species.  $H^*$  values close to 1 (dark blue) indicate evidence for climatic adaptation (see text for details). Colour intensity is proportional to  $H^*$  values and correlation coefficients, in a blue scale for positive values and in a red scale for negative values. Significant ( $p < .05$ )  $r$ -coefficients are underlined. Only traits showing a signal of selection greater than 0.5 were considered for these analyses. Trait abbreviations as in Figure 3. In the experiment column (Exp): Outdoor, outdoor common garden experiment; GH-WW, greenhouse experiment under well-watered treatment; and GH-D, greenhouse experiment under dry treatment.



**FIGURE 4** Principal component analysis (PCA) of phenotypic traits measured in the greenhouse experiment under dry (red) and well-watered (blue) treatments for the additive mean populations and the estimated ancestral population of *Quercus faginea* (left panel). Small grey circles and large blue/red circles indicate phenotypic trait loadings and population scores for the first two PC axes (PC-phenotype1, PC-phenotype2), respectively. Population codes are described in Table 1. “A” indicates the ancestral population mean estimated following Ovaskainen et al. (2011) and Csilléry, Buchmann, et al. (2020). Phenotypic traits: Specific leaf area (SLA), leaf lamina area (area), leaf thickness (thickness), leaf perimeter-to-area ratio (PA), mass-based stomatal conductance ( $g_{s,\text{mass}}$ ), area-based stomatal conductance ( $g_{s,\text{area}}$ ), maximum quantum yield of photosystem II in light ( $F_v'/F_m'$ ), effective quantum yield of photosystem II ( $\Phi_{\text{PSII}}$ ), nonphotochemical quenching (NPQ), and relative and absolute height growth rate (RGR and AGR). The right panel shows linear regressions between the second axis of the PCA of environmental variables (PC-climate2) and the second axis of the PCA of phenotypic traits (PC-phenotype2) for *Q. faginea* populations. Dots indicate additive population means for PC-phenotype2 in the dry (red) and well-watered (blue) treatments in the greenhouse experiment.  $H^*$  values were obtained following Csilléry, Buchmann, et al. (2020), and  $R^2$  coefficients from Pearson correlations are shown for each treatment. Asterisks for  $R^2$  coefficients indicate  $p < .001$ .

In the Iberian Peninsula, Olalde et al. (2002) found two main maternal lineages differentiating northwestern from southeastern white oak populations, as well as two other minor lineages specific to northeastern populations. The existence of multiple Iberian glacial refugia during the last glacial maximum and posterior recolonization and hybridization between species have been proposed as the main processes driving the current genetic structure in cpDNA in white oaks (Olalde et al., 2002; Petit, Csaikl, et al., 2002). Using biparentally inherited nuclear markers, we did not observe the same pattern. In fact, STRUCTURE and DAPC analyses revealed a very weak genetic structure in both *Q. faginea* and *Q. lusitanica* (Figure 1). Similar contrasting geographical patterns in nuclear versus plastidial markers have been commonly reported in other tree species, including oaks (e.g., Magri et al., 2007; Ortego et al., 2015; Pina-Martins et al., 2019). Chloroplast and nuclear genomes have different forms of inheritance, mutation rates and vectors of dispersion (Petit et al., 2005). The chloroplast is maternally inherited in oaks and dispersed via seeds (Dumolin et al., 1995). Thus, the stronger genetic structure in chloroplast vs. nuclear loci probably reflects shorter dispersal distances of seeds by animals and gravity, relative to pollen dispersal distances by wind (e.g., Dow & Ashley, 1998; Cavender-Bares & Pahlich, 2009; Ortego et al., 2014; Sork et al., 2015). Greater genetic structure in chloroplast loci may also result from their greater sensitivity to genetic drift than nuclear markers, because of their haploid status and, thus, lower effective population sizes (Ortego et al., 2015).

The low population divergence in both *Q. faginea* and *Q. lusitanica* is consistent with that reported in other oak species from temperate regions (e.g., Ramírez-Valiente et al., 2009; Cavender-Bares et al., 2011, 2015; Pina-Martins et al., 2019). In wind-pollinated trees, only strong geographical isolation might effectively restrict gene flow and create marked neutral genetic divergence provided such isolation is maintained through extended periods of time (e.g., Cavender-Bares et al., 2011, 2015). Although the persistence and stability of oak populations from the Iberian Peninsula through the Pleistocene might have potentially contributed to the long-term isolation of local/regional gene pools, large effective population sizes are expected to have buffered genetic drift and resulted in a limited genetic structure across space and time (e.g., Lesser et al., 2013). Thus, the interplay between the demographic history and life-history characteristics of oaks (i.e., long life, overlapping generations, wind pollination, outcrossing mating and large historical effective population size; Petit & Hampe, 2006) probably underlies the weak genetic structure observed in our two focal species.

#### 4.2 | Consistent evidence of divergent selection on leaf morphology

Our results revealed that SLA was the studied trait that exhibited the strongest evidence of divergent selection, for any combination

of species, experimental treatment and garden. In particular, we detected genetically-based trait differentiation among populations, a signal of selection ( $S$ ) close to 1,  $Q_{ST}$  significantly higher than  $F_{ST}$  and a strong association with climate (Figures 2 and 3; Figure S5, Table S5). The importance of SLA in ecological processes and adaptation to resource gradients and drought tolerance has been demonstrated in numerous studies. SLA is part of the so-called Leaf Economics Spectrum, a suite of correlated leaf traits that define the strategies of investment and return of carbon, nitrogen and phosphorous at global scale (Wright et al., 2004; Shipley et al., 2006). In oaks, SLA has been found to be related to growth rates and other fitness components at different organizational scales, supporting its role as a key functional trait (Ramírez-Valiente et al., 2010, 2015). From an evolutionary perspective, leaf morphological traits, including SLA, have been shown to be evolutionarily labile, evolving in response to climatic gradients (Ramírez-Valiente et al., 2020; Hipp et al., 2020; Sancho-Knapik et al., 2021).

There was evidence of divergent selection in other morphological traits across species and common garden experiments, such as leaf lamina area, leaf thickness and perimeter-to-area ratio, although the signal of selection was lower than that detected for SLA, particularly for *Q. lusitanica* (Figure 2). Multivariate analyses for *Q. faginea* also revealed strong correlated selection on leaf morphological traits associated with the climate of the provenances (Figure 4). Similar results have been reported in other oaks, where SLA but also leaf lamina area or leaf anatomical traits such as size and density of stomata appear to have evolved under divergent selection (Ramírez-Valiente et al., 2009, 2018). Support for the adaptive value of SLA and other leaf morphological traits has been found in different plant species. For example, a study on *Pinus canariensis* found that population genetic differentiation in leaf mass per area (LMA, a trait inversely related to SLA) was higher than expectations based on neutral evolution (López et al., 2013). Keller et al. (2011) reported stronger population genetic divergence ( $Q_{ST}$ ) for LMA than for neutral markers ( $F_{ST}$ ) in *Populus balsamifera*, supporting the idea that this trait has evolved in response to divergent selection in this species as well. Despite extensive support for an adaptive value of leaf morphological traits, not all studies have found them to exhibit evidence of climate-driven population divergence. For instance, Gauzere et al. (2020), in a study combining  $Q_{ST}$ - $F_{ST}$  analyses with the method developed by Ovaskainen et al. (2011), reported evidence of divergent selection among *Fagus sylvatica* populations on growth and leaf phenological traits, but not on leaf morphology. Studies on other species have not found signals of divergent selection on SLA or leaf morphology either, suggesting that selective pressures on SLA and leaf morphology are not universal but species- or context-dependent (e.g., Frei et al., 2012; Brouillet et al., 2014; Marin et al., 2020). SLA might be key in species that experience a dry season such as Mediterranean and dry tropical regions. Alternatively, some species might have traits other than leaf morphology, such as those related to phenology and physiological functions, which may allow them to adapt to resource availability gradients and drought (Aitken et al., 2008; Alberto et al., 2013).

For both *Q. faginea* and *Q. lusitanica*, mass-based stomatal conductance ( $g_{s,mass}$ ) also presented a strong signal of divergent selection, although with contrasting species patterns (Figure 2; Table S5). In *Q. faginea*, the highest values of  $g_{s,mass}$  under dry conditions were found for populations from dry and continental areas whereas, in *Q. lusitanica*, the highest values both in dry and well-watered conditions were found in the wettest population, located in the northernmost extreme of the distribution range of the species (Figure 3). Stomatal control is a key response to water availability conditions. Closing stomata under water stress allows plants to maintain plant water potentials but at the cost of reducing carbon assimilation (Medrano et al., 2002; Flexas et al., 2004). Thus, higher values of stomatal conductance under drought conditions might indicate a poor stomatal control, but it could also be related to other mechanisms such as increased root allocation or reduced osmotic potentials that allow plants to maintain gas exchange and, consequently, carbon assimilation under water stress (Sparks & Black, 1999; Otieno et al., 2006; Bartlett et al., 2012; Hanslin et al., 2019). To understand how selection has acted on  $g_{s,mass}$  in our studied species, and why they exhibit opposing climate-trait associations for this trait, other traits related to drought tolerance would be needed. Only very few studies on selection have evaluated stomatal conductance and, to our knowledge, none of them have shown evidence of divergent selection on this trait (e.g., Keller et al., 2011; Brouillet et al., 2014; Ramírez-Valiente et al., 2018). Stomatal conductance is a highly plastic trait and population differences might only be evident under particular environmental conditions. Nevertheless, the observed significant selection on stomatal anatomical traits (Ramírez-Valiente et al., 2018), and the observed fitness benefits of low stomatal conductance under drought in phenotypic selection studies (e.g., Dudley, 1996) suggest a key role of this trait for climate adaptation also in other plant species.

Under well-watered conditions, growth traits (AGR and RGR) also exhibited strong evidence of divergent selection for *Q. faginea*. Populations from rainier and milder climates exhibited higher RGR (Figure 4). It has been hypothesized that larger growth rates are beneficial under mesic environments because they increase the ability to compete for other resources such as light. Previous studies have demonstrated evidence of divergent selection on growth traits for many species (e.g., Keller et al., 2011; Csilléry, Buchmann, et al. 2020; Csilléry, Ovaskainen, et al., 2020; Marin et al., 2020; Gauzere et al., 2020) including oaks (e.g., Ramírez-Valiente et al., 2009, 2018). Growth is considered a trait that integrates responses from different plant functions and is one of the components of plant performance together with reproduction and survival (Viole et al., 2007).

#### 4.3 | Differences between species, common gardens and watering treatments in patterns of selection

In the greenhouse experiment, where *Q. faginea* and *Q. lusitanica* plants were grown under the same environmental conditions, the two species exhibited a contrasting number of traits with evidence

of selection ( $S$ ) and associations with climate (Figures 2 and 3). Specifically, for *Q. faginea*, seven out of 11 traits exhibited a signal of selection higher than 0.9 and eight of them presented associations with climate (PC-climate2). In contrast, for *Q. lusitanica*, only SLA and  $g_{s,\text{mass}}$  had strong evidence of selection ( $S > 0.9$ ). Similar differences between species were found based on  $Q_{ST}$ - $F_{ST}$  analyses (Table S5). The weaker evidence of divergent selection and weaker associations between trait population divergence and climate in *Q. lusitanica* could be related to the fact that this species occupies a niche with lower climatic and environmental variability compared to *Q. faginea*. For example, *Q. faginea* populations are found in ranges of annual temperature of 7.2°C (9.0–16.2°C), minimum temperatures of the coldest month of 8.8°C (-2.9 to 5.7°C) and annual precipitation of 549 mm (386–935 mm). In contrast, *Q. lusitanica* exhibits a more restricted climatic niche, with variation among populations in annual temperature, minimum temperature and annual precipitation of 2.3°C (14.3–16.6°C), 5.5°C (2.6–8.1°C) and 370 mm (638–1008 mm), respectively.

On the other hand, the strength of the signal of selection ( $S$ ) differed between growing environments in growth rates (AGR and RGR) for both species (Figure 2).  $Q_{ST}$ - $F_{ST}$  comparisons also provided contrasting patterns of selection between treatments in RGR for *Q. lusitanica* (Table S5). These results are consistent with  $Q_{ST}$ - $F_{ST}$  analyses in other species, which have reported genotype-by-environment ( $G \times E$ ) interactions and different levels of population genetic differentiation across experimental environmental conditions. For example, in a riparian tree, *Populus fremontii*, Cooper et al. (2022) showed that  $Q_{ST}$  for height varied from 0.14 to 0.44 across three planting sites. Furthermore, they calculated  $Q_{ST}$  for plasticity in height across sites, being significantly higher than  $F_{ST}$ , supporting divergent selection for plasticity in growth rates. In a study on a dry tropical oak, *Q. oleoides*, Ramírez-Valiente et al. (2018) showed  $Q_{ST} > F_{ST}$  under drought but not under well-watered conditions or in a field common garden experiment. In maritime pine, *P. pinaster*,  $Q_{ST}$  values for height differed strongly among common garden experiments, suggesting that selection signals are highly determined by the experimental environment (Rodríguez-Quilón et al., 2016; de Miguel et al., 2022). These results reveal the importance of evaluating phenotypes under contrasting environments to elucidate climatic adaptation.

## 5 | CONCLUSIONS

Our study demonstrates the importance of integrating genomic data and phenotypic information from common garden experiments to understand patterns of adaptation to climate. It should be borne in mind that although the method developed by Ovaskainen et al. (2011) and Karhunen et al. (2013) addresses some limitations of the classical  $Q_{ST}$ - $F_{ST}$  analysis, it still assumes a normal distribution of traits, low mutation rates and additive genetic effects. Although our study used normally distributed traits and SNP markers, which might help in approximating the two first assumptions, future developments should account for additional sources of variation, such as dominance,  $G \times E$  interactions and non-normally distributed traits

(Karhunen et al., 2013). Collectively, our results support an important role of leaf morphology, stomatal conductance and growth rates to adapt to climate heterogeneity in two Mediterranean species. Lower levels of functional divergence in the narrowly distributed shrub *Quercus lusitanica* compared to *Q. faginea* suggest that climatic niche and life history traits might be key aspects in shaping the genetic structure in genes involved in plant functions. These results highlight the need for more multidisciplinary studies that integrate a thorough functional characterization of traits important to drought tolerance and genomic data to understand population responses to climate change.

## AUTHOR CONTRIBUTIONS

J.A.R-V. and J.O. conceived the idea. A.S-M., J.A.R-V. and J.J.R-A. contributed to the experimental design, management and data collection. J.A.R-V., J.O. and J.J.R-A. analysed the data. J.A.R-V. wrote the manuscript in collaboration with all the authors.

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## CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

## DATA AVAILABILITY STATEMENT

Raw Illumina reads have been deposited at the NCBI Sequence Read Archive (SRA) under BioProject PRJNA890904. Phenotypic data and input files for all analyses are available in the Zenodo repository (<https://doi.org/10.5281/zenodo.7377140>).

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## SUPPORTING INFORMATION

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