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Oak leaf morphology may be more strongly shaped by climate than by phylogeny

Rubén Martín-Sánchez[1*], Domingo Sancho-Knapik[1,2], David Alonso-Forn[3], Ana López-Ballesteros[1], Juan Pedro Ferrio[1,4], Andrew L. Hipp[5,6], José Javier Peguero-Pina[1,2] and Eustaquio Gil-Pelegrín[7]

Abstract

Key message Despite been grown under the same climate, oak species are able to correlate with looser, but still identifiable, leaf morphological syndromes, composed by morphological traits with an ecological role in their respective macroclimates.

Context Environmental restrictions imposed by climate have been shown to modulate leaf morphology. A reduction of leaf area in hot and dry climates reduces overheating because of a thinner boundary layer. Lobed shapes enhance hydraulic conductivity and faster cooling. Elongated leaves drain more quickly under high precipitation. Trichomes may help to reduce the effects of excessive sun exposure in hot and dry environments. Leaf mass per area (LMA) increases in stressful environments.

Aim To assess the influence of global climate and clade on oak leaf syndromes comprising morphological traits with ecological roles.

Methods We analyzed seven morphological traits in 141 oak species grown in a botanical garden, characterized into five macroclimates, and explored the partial effects of clade and climate.

Results We found significant associations between macroclimate and every morphological trait measured. Temperate species tend to have large, obovate, lobed, malacophyllous leaves. Species occurring in dry habitats usually present small, rounded, pubescent, sclerophyllous leaves. Warm and wet climates induce the development of slender, lanceolate, glabrous leaves with an acuminate apex.

Conclusion The functional roles of the different morphological traits are partially confirmed in genus *Quercus* as a response to the different macroclimates, where different leaf syndromes can be distinguished.

Keywords *Quercus*, Macroclimate, Functional traits, Factor Analysis of Mixed Data, Leaf syndrome

*Correspondence:
Rubén Martín-Sánchez
rmartin@cita-aragon.es
[1] Departamento de Sistemas Agrícolas, Forestales y Medio Ambiente, Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), Avda. Montañana 930, Zaragoza 50059, Spain
[2] Instituto Agroalimentario de Aragón -IA2- (CITA-Universidad de Zaragoza), Zaragoza, Spain
[3] Research Group On Plant Biology Under Mediterranean Conditions, Department of Biology, University of Balearic Islands (UIB), Ctra Valldemossa Km 7.5, Palma 07122, Spain
[4] Aragon Agency for Research and Development (ARAID), Zaragoza 50018, Spain
[5] The Morton Arboretum, Lisle, IL 60532-1293, USA
[6] The Field Museum, Chicago, IL 60605, USA
[7] Estación Experimental de Aula Dei, Consejo Superior de Investigaciones Científicas (EEAD-CSIC), Avda. Montañana 1005, Zaragoza 50059, Spain

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1 Introduction

Leaves are the primary photosynthetic organs of plants. Their morphology should be adapted to environmental conditions (Traiser et al. 2005), as seemingly fine differences in leaf form may strongly affect the capacity of plants to grow and survive under different environments (Givnish 1987; Desmond et al. 2021). It has long been recognized that natural selection by climatic restrictions may influence leaf morphology (Stahl 1880, 1883; Schimper 1903). In the past two decades, numerous studies have found correlations between leaf morphological traits (e.g. leaf size and shape) and environmental factors (mainly annual mean temperature and precipitation) from global to local scales (Royer et al. 2005; Traiser et al. 2005; Adams et al. 2008; Peppe et al. 2011; Li and Wang 2021). Leaf area, shape, pubescence, and dry mass per area in particular have proven to be of high functional significance (Alonso-Forn et al. 2020; Cavender-Bares and Ramírez-Valiente 2017; Du et al. 2022; Sancho-Knapik et al. 2021).

Leaf area varies and evolves in response to water availability, temperature, and other aspects of climate (Webb 1968; Dolph and Dilcher 1980; Givnish 1984; Peppe et al. 2011; Li et al. 2020). Small leaves have been linked to harsh conditions such as hot, dry and light-saturating environments (Peguero-Pina et al. 2020; Nicotra et al. 2011) due to the thinner boundary layer of small leaves (Yates et al. 2010), which facilitates heat loss (Roth-Nebelsick 2001). Leaf temperature tends to be higher in large leaves due to their thicker boundary layers (Vogel 1970; Parkhurst and Loucks 1972; Givnish 1987; Vogel 2009).

Leaf shape—e.g. length–width ratio, toothing and lobation, apex structure—interacts with leaf area in adapting plants to climate and habitat. Leaf shape has various functional roles, including thermoregulation, hydraulic constraints, and leaf expansion patterns in deciduous species (Nicotra et al. 2011). Plants in cool regions tend to have broader leaves with lower length–width ratios to increase the thickness of their boundary layers and prevent wind cooling, maintaining higher leaf temperatures for photosynthesis (Wright et al. 2017; Li et al. 2020). In contrast, plants in warm and humid regions often have elliptic leaves with higher length–width ratios to avoid overheating and enhance water transport (Wright et al. 2017; Hu and Hu 2015), analogous to the effect of having smaller leaves. Mean annual temperature has long been shown to influence leaf toothing and lobation, with narrow and deeply lobated leaves better adapted to and more frequent in warmer climates due to thinner boundary layers and enhanced hydraulic efficiency (Sisó et al. 2001; Sack and Tyree 2005; Leigh et al. 2017). However, toothed leaves are more common in cool climates, where

they are considered an adaptation that enhances sap flow and carbon gain early in the growing season (Bailey and Sinnott 1916; Baker-Brosh and Peet 1997; Royer and Wilf 2006; Nicotra et al. 2011; Peppe et al. 2011; MacKee et al. 2019; Li and Wang 2021).

Leaf dry mass per unit area (LMA) is a leaf morphological trait related to shape widely used as a proxy for sclerophylly (see Alonso-Forn et al. 2020 and references therein). LMA commonly varies along environmental gradients (Niinemets 2001; Poorter et al. 2009). It is widely recognized that an increase in LMA is a general response to environmental stress (Onoda et al. 2011; Alonso-Forn et al. 2020), such as drought (Turner 1994; Reich et al. 1999; Jordan et al. 2005) or low winter temperatures (Ogaya and Peñuelas 2007; González-Zurdo et al. 2016; Niinemets 2016). Recently, Sancho-Knapik et al. (2021) showed that LMA in deciduous oak species may be modulated by aridity, whereas diversification of LMA within evergreen oaks seems modulated by both aridity and winter cold.

The functions of leaf pubescence are more ambiguous. Functions that have been demonstrated include providing resistance against biotic or abiotic damages, absorbing nutrients and water, and protecting against excessive solar radiation (Ehleringer et al. 1976; Sakai et al. 1980; Ehleringer 1981; Werker 2000; Morales et al. 2002; Agrawal et al. 2009; Fernández et al. 2014). Adaxial trichomes may increase leaf reflectance, which could be advantageous in dry and hot climates by reducing heat load and leaf temperature without increasing water loss through transpiration (Ehleringer and Björkman 1978; Ehleringer and Mooney 1978; Pérez-Estrada et al. 2000; Abdulrahaman and Oladele 2011; Bickford 2016). Additionally, trichomes have been explored for their role in repelling water from the leaf surface to keep stomata dry and ensure free gas exchange (Brewer and Smith 1997; Pierce et al. 2001). However, some studies have found conflicting results regarding the role of trichomes in regulating leaf transpiration and coping with aridity, indicating that further research is needed to understand better their functional significance and possible context-dependence of different roles (Aronne and DeMicco 2001; Haworth and McElwain 2008).

There are few studies within Fagaceae genera that analyze the relationships between morphological traits and their response to climatic variables in a broad set of species and climatic conditions. For example, Chen and Kohyama (2022) studied in 91 *Lithocarpus* species how fruit and vegetative traits correlate with temperature and wetness, however, *Lithocarpus* is a genus with little morphological and climatic variation compared to other Fagaceae genera. Most of the research focuses on other proxies, such as how a reduced set of species, or even a

single species, responds to a climatic gradient or to climate change, typically using the most common species (e.g. *Fagus sylvatica* or *Castanea sativa*). *Quercus* (oaks) is the genus with more research in this matter since it is an excellent system for investigating how co-variation in leaf traits reflects complex adaptations to varying environments (Ramírez-Valiente et al. 2020; Cavender-Bares 2019). Oaks show considerable variation among-species in leaf morphological traits, climatic niches and geographical range (Gil-Pelegrín et al. 2017; Cavender-Bares 2019; Jiang et al. 2019; Sancho-Knapik et al. 2021). Although some studies have explored the relationships between climate and morphology within oaks (Kaproth et al. 2023; Ramírez-Valiente et al. 2020) they are limited by the number of species or the biogeographical range. To our knowledge, there is not any study taking into account a great number of species, representing almost every biome occupied by oak species and involving species from each infrageneric clade.

The genus is divided into two monophyletic subgenera with eight monophyletic sections: subgenus *Quercus* (Sect. *Lobatae*, *Protobalanus*, *Ponticae*, *Virentes* and *Quercus*), with a hypothesized Nearctic origin (Cavender-Bares 2019; Hipp et al. 2020); and subgenus *Cerris* (Sect. *Cyclobalanopsis*, *Cerris* and *Ilex*), which likely evolved in warm-temperate east Asia (Denk et al. 2023) and the northern Paleotropics (Deng et al. 2018; Jiang et al. 2019).

The study aims to assess in a wide range of oak species (141) to what extent leaf morphological traits (leaf area, leaf mass per area, length–width ratio, index of lobation, leaf shape, leaf apex, leaf margin, pubescence and leaf habit) are associated with the prevailing macroclimate within the species distribution. The study also includes clade as a covariate to investigate the partial effects of clade and climate in the evolution of oak leaf syndromes. These analyses help illuminate the relative importance of phylogenetic niche conservatism and adaptation in the broad evolution of oak leaf syndromes.

2 Material and methods

2.1 Plant material

One hundred and forty-one oak species (*Quercus* spp.) (Martín-Sánchez et al. 2024, Table S1) were sampled during midsummer from living collections in the Jardín Botánico de Iturrarán (43°130N, 02°010W, 70 m asl, Gipuzkoa, Spain, www.iturraran.org). Using of the botanical garden as a quasi-common garden in our study enables interspecific comparisons of traits that result from long-term adaptation to the species' native climate without conflating that adaptive history with the short-term plastic response of each tree to the climate where it occurs naturally. Note that some variation we observe is plastic in response to site conditions within the botanic garden, but this variation is essentially irrelevant to our question about among-biome adaptation. Ten fully developed leaves per species (3–4 leaves per tree × 3 different trees) were collected from mature trees (15–25 years old) in midsummer. Leaves were sampled from south-exposed branches during early morning (07:00–09:00 h, solar time) and they were stored in sealed plastic bags and carried to the laboratory in portable coolers to measure both quantitative and qualitative morphological traits.

2.2 Morphological traits

In this study, we considered five qualitative variables: overall shape, apex shape, margin, pubescence and leaf habit. We also measured four quantitative variables: leaf area (LA), leaf mass per area (LMA), length–width ratio (LW) and index of lobation (ILB). Leaf shape, apex and margin were classified according to Ellis et al. (2009) as one of six shapes (obovate, elliptic, oblong, ovate, lanceolate or circular), one of four apex shapes (rounded, acute, acuminate or straight), and one of six margin types (entire, serrate, dentate, crenate, undulate or spinose). Although quantitative variables are always preferred, we decided to include qualitative traits to support the continuous traits. The variability in nominal margin is gathered by the continuous ILB. In the same way, LW strongly overlaps with the shape. However, nominal traits may explain nuances that are not achieved with these continuous traits. Regarding pubescence, we followed Gil-Pelegrín et al. (2017), classifying species into three categories: glabrous (or with scattered trichomes), pubescent, and densely pubescent, according to the botanical description of the species in their respective floras (Flora of North America, Nixon 1993; Flora of China, Huang et al. 1999; and Flora Europaea, Schwarz 1964). The same floras were taken into account to classify species as deciduous or evergreen.

Leaves were digitized and processed using *ImageJ* (http://rsb.info.nih.gov/nih-image/) to measure LA, length and width (for LW) and perimeter (for ILB, calculated as perimeter / square root of LA) (Valladares et al. 2000). Afterwards, leaves were oven dried for 3 d at 70 °C and weighed using an analytical balance (Sartorius AG Model BP221S; Sartorius AG, Goettingen, Germany). LMA is calculated as the ratio of the foliage dry mass to foliage area. For 85 of our 141 species, LA and LMA data were already published (Sancho-Knapik et al. 2021; Kattge et al. 2020; http://doi.org/https://doi.org/10.17871/TRY.64.).

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2.3 Climatic and geographic data

Climate analysis for each studied species were carried out following a similar procedure used by Gil-Pelegrín et al. (2017). We first obtained geographical distribution coordinates for each species individually from GBIF (https://www.gbif.org/) taking into account their distribution ranges (when available, see Euforgen and US Tree Atlas databases) or the geographical descriptions in the beforementioned floras to crop the areas from where points were downloaded. Then, the downloaded datasheets were thinned using SDMtune R package so that redundant points within a square kilometre were removed (Vignali et al. 2022). The resultant information (see Martín-Sánchez et al. 2024, Table S2) was used to obtain the values of each climatic variable from the WorldClim version 2.1 database (Fick and Hijmans 2017. WorldClim 2. https://www.worldclim.org/) for each presence point using Raster R package. We calculated the annual precipitation range (bio20) as BIO16 minus BIO17 (see Martín-Sánchez et al. 2024, Table S3 for the meaning of each bioclimatic variable). Points were also overlaid on a climatic Köppen map (Köppen 1936) to extract their Köppen climate (Martín-Sánchez et al. 2024, Table S2). Köppen climates inhabited by *Quercus* were grouped into five macroclimates: Arid, Mediterranean, Winter-dry temperate to Tropical, Warm Temperate and Cold Temperate, similar to the schema used by Gil-Pelegrín et al. (2017) (Fig. 1). Arid macroclimate (ARID) is defined by climates where annual precipitation is lower than evapotranspiration (BSk and BSh primarily, with some peripheral populations in BWk or BWh). Mediterranean macroclimate (MED) comprises climates with the dry period in summer (Csa, Csb, Csc, Dsa and Dsb). Winter-dry temperate to Tropical macroclimate (WdT-TROP) includes warm and temperate climates with a dry season other than summer (Aw, Am, Cwa

and Cwb). Warm Temperate macroclimate (WARM_T) comprises Cfa Köppen climates, since it represents a climate widely colonized by *Quercus* species, especially in southeastern regions of the United States and China. The Cold Temperate (COLD_T) macroclimate comprises Dfa, Dfb, Dwa, Dwb, Cfb, and Cfc primarily, with some peripheral populations in Dsc, Dsd, Dwc, Dwd, Dfc or Dfd. Several species of oaks, especially some *Cyclobalanopsis* species, live in an equatorial climate (Af), which was not treated in this study as these last species are not considered in this study (for a more detailed definition of the Köppen climate categories see Martín-Sánchez et al. 2024, Table S4). Species were assigned to each macroclimate according to where most of their occurrences fall compared with the rest of the other macroclimates (Appendix Fig. 8).

2.4 Statistical analysis

ANOVAs were performed to quantify the variance explained by macroclimate, subgenus, section—nested into subgenus—on each quantitative variable (LA, LMA, LW and ILB) (Table 1). We also performed ANOVAs to compare quantitative traits among macroclimates. Multiple comparisons were carried out among macroclimates for LA, LMA, LW and ILB using the post-hoc Tukey's honestly significant difference test. Heteroscedasticity and normality of the data were checked throughout analyses of the residuals, and Kolmogorov–Smirnov tests were carried out using the DHARMa R package (Hartig 2022). In addition, contingency tables based on pairwise χ^2 tests—corrected by Holm-Bonferroni method for multiple comparisons—were used to test the correlation between macroclimate and the qualitative traits: margin, apex, shape, pubescence and leaf habit.

Factor analysis of mixed data (FAMD) was carried out to summarize the multivariate relationships among
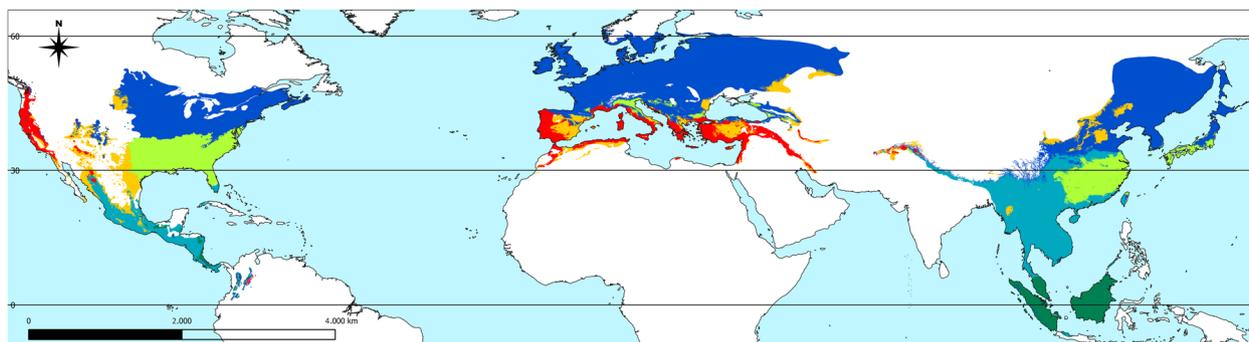
Fig. 1 Map of the five macroclimates considered in this study: Arid in yellow (BSk, BSh, according to Köppen Classification), Mediterranean in red (Csa, Csb, Csc, Dsa, Dsb), Winter-dry to Tropical in cyan (Aw, Am, Cwa, Cwb), Warm Temperate in light green (Cfa) and Cold Temperate in dark blue (Cfb, Cfc, Dfa, Dfb, Dwa, Dwb). Colored areas represent the regions where *Quercus* species are present. The Equatorial climate (not considered in this study) is represented by Tropical Rainforest Climate (Af) and coloured with dark green

Table 1 Summary of percentage of variance explained by ANOVAs performed for each variable separately: Leaf area (LA), length–width ratio (LW), index of lobation (ILB), leaf mass per area (LMA) and altogether (PC1 scores from FAMD). Each row represents a separate ANOVA. All models include all factors: macroclimate, subgenus and section nested in subgenus. Number of asterisks represents the level of significance ($P < 0.001$ ***, $P < 0.01$ **, $P < 0.05$ *). The proportion of variance is calculated dividing the sum squares for each factor by the total sum of squares

	Macroclimate		Subgenus		Section		Residuals
LA	30.0	***	1.47		3.70		64.8
LW	18.1	***	1.71		9.64	**	70.6
ILB	11.3	**	0.25		5.49		83.0
LMA	6.64	*	6.61	***	16.4	***	70.3
Altogether	29.9	***	4.79	**	3.90		61.4

morphological traits. FAMD has been proved to be an accurate statistical method used to analyze a data set containing both quantitative and qualitative variables (Pagès 2004) since they are normalized during the analysis to balance the influence of each set of variables. It was performed using FactoExtra and FactoMineR R packages (Josse and Husson 2008; Kassambara and Mundt 2020). First dimension scores (PC1 Morphology) were treated as a generalized leaf morphological response variable influenced by all four quantitative traits together. An additional ANOVA was performed to quantify the variance explained by macroclimate, subgenus, and section when all traits are taken into account altogether (Table 1). Principal components analysis (PCA) was performed with climatic variables extracted from Worldclim database, with a previous selection of those that accounted for more variance in the first two principal components via the FactoExtra R package (Kassambara and Mundt 2020). Every data included in both FAMD and climatic PCA represent the mean value for each variable and species.

Finally, we did cross-correlations between the first and second dimensions of both FADM and climatic PCA to check if there is any significance between both analyses and to explore the existence of relationships between morphological traits and climatic variables. We first tested if there was a phylogenetic signal performing phylogenetic generalized least squares (PGLS) regressions using the most complete published phylogenetic tree for *Quercus* (Hipp et al. 2020). Section and subgenus names correspond to named clades in Hipp et al. (2020). Ninety-nine species intersected between phylogenetic and trait datasets. We first performed a PGLS with all species. The results do not allow us to refuse the lack of phylogenetic signal. However, since the slopes of both regressions: ordinary least squares (OLS) and PGLS, are minimally different (Appendix Fig. 9) and we want to include all taxa, we decided to present the results of the OLS regression. We also performed additional PGLS within the different clades: both subgenera and the two sections with

more species, i.e. *Quercus* and *Lobatae* (Martín-Sánchez et al. 2024, Table S5).

3 Results

ANOVAs reveal that subgenus and macroclimate significantly ($P < 0.001$) account for 6.9% and 35.4% of the morphological variance when traits are analyzed altogether (Table 1). The taxonomic section is a non-significant predictor of 3.2% of the variance ($P = 0.18$). 54.5% of variance was unexplained. By contrast, when traits are analysed separately, we find that macroclimate explains more variance for LA (29%), LW (20.3%) and ILB (14.4%) than any other factor. However, in the case of LMA, not only macroclimate is significant, but also subgenus and section, with this last one explaining the highest percentage of variance (16.4%) (Table 1).

All quantitative traits differ significantly among macroclimates according to Tukey tests (Fig. 2). LMA shows the least variation among climates, reaching its highest values in drier climates, but only showing a significant difference in its smaller values in Cold Temperate species (8.33 ± 1.47 g m^{-2}) compared with the rest of the macroclimates (Fig. 2b). By contrast, LA is the most variable trait among macroclimates, ranging from a mean value of 7.62 ± 3.86 cm^2 in Arid species to 30.38 ± 28.17 cm^2 in Warm Temperate species, doubling to 64.71 ± 40.64 cm^2 in Cold Temperate species (Fig. 2a). LW varies from rounder in Cold Temperate (1.81 ± 0.41), Mediterranean (1.79 ± 0.34) and Arid (1.96 ± 0.49) species to more elongate in Warm Temperate (2.25 ± 0.97) and Winter-dry temperate to Tropical species (2.56 ± 0.76) (Fig. 2c). Cold Temperate species display the highest lobation (ILB $= 6.43 \pm 1.44$), Winter-dry temperate to Tropical species the lowest (5.11 ± 0.56) (Fig. 2d).

Contingency tables show significant associations between the five qualitative morphological traits and macroclimate. In terms of shape, Obovate leaves relate to Cold Temperate ($P = 0.003$) and Warm Temperate ($P < 0.001$) species; besides, elliptic leaves to
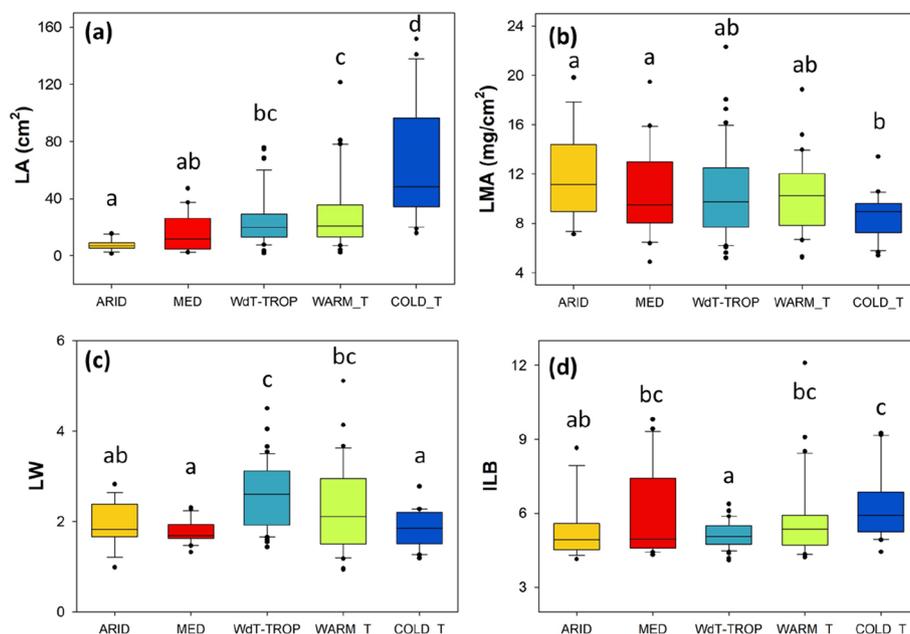
**Fig. 2** Box-plot representation of the four quantitative traits measured: Leaf area (LA) (**a**), leaf mass per area (LMA) (**b**), length–width ratio (LW) (**c**) and index of lobation (ILB) (**d**) in the five macroclimates considered: Arid (ARID), Mediterranean (MED), Winter-dry to Tropical (WdT-TROP), Warm Temperate (WARM_T) and Cold Temperate (COLD_T). Letters indicate statistically significant differences across macroclimates (Tukey test, $P < 0.05$)
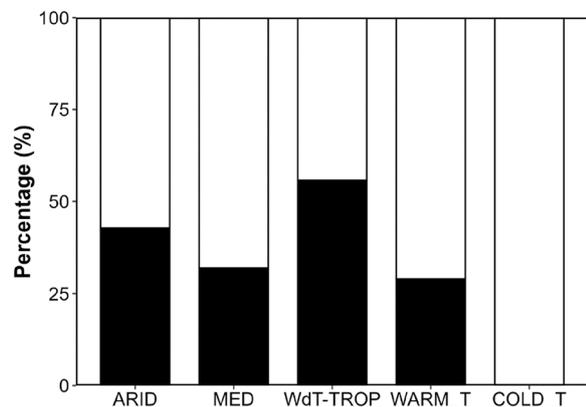
Winter-dry temperate to Tropical species ($P < 0.001$) (Fig. 3a). For apex, acute ($P < 0.001$) and acuminate ($P = 0.01$) apices are linked to Winter-dry temperate to Tropical species (Fig. 3b). Lobated margins are associated with Cold Temperate ($P = 0.014$) and Warm Temperate ($P < 0.001$) species, entire margins with Warm Temperate ($P = 0.014$) and Winter-dry temperate to Tropical species ($P < 0.014$) and serrate margins with Winter-dry temperate to Tropical species ($P < 0.001$) (Fig. 3c). Glabrous leaves are associated with Winter-dry temperate to Tropical species ($P < 0.001$) (Fig. 3d). Finally, deciduousness is significantly correlated with Cold Temperate ($P = 0.008$) and Warm Temperate species ($P < 0.001$) (Fig. 3).

FAMD on the combined morphological groups species together by macroclimate, but with strong overlap (Fig. 5a). Traits cluster loosely by climate into three groups: (1) obovate, lobated leaves with large areas corresponding mainly to species from Cold Temperate climates, with some Warm Temperate and deciduous Mediterranean species; (2) elongate leaves with acuminate apices, lacking abaxial trichomes tend to be associated with Winter-dry temperate to Tropical and Warm Temperate species; and (3) oblong and undulate leaves with dentate margins, rounded apices, a dense tomentum abaxially and a high LMA are concentrated in Arid and evergreen Mediterranean species (Fig. 5a).

Climatic PCA clusters species into well-defined groups, with the most overlap between Arid and Mediterranean species (Fig. 5b). Cold Temperate species, are defined by a high temperature annual range (BIO3, BIO7), with very cold winters (BIO6). By contrast, Winter-dry temperate to Tropical species are characterized by warmer winters (BIO6) and more temperature-stable climates (BIO3 and BIO4) but pronounced seasonality in precipitation regimes (BIO15, annual precipitation range), with high rainfall during the wet season (BIO16, BIO18, annual precipitation range). Warm temperate species do not suffer a great reduction in precipitation during their driest month. Arid and Mediterranean species occupy climates with a very dry season during the warmest period of the year (BIO18, annual precipitation range) and low annual precipitation (BIO12). This relationship between climate and morphology is supported as well by the OLS regression between morphology PCA axis 1 (PC1-Morph) and climate PCA axis 1 (PC1-Clim), which is significant ($r = 0.543$, $P < 0.001$) (Fig. 6). The same correlation performing a PGLS shows a similar slope, significance ($P < 0.001$) and a low phylogenetic signal ($\lambda = 0.28$) (Appendix Fig. 9). The additional PGLS within the different clades: subgenus *Cerris*, subgenus *Quercus*, section *Lobatae* and section *Quercus* (clades named as in Hipp et al. 2020) revealed that we cannot claim that both subgenera are
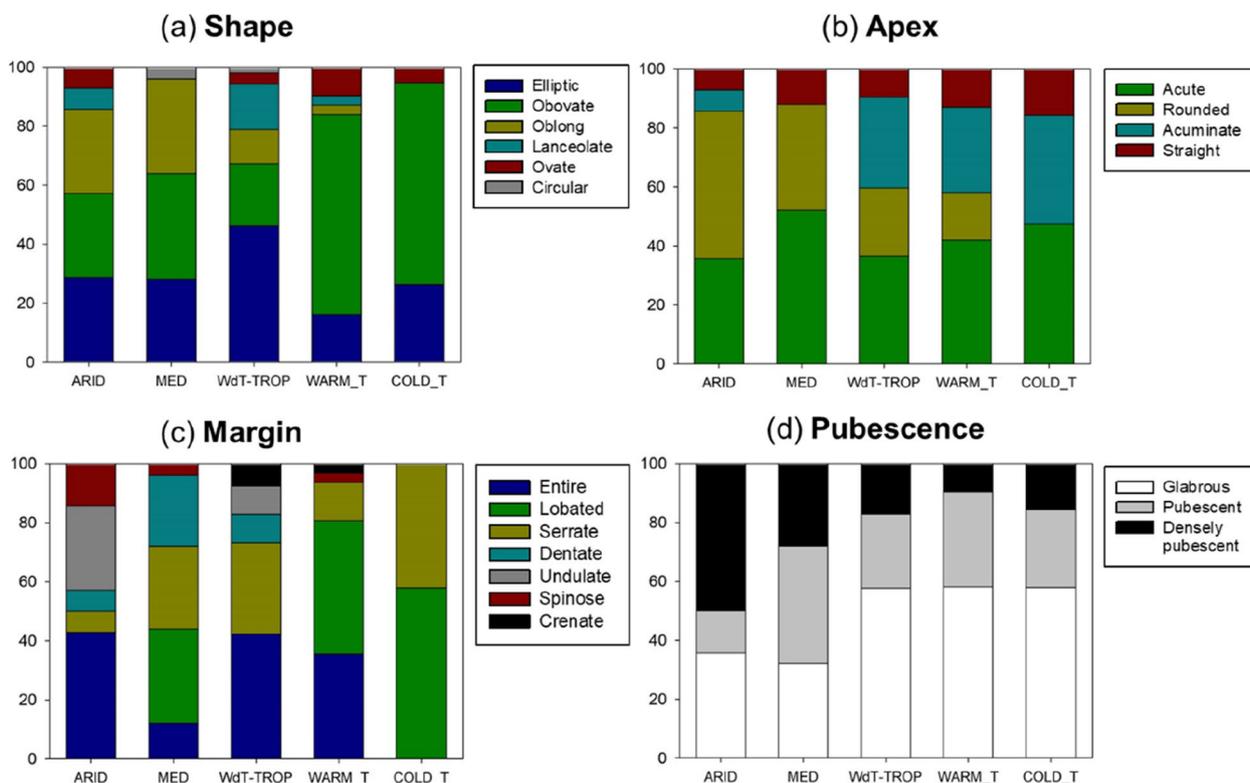
Fig. 3 Relative percentage of species presenting the different qualitative traits in the five macroclimates considered: Leaf shape (**a**), apex (**b**), margin (**c**) and abaxial pubescence (**d**) in Arid (ARID), Mediterranean (MED), Winter-dry Temperate to Tropical (WdT-TROP), Warm Temperate (WARM_T) and Cold Temperate (COLD_T) macroclimates


Fig. 4 Relative percentage of species according to their leaf habit in the five macroclimates considered: Deciduous (white) and evergreen (black) in Arid (ARID), Mediterranean (MED), Winter-dry Temperate to Tropical (WdT-TROP), Warm Temperate (WARM_T) and Cold Temperate (COLD_T) macroclimates

significantly different, since the confidence intervals of the slope overlap. However, at section level significant differences can be found between sections *Quercus* and *Lobatae*. (Martín-Sánchez et al. 2024, Table S5).

4 Discussion

4.1 Individual leaf traits correlate with climate

Leaf area exhibits high evolutionary lability in some clades, with high potential to evolve in response to climate variation (Jones et al. 2009; Peppe et al. 2011). Our work shows a strong negative correlation between leaf size, growing season temperature, and aridity. Thus, the smallest leaves are found in Arid and Mediterranean climates, the largest ones in Cold Temperate climates (Fig. 2a). This may be a result of oaks reducing LA to decrease their boundary layer, enhancing heat loss (Vogel 1970; 2009; Givnish 1987). A reduced LA also contributes to a higher leaf-specific conductivity (LSC) of the shoot which is advantageous in dry habitats (Peguero-Pina et al. 2014, 2016). Conversely, larger leaves confer to species a greater photosynthetic rate when compared with smaller leaves. However, many small-leaf species usually compensate for this reduction of photosynthetic tissue with a higher ability to photosynthesize throughout a higher carboxylation rate, mesophyll and stomatal conductance (Peguero-Pina et al. 2016). Of leaf shape traits, lobation, length−width ratio, and leaf apex exhibit the strongest correlation with climate. Lobing assessed qualitatively and higher ILB, both
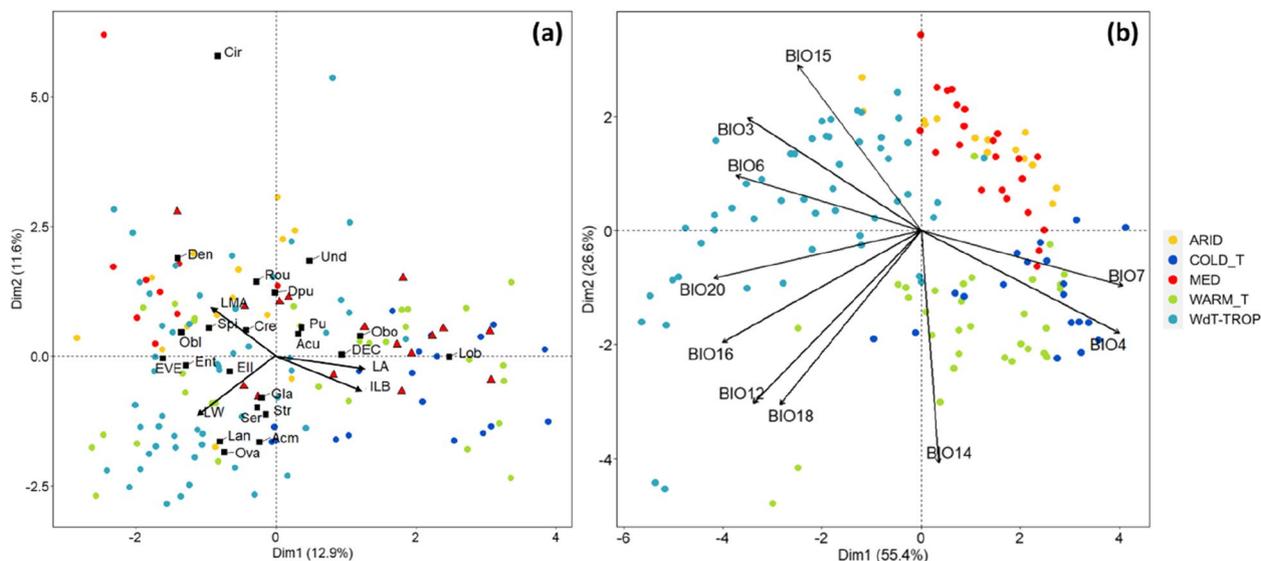
Fig. 5 Factor Analysis of Mixed Data (FAMD) performed with morphological traits (**a**) and Principal component analysis (PCA) performed with climatic data (**b**), showing the first two principal components (Dim1 and Dim2). In FAMD (**a**), Dim1 and Dim2 account for 12.9% and 11.6% of the variance respectively. The FAMD was elaborated with the four quantitative traits: Leaf area (LA), leaf mass per area (LMA), length–width ratio (LW), index of lobation (ILB) and the qualitative traits regarding shape (Cir = circular, Ell = elliptic, Lan = lanceolate, Obl = oblong, Obo = obovate, Ova = Ovate), apex (Acm = acuminate, Acu = Acute, Rou = rounded, Str = straight), margin (Den = dentate, Cre = crenate, Ent = entire, Lob = lobated, Ser = Serrate, Spi = spinose, Und = undulate), pubescence (Gla = glabrous, Pu = pubescent, Dpu = densely pubescent) and leaf habit (DEC = deciduous, EVE = evergreen). In climatic PCA (b), Dim1 and Dim2 account for 55.4% and 26.6% of the variance respectively. The PCA was elaborated with the next variables: BIO3, BIO4, BIO6, BIO7, BIO12, BIO14, BIO15, BIO16, BIO18 and annual precipitation range (see Martín-Sánchez et al. 2024, Table S3 for meaning). Individual species are represented by dots and their colour represent the macroclimate which they have been classified: Arid (yellow), Mediterranean (red, with triangles deciduous Mediterranean species and dots for evergreen Mediterranean, see discussion for further details), Winter-dry Temperate to Tropical (cyan), Warm Temperate (light green) and Cold Temperate (dark blue)
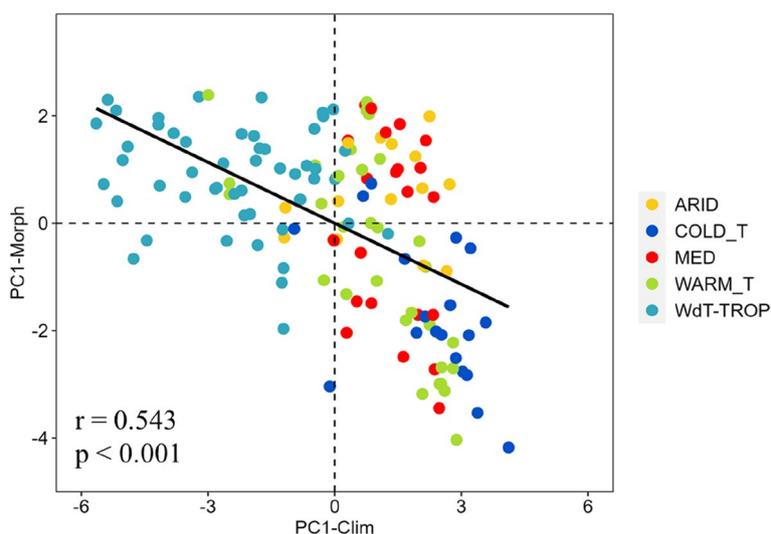
Fig. 6 Correlations between PC1 scores resulting from FAMD (PC1-Morph) and climatic PCA (PC1-Clim). Individual species are represented by dots and their colour represent the macroclimate which they have been classified: Arid (yellow), Mediterranean (red), Winter-dry temperate to Tropical (cyan), Warm Temperate (light green) and Cold Temperate (dark blue). A linear model was adjusted and showed a significant *p*-value (*P* < 0.001)

were associated significantly with temperate climates (Fig. 2d, Fig. 3c). Lobation is also exhibited in sub-Mediterranean species, which occupy a climate transitional between Mediterranean and temperate (Abadía et al. 1996; Himrane et al. 2004; Gil-Pelegrín et al. 2017).

Cold Temperate, sub-Mediterranean and some of the Warm Temperate (primarily American) species gather under the same group in the FAMD (Fig. 5a), which is defined by four traits: high ILB, high LA and obovate and lobated shapes. Leaf lobation may enhance hydraulic efficiency by reducing the ratio of mesophyll to conductive tissue (Sisó et al. 2001; Sack and Tyree 2005) and decreasing the boundary layer to speed leaf cooling (Vogel 1970, 2009; Givnish 1987). Both potential benefits of lobation are especially relevant in sub-Mediterranean species, which experience very high, dry temperatures during summer compared to temperate species. The sub-Mediterranean species in our study also compensate for the reduction of photosynthetic tissue in their relatively small and / or lobated leaves by increasing net CO_2 assimilation per unit leaf area, as Peguero-Pina et al. (2016) showed when comparing the sub-Mediterranean *Q. faginea* with the cold temperate *Q. robur*. In temperate environments, by contrast, leaf lobation may play a role in bud packing, which is crucial for competitive growth in winter-freezing environments (Edwards et al. 2016).

By contrast, Winter-dry temperate to Tropical and some Warm Temperate species exhibit a high LW (Fig. 2c), besides a significant percentage of lanceolate shapes (Fig. 3a) and acuminate apices (Fig. 3b). These three traits associate in the FAMD with Winter-dry temperate to Tropical and Warm Temperate species (Fig. 5a). Together with a tilted leaf position, these traits have been hypothesized to limit rainfall damage (Wang et al. 2020). High rains in the tropics and Warm Temperate regions can slow photosynthesis (Ishibashi and Terashima 1995), leach minerals from the leaves (Minorsky 2019), damage leaves mechanically (Wang et al. 2020), and promote pathogens (Burd 2007). The morphological mechanisms we document, which evacuate water from the leaf surface, are particularly needed in the Warm Temperate and Winter-dry temperate to Tropical climates where these traits dominate. These climates, in the *Quercus* distribution, are most characterized by high precipitation (Fig. 5b).

LMA follows a completely different pattern in our study. Although it is significantly modulated by climate (Table 1), the major clade in which a species occurs plays a more significant role in explaining differences in LMA among species (Table 1) (cf. Sancho-Knapik et al. 2021). The effect of clade at section level is likely explained by the LMA-homogeneous sections, i.e., *Ilex*, *Protobalanus* and *Virentes*. After accounting for the clade, Arid

and Mediterranean species tend to have a higher LMA than the rest of the species, although not significant (Fig. 2b). This relatively weak effect of climate is particularly remarkable given the plasticity that LMA presents in response to several environmental factors, such as drought (Niinemets 2001), exposure to light (Poorter et al. 2006) or herbivory (Mediavilla et al. 2018; Onoda et al. 2011). For example, Sancho-Knapik et al. (2021) demonstrated that oak species with long cold periods and/or humid climates have lower LMA.

Despite the poor correlation between climate and LMA, we find a significant correlation between sclerophylly—which is strongly correlated with LMA—and continuous climatic variables (Alonso-Forn et al. 2020). Species with low LMA are usually deciduous and malacophyllous. In contrast, species with a high LMA tend to be evergreen and sclerophyllous (Mediavilla et al. 2008) (Appendix Fig. 10). Thus, Arid, evergreen Mediterranean, most of Winter-dry temperate to Tropical and some Warm Temperate species are located at low Dim1 values in the FAMD (Fig. 5a). By contrast, deciduous Mediterranean, Cold Temperate and the rest of Warm Temperate species are located at high Dim1 values.

The increment in LMA, both via increasing leaf density or leaf thickness, is a major resource investment for the plant. Species which make a higher investment per leaf must keep their photosynthetic tissues active for longer to recover the resources expended according to the worldwide leaf economics spectrum (Wright et al. 2004). However, there are several factors within an individual to take into account in the construction costs and payback time, such as light exposure of the leaves, chemical compounds and leaf habit. Poorter et al. (2006) showed how shade leaves present longer payback times than light leaves, in spite of the fact that these long times can be partially compensated by decreasing the LMA. Differences in payback time can be found between deciduous and evergreen species too (Villar and Merino 2001). Thus, species with a higher LMA—correlated with sclerophylly and longer payback times—tend to be evergreen. Winter-dry temperate to Tropical and some Warm Temperate species can develop sclerophyllous leaves increasing their LMA more than temperate deciduous species but less than Arid and Mediterranean (Alonso-Forn et al. 2020), as the high contribution of LMA to Dim2 of FAMD reflects (Appendix Fig. 11b). This might be related to the two kinds of sclerophyllous leaves: pycnophylls and pachyphylls (Grubb 1986). Differences between them are found in the mesophyll since sclerophylly is mainly defined by an investment to reinforce the epidermis, which is shared by both types. Pycnophyll leaves present a very compact mesophyll, usually small cells with little space among them, i.e., an increase in LMA via

investment in leaf density. By contrast, pachyphyll leaves are characterized by a larger, laxer and spongier mesophyll, i.e., an increase in LMA via leaf thickness (Grubb 1986). Thereby, Winter-dry temperate to Tropical and Warm Temperate species might be mainly pachyphylls, exhibiting thick leaves with low density; meanwhile, Arid and evergreen Mediterranean might be pycnophylls.

Leaf water relations relations are also strongly influenced by pubescence, which appears in our study to vary independently of macroclimate, except glabrous leaves associated with Winter-dry temperate to tropical climates ($P < 0.001$). Previous studies have suggested that abaxial pubescence in oaks could be a xeromorphic trait in response to drier climates (Fig. 3d) (Hardin 1979). This trend is observed in a major presence of pubescent species in Arid and Mediterranean species, although not significant ($P = 0.41$). (Fig. 3d). The functional roles of trichomes in the genus may be broader, including protection against a range of biotic and abiotic factors (Zvereva et al. 1998; Sato and Kudoh 2015; Ehleringer et al. 1976; Coltharp et al. 2021). A trichome layer may act as a protection against some environmental factors, especially excessive solar radiation, by modifying the leaf's optical properties, lowering heat load, and reducing leaf temperature and transpiration (Ehleringer et al. 1976; Ehleringer 1981; Sandquist and Ehleringer 1997; Pérez-Estrada et al. 2000; Abdulrahaman and Oladele 2011; Bickforf 2016). Nonetheless, the hydrophobic nature of some trichomes may also be advantageous under wet climates, since they can keep the stomata dry and ensure the free exchange of CO_2 (Brewer and Smith 1997; Pierce et al. 2001). However, trichomes do not necessarily have to be hydrophobic, as Fernández et al. (2014) demonstrated in *Quercus ilex* subsp. *rotundifolia*, whose adaxial trichomes seem to help leaves to rehydrate during summer rainfall events. This double behaviour of trichomes and the lack of significance in our results indicate that their role as xeromorphic trait cannot be taken for granted, and further research to clarify the role of pubescence is needed.

4.2 Multivariate leaf syndromes associate with macroclimate

Our study demonstrates that the world's oak species assemble into well-defined climatic groups that correlate with loose but still identifiable leaf morphological syndromes. The Arid and Mediterranean species are an exception, as they mainly overlap in climatic space (Fig. 5b). Because species were classified into their macroclimate according to their presence within a specific Köppen climate, the climatic separation is a result of the Köppen system itself: PC1 and PC2 together account for 82% of the explained variance, which suggests that

Köppen climates are well defined by WorldClim climatic variables.

A more interesting finding is the significant correlation of climatic PC1 with morphological PC1 (Fig. 6). Morphological PC1 is explained most strongly by lobation (ILB and lobated shapes), LA and Obovate shapes (Appendix Fig. 11a). In contrast, climatic variables with the highest scores are the difference in precipitation between the wettest month and the driest one (annual precipitation range), temperature annual range (BIO7), temperature seasonality (BIO4) and precipitation of the wettest quarter (BIO16) (Appendix Fig. 12a). Climatic PC1 thus separates most strongly between Winter-dry temperate to Tropical (with negative values) and the rest of the species (with positive ones) (Fig. 5b). The segregation of species along this climatic gradient represents both an ecological split and a phylogenetic split replicated twice in the Americas (Hipp et al. 2018) and between the primarily Mediterranean to temperate sections *Cerris* and *Ilex* of Eurasia versus section *Cyclobalanopsis* of the southeast Asia's broad-leaved evergreen forests (Deng et al. 2018).

Mediterranean, Arid, and temperate species live in climates with a marked thermal seasonality. The stressful dry or cold periods that mark these climates are much reduced in Winter-dry temperate to Tropical climates. Winter-dry temperate to Tropical, Arid and Mediterranean species—three macroclimates with higher temperatures, on average, than temperate climates—are represented with negative values in PC1 of FAMD (Fig. 4a). Simultaneously, temperate and sub-Mediterranean species appear together and are characterized by a high lobation, and large, obovate leaves (Fig. 5a), which are the four variables with highest loadings in PC1 (Appendix Fig. 11a). Thus, we see once again a correlation between area and lobation with temperature.

The significant regression between climate and morphology PC2 (Appendix Fig. 13) shows the correlation of glabrous, slender leaves with drip-tips with higher-precipitation climates and pubescent rounded leaves with dry climates (Appendix Fig. 11b and 12b). As morphological and climatic PC2 represents a water-availability gradient, Mediterranean, Arid, and Winter-dry temperate to Tropical with monsoon-dry season cluster in the FAMD separate from most Warm Temperate and the rest of the Winter-dry temperate to Tropical species (Fig. 5a).

Cold Temperate species are generally characterized by large, obovate, deciduous leaves with complex—generally lobated—margins and low LMA (Fig. 7). Winter-dry temperate to Tropical species are defined by long, lanceolate and acuminate leaves, generally glabrous, evergreen or tardily-deciduous and sclerophyllous, likely pachypyll-type (anatomical studies in further researches are needed) (Fig. 7). Warm Temperate species tend to share or present
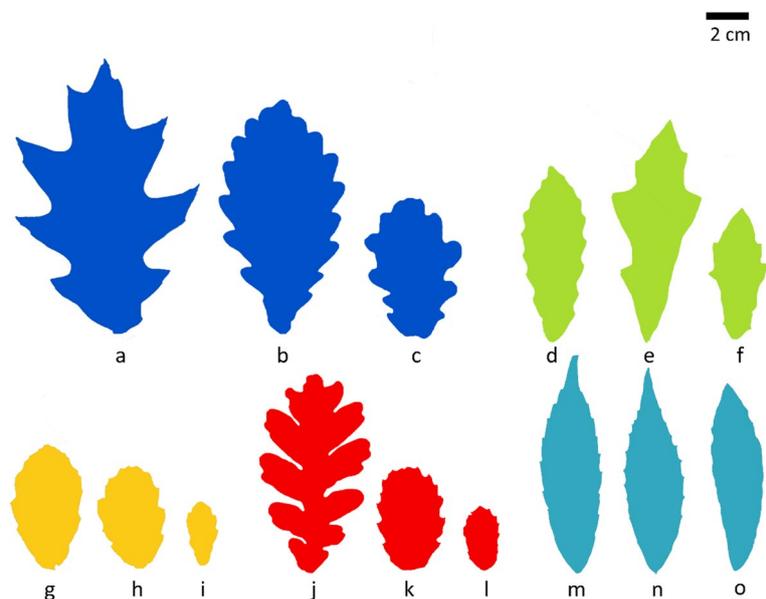
Fig. 7 Type-leaves of three different species as representatives of the different macroclimates studied. Cold Temperate (in dark blue): **a** *Quercus rubra*, **b** *Q. mongolica*, **c** *Q. robur*. Warm Temperate (in light green): **d** *Q. fabri*, **e** *Q. nigra*, **f** *Q. virginiana*. Arid (in yellow): **g** *Q. hintoniorum*, **h** *Q. hypoxantha*, **i** *Q. grisea*. Mediterranean: **j** *Q. pyrenaica* (sub-Mediterranean), **k** *Q. faginea*, **l** *Q. rotundifolia*. Winter-dry temperate to tropical (in cyan): **m** *Q. stenophylloides*, **n** *Q. leucotrichophora*, **o** *Q. pinnativenulosa*

intermediary traits between Cold Temperate and Winter-dry temperate to Tropical species (Fig. 7). Arid and Mediterranean species exhibit the smallest leaves, short, generally with a LW ratio near 1, and entire or relatively simple margins. They are usually evergreen or semi-evergreen, with the highest values of LMA probably due to their hypothetical sclerophyll pycnophyll-type condition (also further researches are needed) (Fig. 7). Finally, sub-Mediterranean species (e.g. *Q. pyrenaica*, *Q. frainetto* or *Q. cerris*) are characterized by larger leaves than their genuine Mediterranean relatives, with deeply lobated margins, usually obovate, deciduous and pubescent (Fig. 7). Overall, our work shows that we can relate leaf syndromes in oaks to broad-scale patterns in global climate.

Finally, although climate explains most of the variance found in oak leaf morphology, clade seems to have a minor but still significative effect since we cannot reject the presence of a phylogenetic signal in these morphological traits, especially in LMA. This phylogenetic signal could be appearing due to some morphologically homogeneous clades, e.g. Sect. *Protobalanus*, *Virentes* or *Ilex*.

5 Conclusion

In this study we analysed 141 *Quercus* species across a broad range of northern hemisphere biomes, ca. 25% of the species in the genus. Although we cannot refuse the presence of a phylogenetic signal, our results strongly suggest that leaf morphological traits of oaks are adapted to their macroclimates, as previous work has demonstrated for leaf anatomy (Sancho-Knapik et al. 2021; Alonso-Forn et al. 2023). Our study demonstrates that even in a common garden, growing under the same climatic conditions, a wide sample of the world's oak species develop a set of morphological traits with different ecological roles that conclude into recognisable leaf syndromes according to the climate that these species occupy. Except for Mediterranean species that, on the one hand, tend to develop a common leaf syndrome with Arid species. On the other hand, another group composed by sub-Mediterranean species gathers into a temperate syndrome.

Even though climate explains most of the variance found in leaf morphology, the partial effects of clade cannot be disregarded, as we see within the LMA, which resulted to be an outlier trait in this study. LMA is still explained significantly by climate; however, section explains twice variance as much as the climate for this trait.

Finally, pubescence did not show significant enough results to be considered a xeromorphic trait, as many authors have proposed.

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Appendix
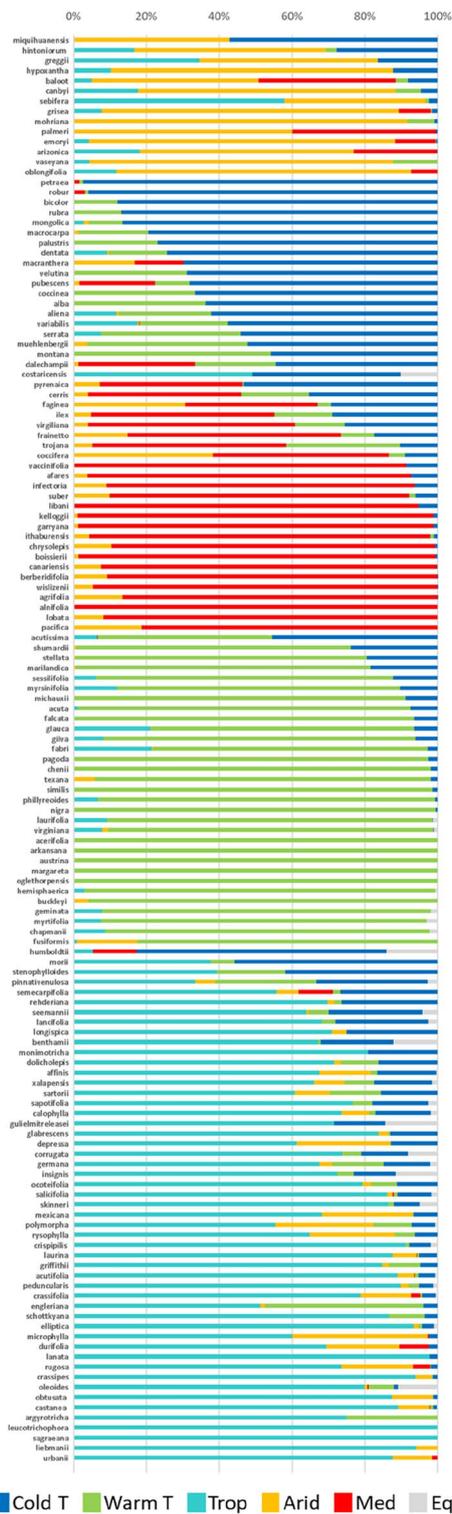
Fig. 8 Percentage of presence points in each of the five macroclimates for each species: Arid (yellow), Mediterranean (red), Winter-dry temperate to Tropical (cyan), Warm Temperate (light green) and Cold Temperate (dark blue). Equatorial climate, not considered in this study, is represented in grey
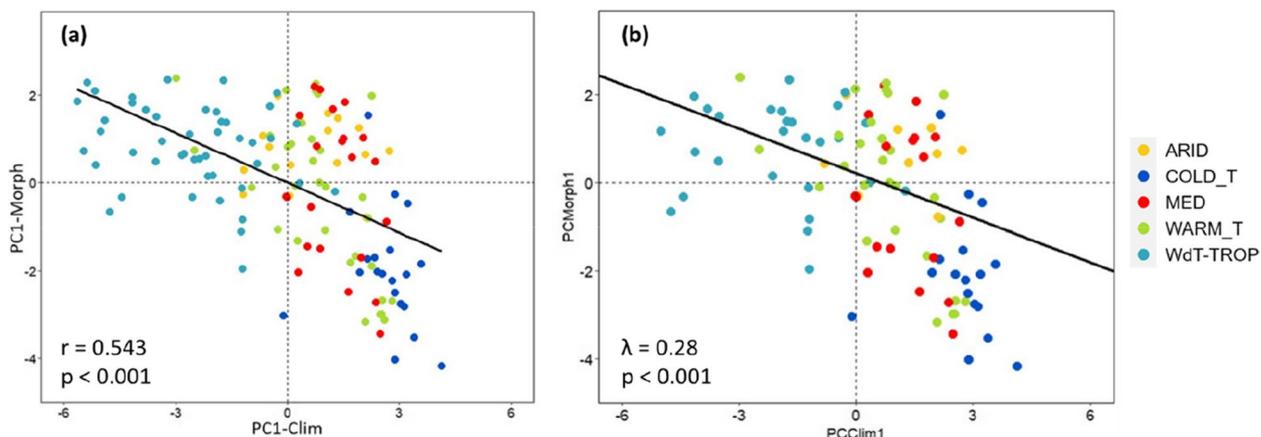
Fig. 9 Comparison between ordinary least squares regression (*N* = 138, Fig. 5 in the main text), and the phylogenetic generalized least square regression (*N* = 99). Phylogenetic signal showed with lambda (λ)
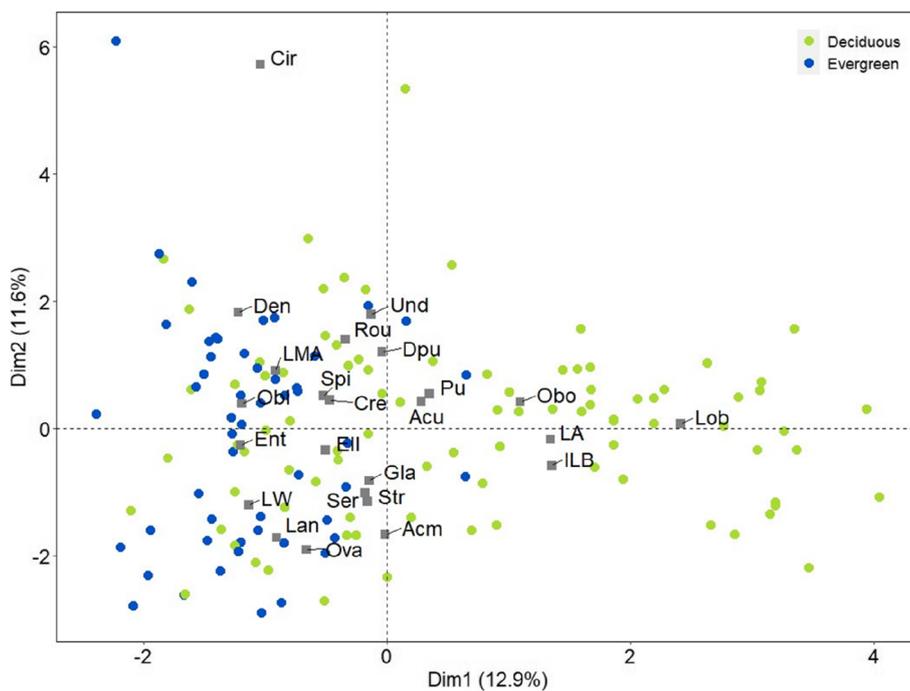
Fig. 10 Factor Analysis of Mixed Data (FAMD) performed with morphological traits. Same FAMD that Fig. 5a, but colouring species according to their leaf habit: Evergreen (dark blue) or deciduous (green)
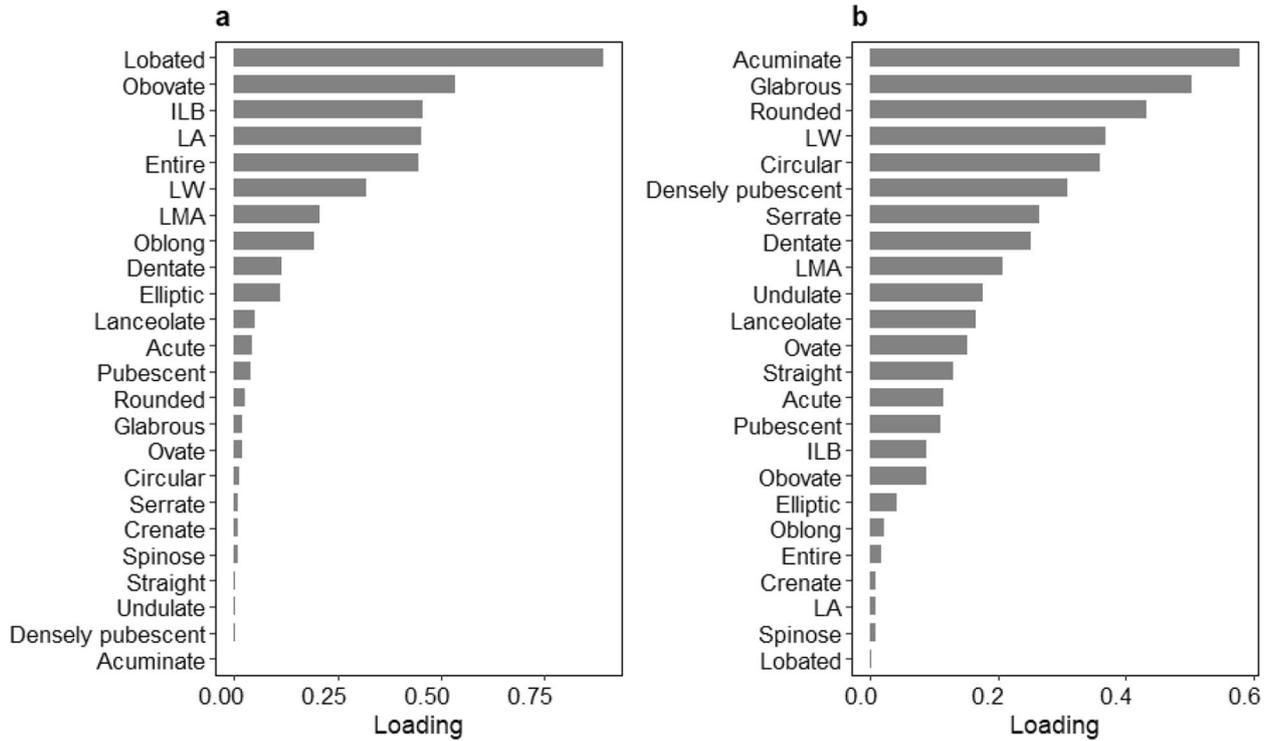
Fig. 11 Bar plots of the loadings of the first (a) and second (b) principal components computed via Factor Analysis of Mixed Data (FAMD) for all morphological traits
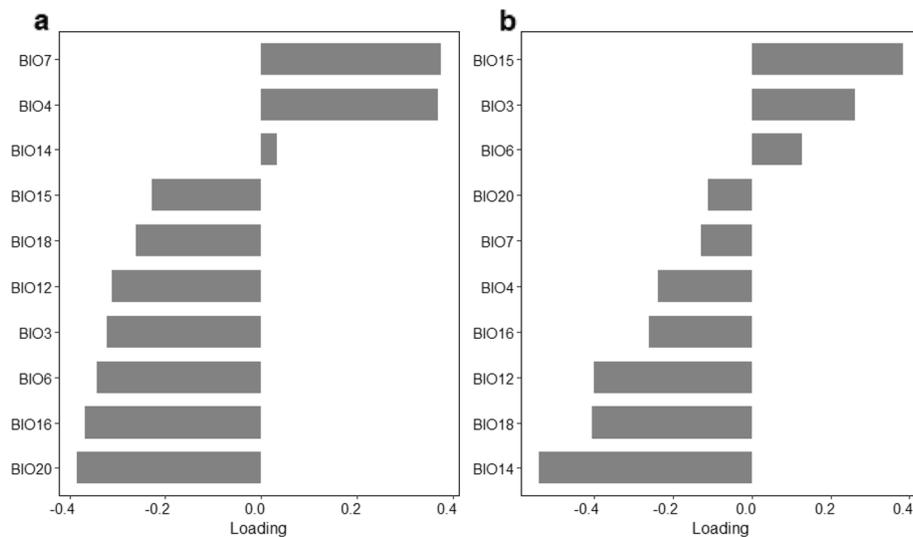
Fig. 12 Bar plots of the loadings of the first (a) and second (b) principal components computed via Principal Component Analysis (PCA) for climatic variables with a total number of 10 variables
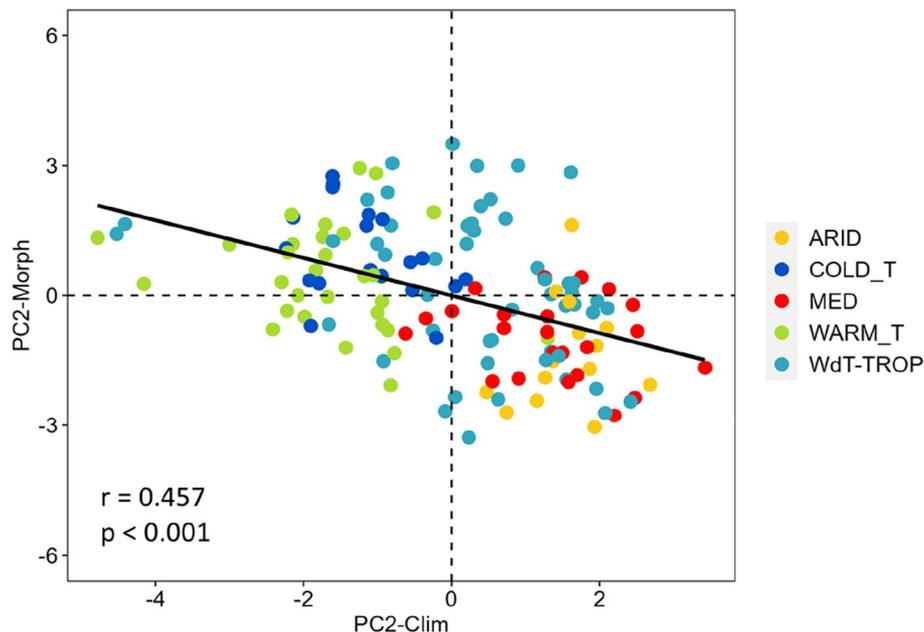
Fig. 13 Correlations between PC2 scores resulting from FAMD (PC2-Morph) and climatic PCA (PC2-Clim). Individual species are represented by dots and their colour represent the macroclimate which they have been classified: Arid (yellow), Mediterranean (red), Winter-dry temperate to Tropical (cyan), Warm Temperate (light green) and Cold Temperate (dark blue). A linear model was adjusted and showed a significant *p*-value ($P < 0.001$)

Availability of data and materials
The datasets generated and/or analyzed during the current study are available at https://doi.org/https://doi.org/10.5281/zenodo.10633924.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All authors gave their informed consent to this publication and its content.

Competing interests
The authors declare no conflict of interest.

References
Abadía A, Gil F, Morales F, Montañés L, Montserrat G, Abadía J, Abadía J (1996) Marcescence and senescence in a submediterranean oak (*Quercus subpyrenaica* E.H. del Villar): photosynthetic characteristics and nutrient composition. Plant Cell Environ 19(6):685–694. https://doi.org/10.1111/j.1365-3040.1996.tb00403.x

Abdulrahaman AA, Oladele FA (2011) Response of trichomes to water stress in two species of *Jatropha*. Insight Botany 1(2):15–21

Adams JM, Green WA, Zhang Y (2008) Leaf margins and temperature in the North American flora: recalibrating the paleoclimatic thermometer. Global Planet Change 60:523–534. https://doi.org/10.1016/j.gloplacha.2007.07.001

Agrawal AA, Fishbein M, Jetter R, Salminen JP, Goldstein JB, Freitag AE, Sparks JP (2009) Phylogenetic ecology of leaf surface traits in the milkweeds

(*Asclepias* spp.): Chemistry, ecophysiology, and insect behavior. New Phytol 183:848–867. https://doi.org/10.1111/j.1469-8137.2009.02897.x

Alonso-Forn D, Sancho-Knapik D, Ferrio JP, Peguero-Pina JJ, Bueno A, Onoda Y, Cavender-Bares J, Niinemets Ü, Jansen S, Cornelissen RM, JHC, Chai Y, Gil-Pelegrín E, (2020) Revisiting the functional basis of sclerophylly within the leaf economics spectrum of oaks: different roads to Rome. Current Forestry Reports 6:260–281

Alonso-Forn D, Sancho-Knapik D, Fariñas MD, Nadal M, Martín-Sánchez R, errio JP, Resco de Dios V, Peguero-Pina JJ, Onoda Y, Cavender-Bares J, Álvarez-Arenas TEG, Gil-Pelegrín E (2023) Disentangling leaf structural and material properties in relationship to their anatomical and chemical compositional traits in oaks (*Quercus* L.). Ann Bot 131(5):789–800. https://doi.org/10.1093/aob/mcad030

Aronne G, De Micco V (2001) Seasonal dimorphism in the Mediterranean *Cistus incanus* L. subsp. *incanus*. Ann Bot 87:789–794. https://doi.org/10.1006/anbo.2001.1407

Bailey IW, Sinnott EW (1916) The climatic distribution of certain types of angiosperm leaves. Am J Bot 3:24–39. https://doi.org/10.2307/2435109

Baker-Brosh KF, Peet RK (1997) The ecological significance of lobated and toothed leaves in temperate forest trees. Ecology 78:1250–1255. https://doi.org/10.1890/0012-9658(1997)078[1250:TESOLA]2.0.CO;2

Bickford CP (2016) Ecophysiology of leaf trichomes. Funct Plant Biol 43:807–814. https://doi.org/10.1071/FP16095

Brewer CA, Smith WK (1997) Patterns of leaf surface wetness for montane and subalpine plants. Plant, Cell Environ 20:1–11. https://doi.org/10.1046/j.1365-3040.1997.d01-15.x

Burd M (2007) Adaptive function of drip tips: a test of the epiphyll hypothesis in *Psychotria marginata* and *Faramea occidentalis* (Rubiaceae). J Trop Ecol 23(4):449–455. https://doi.org/10.1017/S0266467407004166

Cavender-Bares J (2019) Diversification, adaptation, and community assembly of the American oaks (*Quercus*), a model clade for integrating ecology and evolution. New Phytol 221(2):669–692. https://doi.org/10.1111/nph.15450

Cavender-Bares J, Ramírez-Valiente JA (2017). Physiological evidence from common garden experiments for local adaptation and adaptive plasticity to climate in American live oaks (*Quercus* Section Virentes): implications for conservation under global change. In: Gil-Pelegrín E, Peguero-Pina JJ, Sancho-Knapik D (eds) Oaks physiological ecology. Exploring the functional diversity of genus *Quercus* L. Cham, Switzerland: Springer International, pp 107–135.

Chen X, Kohyama TS (2022) Variation among 91 stone oak species (Fagaceae, *Lithocarpus*) in fruit and vegetative morphology in relation to climatic factors. Flora 286:151984. https://doi.org/10.1016/j.flora.2021.151984

Coltharp E, Knowd C, Abelli-Amen E, Abounayan A, Alcaraz S, Auer R, Beilman S, Breit E, Brennan J, Brown H … Weber M (2021). Leaf hair tufts function as domatia for mites in *Quercus agrifolia* (Fagaceae). Madroño 67. https://doi.org/10.3120/0024-9637-67.4.165

Deng M, Jiang XL, Hipp AL, Manos PS, Hahn M (2018) Phylogeny and biogeography of East Asian evergreen oaks (*Quercus* section Cyclobalanopsis; Fagaceae): Insights into the Cenozoic history of evergreen broad-leaved forests in subtropical Asia. Mol Phylogenet Evol 119:170–181. https://doi.org/10.1016/j.ympev.2017.11.003

Denk T, Grimm GW, Hipp AL, Bouchal JM, Schulze ED, Simeone MC (2023) Niche evolution in a northern temperate tree lineage: biogeographic legacies in cork oaks (*Quercus* sect. Cerris). Ann Bot 161(5):769–787. https://doi.org/10.1093/aob/mcad032

Desmond SC, Garner M, Flannery S, Whittemore AT, Hipp AL (2021) Leaf shape and size variation in bur oaks: an empirical study and simulation of sampling strategies. Am J Bot 108(8):1540–1554. https://doi.org/10.1002/ajb2.1705

Dolph GE, Dilcher D (1980) Variation in leaf size with respect to climate in Costa Rica. Biotropica 12:91–99. https://doi.org/10.2307/2387724

Du FK, Qi M, Zhang YY, Petit RJ (2022) Asymmetric character displacement in mixed oak stands. New Phytol 236(3):1212–1224. https://doi.org/10.1111/nph.18311

Edwards EJ, Spriggs EL, Chatelet DS, Donoghue MJ (2016) Unpacking a century-old mystery: Winter buds and the latitudinal gradient in leaf form. Am J Bot 103:975–978. https://doi.org/10.3732/ajb.1600129

Ehleringer J (1981) Leaf absorptances of Mohave and Sonoran desert plants. Oecologia 49:366–370. https://doi.org/10.1007/BF00347600

Ehleringer J, Björkman O (1978) Pubescence and leaf spectral characteristics in a desert shrub, *Encelia farinosa*. Oecologia 36:151–162. https://doi.org/10.1007/BF00349805

Ehleringer J, Mooney HA (1978) Leaf hairs: effects on physiological activity and adaptive value to a desert shrub. Oecologia 37:183–200. https://doi.org/10.1007/BF00344990

Ehleringer J, Björkman O, Mooney HA (1976) Leaf pubescence: effects on absorptance and photosynthesis in a desert shrub. Science 192:376–377. https://doi.org/10.1126/science.192.4237.376

Ellis B, Daly DC, Hickey LJ, Johnson KR, Mitchell JD, Wilf P, Wing SL (2009) Manual of leaf architecture. Cornell University Press, Ithaca, NY, USA

Fernández V, Sancho-Knapik D, Guzmán P, Peguero-Pina JJ, Gil L, Karabourniotis G, Khayet M, Fasseas C, Heredia-Guerrero JA, Heredia A, Gil-Pelegrín E (2014) Wettability, polarity, and water absorption of holm oak leaves: effect of leaf side and age. Plant Physiol 166:168–180. https://doi.org/10.1104/pp.114.242040

Fick SE, Hijmans RJ (2017) WorldClim 2: new 1km spatial resolution climate surfaces for global land areas. Int J Climatol 37(12):4302–4315

Gil-Pelegrín E, Saz MA, Cuadrat JM, Peguero-Pina JJ, Sancho-Knapik D (2017). Oaks under mediterranean-type climates: functional response to summer aridity. In: Gil-Pelegrín E, Peguero-Pina JJ, Sancho-Knapik D (eds) Oaks physiological ecology. Exploring the functional diversity of genus *Quercus* L. Cham, Switzerland: Springer International, pp 137–193.

Givnish TJ (1984) Leaf and canopy adaptations in tropical forests. In: Medina E, Mooney HA, Vasquez-Yanes C (eds) Physiological ecology of plants of the wet tropics. Dr. W. Junk Publishers, The Hague, the Netherlands, pp 51–84

Givnish TJ (1987) Comparative studies of leaf form: assessing the relative roles of selective pressures and phylogenetic constraints. New Phytol 106:131–160. https://doi.org/10.1111/j.1469-8137.1987.tb04687.x

González-Zurdo P, Escudero A, Babiano J, García-Ciudad A, Mediavilla S (2016) Costs of leaf reinforcement in response to winter cold in evergreen species. Tree Physiol 36:273–286. https://doi.org/10.1093/treephys/tpv134

Grubb PJ (1986) Sclerophylls, pachyphylls and pycnophylls: the nature and significance of hard leaf surfaces. In: Juniper B, Southwood R (eds) Insects and the plant surface. Edward Arnold, London, pp 137–150

Hardin JW (1979) Patterns of variation in foliar trichomes of eastern North American *Quercus*. Am J Bot 66(5):576–585. https://doi.org/10.1002/j.1537-2197.1979.tb06260.x

Hartig F. (2022). DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. R package version 0.4.6. https://cran.r-project.org/web/packages/DHARMa

Haworth M, McElwain J (2008) Hot, dry, wet, cold or toxic? Revisiting the ecological significance of leaf and cuticular micromorphology. Palaeogeogr Palaeoclimatol Palaeoecol 262(1–2):79–90. https://doi.org/10.1016/j.palaeo.2008.02.009

Himrane H, Camarero JJ, Gil-Pelegrín E (2004) Morphological and ecophysiological variation of the hybrid oak *Quercus subpyrenaica* (*Q. faginea* × *Q. pubescens*). Trees 18:566–575. https://doi.org/10.1007/s00468-004-0340-0

Hipp AL, Manos PS, Hahn M, Avishai M, Bodénès C, Cavender-Bares J, Valencia-Ávalos S (2020) Genomic landscape of the global oak phylogeny. New Phytologist 226(4):1198–1212. https://doi.org/10.1111/nph.16162

Hipp AL, Manos PS, González-Rodríguez A, Hahn M, Kaproth M, McVay JD, Avalos SV, Cavender-Bares J (2018) Sympatric parallel diversification of major oak clades in the Americas and the origins of Mexican species diversity. New Phytologist 217(1):439–452. https://doi.org/10.1111/nph.2018.217.issue-110.1111/nph.14773

Hu J, Hu H (2015) Viewing leaf shape and size variation in tobacco plants under different temperatures from a hydraulic perspective. Plant Diversity and Resources 37:168–176

Huang C, Zhang Y, Bartholomew B (1999). Fagaceae: *Quercus*. In: Flora of China 4: 370–380. Published on the Internet http://www.efloras.org [accessed 20 August 2022] Missouri Botanical Garden, St. Louis, MO and Harvard University Herbaria, Cambridge, MA.

Ishibashi M, Terashima I (1995) Effects of continuous leaf wetness on photosynthesis: adverse aspects of rainfall. Plant, Cell Environ 18(4):431–438. https://doi.org/10.1111/j.1365-3040.1995.tb00377.x

Jiang XL, Hipp AL, Deng M, Su T, Zhou ZK, Yan MX (2019) East Asian origins of European holly oaks (*Quercus* section Ilex Loudon) via the Tibet-Himalaya. J Biogeogr 46:2203–2214. https://doi.org/10.1111/jbi.13654

Jones CS, Bakker FT, Schlichting CD, Nicotra AB (2009) Leaf shape evolution in the south African genus *Pelargonium* L'Hér. (Geraniaceae). Evolution 63:479–497

Jordan GJ, Dillon RA, Weston PH (2005) Solar radiation as a factor in the evolution of scleromorphic leaf anatomy in Proteaceae. Am J Bot 92:789–796

Josse LS, Husson F (2008) FactoMineR: An R package for multivariate analysis. J Stat Soft 25(1):1–18. https://doi.org/10.18637/jss.v025.i01

Kaproth MA, Fredericksen BW, González-Rodríguez A, Hipp AL, Cavender-Bares J (2023) Drought response strategies are coupled with leaf habit in 35 evergreen and deciduous oak (*Quercus*) species across a climatic gradient in the Americas. New Phytol 239(3):888–904. https://doi.org/10.1111/nph.19019

Kassambara A, Mundt F (2020). Factoextra: Extract and Visualize the Results of Multivariate Data Analyses. R package version 1.0.7, https://CRAN.R-project.org/package=factoextra

Kattge J, Bönisch G, Díaz S, Lavorel S, Prentice IC, Leadley P, Tautenhahn S, Werner GDA, Aakala T, Abedi M et al (2020) TRY plant trait database – enhanced coverage and open access. Glob Change Biol 26:119–188. https://doi.org/10.1111/gcb.14904

Köppen W (1936). Das geographische system der klimate. In: Köppen W, Geiger R (eds) Handbuch der Klimatologie 3. Gebrueder Borntraeger, Berlin.

Leigh A, Sevanto S, Close JD, Nicotra AB (2017) The influence of leaf size and shape on leaf thermal dynamics: Does theory hold up under natural conditions? Plant, Cell Environ 40:237–248. https://doi.org/10.1111/pce.12857

Li Y, Zou D, Shrestha N, Xu X, Wang Q, Jia W, Wang Z (2020) Spatiotemporal variation in leaf size and shape in response to climate. Journal of Plant Ecology 13(1):87–96. https://doi.org/10.1093/jpe/rtz053

Li YQ, Wang ZH (2021) Leaf morphological traits: ecological function, geographic distribution and drivers. Chin J Plant Ecol 45(10):1154–117. https://doi.org/10.17521/cjpe.2020.0405

Martín-Sánchez R, Sancho-Knapik D, Alonso-Forn D, López-Ballesteros A, Ferrio, JP, Hipp A, Peguero-Pina JJ, Gil-Pelegrín E. (2024). Oak morphology dataset from Iturrarán Botanical Garden and supplementary files. . Zenodo. V2. https://doi.org/10.5281/zenodo.10633924

Mediavilla S, Garcia-Ciudad A, Garcia-Criado B, Escudero A (2008) Testing the correlations between leaf life span and leaf structural reinforcement in 13 species of European Mediterranean woody plants. Funct Ecol 22(5):787–793. https://doi.org/10.1111/j.1365-2435.2008.01453.x

Mediavilla S, Babiano J, Martínez-Ortega M, Escudero A (2018) Ontogenetic changes in anti-herbivore defensive traits in leaves of four Mediterranean co-ocurring *Quercus* species. Ecol Res 33:1093–1102. https://doi.org/10.1007/s11284-018-1622-0

Minorsky PV (2019) The functions of foliar nyctinasty: a review and hypothesis. Biol Rev 94(1):216–229. https://doi.org/10.1111/brv.12444

Morales F, Abadía A, Abadía J, Montserrat G, Gil-Pelegrín E (2002) Trichomes and photosynthetic pigment composition changes: responses of *Quercus ilex* subsp. *ballota* (Desf.) Samp. and *Quercus coccifera* L. to Mediterranean stress conditions. Trees 16:504–510. https://doi.org/10.1007/s00468-002-0195-1

Nicotra AB, Leigh A, Boyce CK, Jones CS, Niklas KJ, Royer DL, Tsukaya H (2011) The evolution and functional significance of leaf shape in the angiosperms. Funct Plant Biol 38(7):535–552. https://doi.org/10.1071/FP11057

Niinemets Ü (2001) Global-scale climatic controls of leaf dry mass per area, density, and thickness in trees and shrubs. Ecology 82:453–469. https://doi.org/10.1890/0012-9658(2001)082[0453:GSCCOL]2.0.CO;2

Niinemets Ü (2016) Does the touch of cold make evergreen leaves tougher? Tree Physiol 36:267–272. https://doi.org/10.1093/treephys/tpw007

Nixon KC (1993). *Quercus*. In: Flora of North America Editorial Committee, eds. Flora of North America North of Mexico [Online]. 22+ vols. New York and Oxford. Vol. 3. http://beta.floranorthamerica.org/Quercus. Accessed [07/28/2022].

Ogaya R, Peñuelas J (2007) Leaf mass per area ratio in *Quercus ilex* leaves under a wide range of climatic conditions. The importance of low temperatures. Acta Oecologica 31:168–173. https://doi.org/10.1016/j.actao.2006.07.004

Onoda Y, Westoby M, Adler PB, Choong AMF, Clissold FJ, Cornelissen JHC, Díaz S, Dominy NJ, Elgart A, Enrico L, … Yamashita N (2011). Global patterns of leaf mechanical properties. Ecology Letters 14: 301–312. https://doi.org/10.1111/j.1461-0248.2010.01582.x

Pagès J (2004) Analyse factorielle de données mixtes. Revue De Statistique Appliquée 52(4):93–111

Parkhurst DF, Loucks OL (1972) Optimal leaf size in relation to environment. J Ecol 60:505–537. https://doi.org/10.2307/2258359

Peguero-Pina JJ, Vilagrosa A, Alonso-Forn D, Ferrio JP, Sancho-Knapik D, Gil-Pelegrín E (2020) Living in Drylands: Functional Adaptations of Trees and Shrubs to Cope with High Temperatures and Water Scarcity. Forests 11:1028. https://doi.org/10.3390/f11101028

Peguero-Pina JJ, Sisó S, Sancho-Knapik D, Díaz-Espejo A, Flexas J, Galmés J, Gil-Pelegrín E (2016) Leaf morphological and physiological adaptations of a deciduous oak (*Quercus faginea* Lam.) to the Mediterranean climate: a comparison with a closely related temperate species (*Quercus robur* L.). Tree Physiol 36(3):287–299. https://doi.org/10.1093/treephys/tpv107

Peppe DJ, Royer DL, Cariglino B, Oliver SY, Newman S, Leight E, Enikolopov G et al (2011) Sensitivity of leaf size and shape to climate: global patterns and paleoclimatic applications. New Phytol 190:724–739. https://doi.org/10.1111/j.1469-8137.2010.03615.x

Pérez-Estrada LB, Cano-Santana Z, Oyama K (2000) Variation in leaf trichomes of *Wigandia urens*: environmental factors and physiological consequences. Tree Physiol 20:629–632. https://doi.org/10.1093/treephys/20.9.629

Pierce S, Maxwell K, Griffiths H, Winter K (2001) Hydrophobic trichome layers and epicuticular wax powders in Bromeliaceae. American Journal of Botsny 88(8):1371–1389. https://doi.org/10.2307/3558444

Poorter H, Niinemets Ü, Poorter L, Wright IJ, Villar R (2009) Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. New Phytol 82:565–588. https://doi.org/10.1111/j.1469-8137.2009.02830.x

Poorter H, Pepin S, Rijkers T, Jong Y, Evans JR, Körner C (2006) Construction costs, chemical composition and payback time of high- and low-irradiance leaves. J Exp Bot 57(2):355–371. https://doi.org/10.1093/jxb/erj002

Ramírez-Valiente JA, López R, Hipp AL, Aranda I (2020) Correlated evolution of morphology, gas exchange, growth rates and hydraulics as a response to precipitation and temperature regimes in oaks (*Quercus*). New Phytol 227(3):794–809. https://doi.org/10.1111/nph.16320

Reich PB, Ellsworth DS, Walters MB, Vose JM, Gresham C, Volin JC, Bowman WD (1999) Generality of leaf trait relationships: a test across six biomes. Ecology 80:1955–1969. https://doi.org/10.1890/0012-9658(1999)080[1955:GOLTRA]2.0.CO;2

Roth-Nebelsick A (2001) Computer-based analysis of steady-state and transient heat transfer of small-sized leaves by free and mixed convection. Plant, Cell Environ 24:631–640. https://doi.org/10.1046/j.1365-3040.2001.00712.x

Royer DL, Wilf P (2006) Why do toothed leaves correlate with cold climates? Gas exchange at leaf margins provides new insights into a classic paleotemperature proxy. Int J Plant Sci 167:11–18

Royer DL, Wilf P, Janesko DA, Kowalski EA, Dilcher DL (2005) Correlations of climate and plant ecology to leaf size and shape: potential proxies for the fossil record. Am J Bot 92:1141–1151. https://doi.org/10.3732/ajb.92.7.1141

Sack L, Tyree MT (2005). Leaf hydraulics and its implications in plant structure and function. In: Holbrook NM, Zwieniecki MA, (eds) Vascular transport in plants. Elsevier Academic Press: Burlington, MA, USA. 93–114. https://doi.org/10.1016/B978-012088457-5/50007-1

Sakai WS, Sanford WG (1980) Ultrastructure of the water-absorbing trichomes of Pineapple (*Ananas comosus*, Bromeliaceae). Ann Bot 46:7–11. https://doi.org/10.1093/oxfordjournals.aob.a085897

Sancho-Knapik D, Escudero A, Mediavilla S, Scoffoni C, Zailaa J, Cavender-Bares J, Álvarez-Arenas TEG, Molins A, Alonso-Forn D, Ferrio JP, Peguero-Pina JJ, Gil-Pelegrín E (2021) Deciduous and evergreen oaks show contrasting adaptive responses in leaf mass per area across environments. New Phytol 230:521–534. https://doi.org/10.1111/nph.17151

Sandquist DR, Ehleringer JR (1997) Intraspecific variation of leaf pubescence and drought response in *Encelia farinosa* associated with contrasting desert environments. N Phytol 135(4):635–644. https://doi.org/10.1046/j.1469-8137.1997.00697.x

Sato Y, Kudoh H (2015) Tests of associational defence provided by hairy plants for glabrous plants of *Arabidopsis halleri* subsp. *gemmifera* against insect herbivores. Ecol Entomol 40(3):269–279. https://doi.org/10.1111/een.12179

Schimper AFW (1903) Plant-geography on a physiological basis. Clarendon Press, Oxford

Schwarz O (1964). *Quercus*. In: Tutin TG, Burges NA, Chater AO, Edmondson JR, Heywood VH, Moore DM, Valentine DH, Walters SM, Webb DA, (eds) Flora Europaea. Cambridge University Press, 1, 72–76

Sisó S, Camarero JJ, Gil-Pelegrín E (2001) Relationship between hydraulic resistance and leaf morphology in broadleaf *Quercus* species: A new interpretation of leaf lobulation. Trees 15:341–345. https://doi.org/10.1007/s004680100110

Stahl E (1880) Ueber den Einfluss der Lichtintensität auf Structur und Anordnung des Assimilationsparenchyms. Botanische Zeitung 38:868–874

Traiser C, Klotz S, Uhl D, Mosbrugger V (2005) Environmental signals from leaves- a physiognomic analysis of European vegetation. New Phytol 166(2):465–484. https://doi.org/10.1111/j.1469-8137.2005.01316.x

Turner IM (1994) Sclerophylly: primarily protective? Funct Ecol 8:669–675. https://doi.org/10.2307/2390225

Valladares F, Martinez-Ferri E, Balaguer L, Perez-Corona E, Manrique E (2000) Low leaf-level response to light and nutrients in Mediterranean evergreen oaks: a conservative resource-use strategy? New Phytologist 148(1):79–91. https://doi.org/10.1046/j.1469-8137.2000.00737.x

Vignali S, Barras AG, Arlettaz R, Braunisch V (2022) SDMtune: An R package to tune and evaluate species distribution models. Ecol Evol 10(20):11488–11506. https://doi.org/10.1002/ece3.6786

Villar R, Merino J (2001) Comparison of leaf construction costs in woody species with differing leaf life-spans in contrasting ecosystems. New Phytologist 151(1):213–226. https://doi.org/10.1046/j.1469-8137.2001.00147.x

Vogel S (1970) Convective cooling at low airspeeds and the shapes of broad leaves. J Exp Bot 21:91–101. https://doi.org/10.1093/jxb/21.1.91

Vogel S (2009) Leaves in the lowest and highest winds: Temperature, force and shape. New Phytol 183:13–26. https://doi.org/10.1111/j.1469-8137.2009.02854.x

Wang T, Si Y, Dai H, Li C, Gao C, Dong Z, Jiang L (2020) Apex structures enhance water drainage on leaves. Proc Natl Acad Sci USA 117(4):1890–1894. https://doi.org/10.1073/pnas.1909924117

Webb LJ (1968) Environmental relationships of the structural types of Australian rain forest vegetation. Ecology 49:296–311. https://doi.org/10.2307/1934459

Werker E (2000) Trichome diversity and development. Adv Bot Res 31:1–35

Wright IJ, Dong N, Maire V, Prentice IC, Westoby M, Díaz S, Gallagher RV, Jacobs BF, Kooyman R, Law EA, Leishman MR, Niinemets Ü, Reich PB, Sack L, Villar R, Wang H, Wilf P (2017) Global climatic drivers of leaf size. Science 357:917–921. https://doi.org/10.1126/science.aal4760

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Villar R (2004) The worldwide leaf economics spectrum. Nature 428(6985):821–827. https://doi.org/10.1038/nature02403

Yates MJ, Verboom GA, Rebelo AG, Cramer MD (2010) Ecophysiological significance of leaf size variation in Proteaceae from the Cape Floristic Region. Funct Ecol 24:485–492. https://doi.org/10.1111/j.1365-2435.2009.01678.x

Zvereva EL, Kozlov MV, Niemelä P (1998) Effects of leaf pubescence in *Salix borealis* on host-plant choice and feeding behaviour of the leaf beetle. Melasoma Lapponica Entomologia Experimentalis Et Applicata 89(3):297–303

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