

Phenotypic plasticity and local adaptation in leaf ecophysiological traits of 13 contrasting cork oak populations under different water availabilities

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Summary Plants distributed across a wide range of environmental conditions are submitted to differential selective pressures. Long-term selection can lead to the development of adaptations to the local environment, generating ecotypic differentiation. Additionally, plant species can cope with this environmental variability by phenotypic plasticity. In this study, we examine the importance of both processes in coping with environmental heterogeneity in the Mediterranean sclerophyllous cork oak *Quercus suber*. For this purpose, we measured growth and key functional traits at the leaf level in 9-year-old plants across 2 years of contrasting precipitation (2005 and 2006) in a common garden. Plants were grown from acorns originated from 13 populations spanning a wide range of climates along the distribution range of the species. The traits measured were: leaf size (LS), specific leaf area (SLA), carbon isotope discrimination ($\Delta^{13}\text{C}$) and leaf nitrogen content per unit mass (N_{mass}). Inter-population differences in LS, SLA and $\Delta^{13}\text{C}$ were found. These differences were associated with rainfall and temperature at the sites of origin, suggesting local adaptation in response to diverging climates. Additionally, SLA and LS exhibited positive responses to the increase in annual rainfall. Year effect explained 28% of the total phenotypic variance in LS and 2.7% in SLA. There was a significant genotype \times environment interaction for shoot growth and a phenotypic correlation between the difference in shoot growth among years and the annual mean temperature at origin. This suggests that populations originating from warm sites can benefit more from wet conditions than populations from cool sites. Finally, we investigated the relationships between functional traits and aboveground growth by several regression models.

Our results showed that plants with lower SLA presented larger aboveground growth in a dry year and plants with larger leaf sizes displayed larger growth rates in both years. Overall, the study supports the adaptive value of SLA and LS for cork oak under a Mediterranean climate and their potentially important role for dealing with varying temperature and rainfall regimes through both local adaptation and phenotypic plasticity.

Keywords: carbon isotope discrimination, drought, natural selection, *Quercus suber*, specific leaf area, water use efficiency.

Introduction

Forest tree species distributed across a wide range of environmental conditions are submitted to differential selective pressures. Long-term selection can lead to the development of morphological and physiological adaptations to the local environment, generating ecotypic differentiation in important functional traits (Kawecki and Ebert 2004, Savolainen et al. 2007). Additionally, forest tree species can cope with this environmental variability if their traits exhibit phenotypic plasticity, i.e., ability to express alternative phenotypes in response to environmental variation (Bradshaw 1965, Sultan 1987, Lortie and Aarssen 1996). These two non-excluding strategies allow species to cope with environmental heterogeneity and shed light on the adaptive role of phenotypic traits in tree species. Thus, if a given trait is adaptive for a particular species, it is expected to show ecotypic differentiation

caused by divergent selection pressures, a large phenotypic plasticity to cope with the environmental heterogeneity or both (Valladares et al. 2000, 2007). Nevertheless, to conclusively support the hypothesis that a trait is adaptive in a given environment, it is necessary to demonstrate that the trait is related to plant performance (e.g., fruit production, vegetative biomass, growth) in that environment and also that this association is weak or absent in other environments where the trait is not expected to be adaptive (e.g., Dudley 1996a, Donovan et al. 2007).

Cork oak is a sclerophyllous tree species with a wide distribution in the western Mediterranean basin that occupies environments of contrasted temperature and rainfall (Díaz-Fernández et al. 1995, Aronson et al. 2009). Thus, it represents a suitable species to study processes of local adaptation. Population divergence has been documented in cork oak for growth form (Gandour et al. 2007) and traits related to tolerance to low temperatures (Aranda et al. 2005). Population differences in traits linked to drought tolerance such as acorn size and leaf characteristics have also been reported (Ramírez-Valiente et al. 2009a, 2009b). However, to our knowledge, the role of phenotypic plasticity on the ability of this species to cope with the environmental heterogeneity has not been explored so far.

In Mediterranean environments, water availability is one of the main factors limiting plant survival, growth and reproduction (Larcher 2000, Gulías et al. 2002). Key traits controlling carbon uptake and water loss such as water use efficiency (WUE), leaf morphology and chemical composition influence plant fitness (Heschel et al. 2002, 2004, Ludwig et al. 2004) and, thus, can be expected to be under selection (Geber and Griffen 2003, Wright et al. 2003). Leaf size (LS), leaf nitrogen content per unit mass (N_{mass}) and specific leaf area (SLA) have been suggested to have an adaptive value for cork oak under short drought periods (Ramírez-Valiente et al. 2009a). However, studies of populations growing for generations under contrasting environments are necessary to support the

adaptive hypothesis for these traits in this species (Dudley 1996a).

The aim of this study was to evaluate the importance of local adaptation and phenotypic plasticity in key functional leaf traits potentially involved in cork oak's drought tolerance. For this purpose, we analysed selected leaf ecophysiological traits in 9-year-old plants established under common environmental conditions during 2 years with contrasting annual and summer precipitation. Plants were sampled from 13 populations characterized by different climates. The traits selected were SLA, LS, N_{mass} and carbon isotope discrimination ($\Delta^{13}\text{C}$) as a surrogate of water use efficiency (Farquhar et al. 1989). These traits have been broadly documented to be involved in drought responses and they have been reported to be under natural selection in dry environments (e.g., Dudley 1996a, Casper et al. 2005, Heschel and Riginos 2005, Donovan et al. 2007). Our specific objectives were (i) to assess inter-population differentiation of selected leaf ecophysiological traits in cork oak and the effect of climate on such differentiation, (ii) to quantify the phenotypic plasticity of these traits in response to rainfall variation and (iii) to estimate the relationships between the ecophysiological traits and aboveground growth in order to explore the potential adaptive value of these traits in cork oak under different water availabilities.

Materials and methods

Common garden and years of study

The study was conducted in a common garden located in the interior of the Iberian Peninsula at Monfragüe National Park (39°51' N, 6°1' W, 375 m, Cáceres, Spain). The climate is typically Mediterranean, characterized by hot and dry summers and cool winters. The average annual precipitation for the last 40 years is 755.2 mm and the average annual temper-

Table 1. Location and climatic characterization of the 13 cork oak populations. Populations were classified in five climatic groups (I–V) according to Ramírez-Valiente et al. (2009a): I-TM, II-CM, III-MW, IV-CW and V-HS. Pa, annual precipitation (in millimetres); Ps, summer precipitation (in millimetres); T, annual mean temperature (in degrees Celsius); MMH, mean maximum temperature of the hottest month (in degrees Celsius); mmc, mean minimum temperature of the coldest month (in degrees Celsius).

Code	Population	Climatic group	Latitude	Longitude	Altitude	Pa	Ps	T	MMH	mmc
CB	Tietar valley	I-TM	40°07' N	4°31' W	677	644	55.1	16.6	36.8	2.4
CA	North Cáceres	I-TM	39°22' N	6°22' W	362	672	42.7	16.8	35.0	4.8
ABU	San Pedro hills	I-TM	39°13' N	7°13' W	500	643	37.6	15.4	31.3	4.1
JC	Western Morena mountain range	I-TM	38°13' N	6°42' W	492	627	39.1	16.1	33.5	4.1
CR	Salamanca	II-CM	40°35' N	6°26' W	653	525	64.1	13.2	31.3	0.8
PAR	Guadarrama mountain range	II-CM	40°31' N	3°45' W	750	474	55.6	13.5	31.4	−0.1
FUE	Eastern Morena mountain range	II-CM	38°24' N	4°16' W	670	432	38.9	14.8	33.3	0.9
HZ	Las Alpujarras region	II-CM	36°47' N	3°18' W	1300	607	24.5	13.0	29.3	1.3
ALM	Los Alcornocales National Park	III-HMW	36°16' N	5°22' W	118	813	20.0	17.4	29.0	7.5
LV	Las Villuercas region	IV-HCW	39°22' N	5°21' W	600	1008	54.9	15.4	34.8	2.2
FIG	Catalonian Pyrenees	V-HS	42°24' N	2°48' E	25	647	101.8	15.7	30.8	3.4
COL	Coastal Catalonia	V-HS	41°54' N	2°30' E	175	805	159.8	15.6	30.5	0.0
POT	La Liebana region	V-HS	43°09' N	4°37' W	270	736	94.6	13.2	26.0	1.4

ature is 17.2 °C (AEMET, Spanish National Meteorological Agency). The common garden followed a design of randomized complete blocks. Thirteen populations were essayed, spanning a wide range of climatic conditions (Table 1). It included 30 blocks and four plants per population within each block. Seedlings were planted following a 3 × 3-m square grid (Figure 1). Seeds were collected from native stands during the autumn and winter of the year 1996 from 20–30 trees per population, separated by more than 100 m to minimize the risk of sampling closely related trees. This distance is enough to avoid familial structures even in open wood formations of cork oak (Soto et al. 2007). Seeds were sown at the beginning of 1997, grown in the nursery for a year under standard conditions of high water and nutrient availability and then planted in the field during the spring of 1998.

Study years and phenotypic measurements

The study was carried out 8 years after plantation in the field. By then, plants were 9 years old, 79.43 ± 2.19 cm in height and 5.14 ± 0.11 cm in diameter (mean and standard deviation [SD], respectively). Saplings were phenotypically characterized in 2 years with contrasting climatic conditions: 2005 (dry) and 2006 (wet). The differences in amount of annual and summer rainfall were particularly remarkable. So, annual rainfall was 468.7 mm in 2005 versus 644.5 mm in 2006. Rainfall during June–September was 27.5 and 79.6 mm for 2005 and 2006, respectively. Temperatures were very similar in both years. Annual mean temperature (AMT) was 17.1 °C in 2005 and 17.4 °C in 2006, mainly due to differences in autumn mean temperatures (October and November) which were 17.0 and 10.8 °C in 2005 and 18.6 and 13.5 °C in 2006 (Figure 2) (AEMET, Spanish National Meteorological Agency).

Phenotypic measurements were carried out during autumn–winter. A total of 20 blocks were randomly selected

and one plant per population was sampled within each block (13 populations × 20 plants per population = 260 plants).

Growth was measured in autumn to ensure that the vegetative season, which extends across spring and summer, had finished. The annual growth was estimated with a ruler from six shoots of the spring flush located in the top third of the crown and covering six different orientations.

To analyse biochemical and morphological traits, 12 sun leaves were sampled from three of the six shoots selected for annual growth measurements (N-, SE- and SW-oriented branches). Spring leaves were sampled and the LS for each leaf was measured prior to scanning. Image analysis was carried out with the image analyser software WINFOLIA v. 2002 (Régent, Quebec, Canada). The leaves were oven dried at 65 °C to a constant weight after scanning. SLA was estimated in one leaf per orientation using the ratio of dry weight to leaf area of one leaf. The rest of the dry material was ground in a ball mill. This was used to determine the isotopic composition of C¹³ ($\delta^{13}\text{C}$) with a Micromass Isochrom mass spectrometer and N_{mass} by the Kjeldahl method (Vapodest 50; Gerhardt).

The $\delta^{13}\text{C}$ values were used to estimate the $\Delta^{13}\text{C}$ as Eq. (1):

$$\Delta(\text{‰}) = \frac{\delta_{\text{a}} - \delta_{\text{p}}}{1000 + \delta_{\text{a}}} \cdot 1000 \quad (1)$$

where δ_{p} (‰) refers to the $\delta^{13}\text{C}$ of bulk leaf material and δ_{a} is the $\delta^{13}\text{C}$ of the air acting as carbon source (it was assumed to be -7.9‰).

Statistical analysis

In order to examine the effects of genetic differentiation on functional leaf traits, to explore the possible climatic influence on population divergence (local adaptation) and to investigate phenotypic plasticity in response to rainfall



Figure 1. Three views of the common garden experiment of the cork oak populations studied. It is located within Monfragüe National Park in central Spain (39°51' N, 6°1' W, 375 m a.s.l.). Plants, which were 9 years old and exhibited contrasting sizes and architectures, were arranged in a 3 × 3-m grid. The whole common garden occupied an extension of 1.40 ha. This figure appears in color in the online version of *Tree Physiology*.

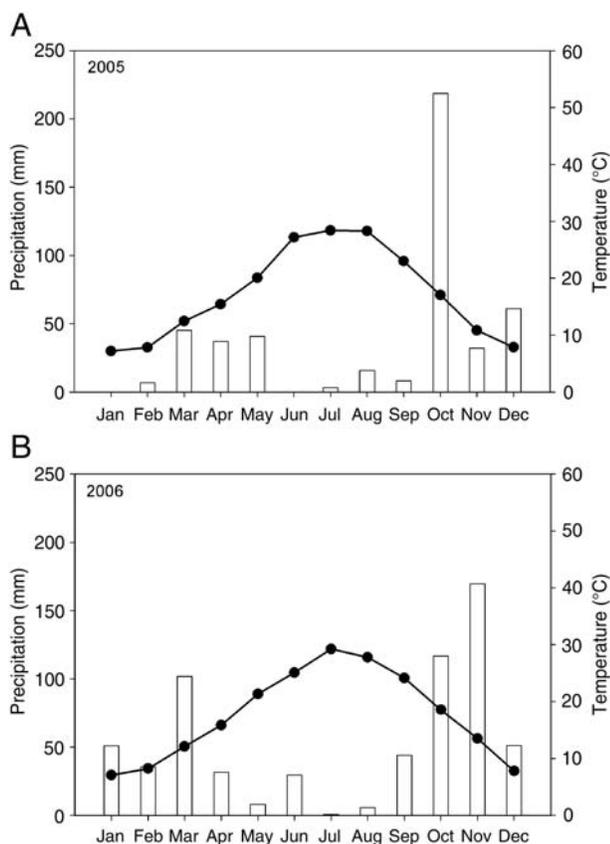


Figure 2. Average monthly rainfall (bars) and temperature (black line and dots) for the two studied years in the common garden: 2005 and 2006. Data were obtained from the nearest meteorological station located 4 km apart from the common garden.

variation, we performed five repeated-measures analyses of variance (one per variable) where year was the repeated factor (subject effect; see model below). Since populations varied over a wide climatic range and the environmental differences among the places of origin were not explained by a single climatic variable, we grouped the populations according to the degree of climatic similarity ('climatic group') following Ramírez-Valiente et al. (2009a). Thus, populations were classified into five climatic groups (I–V): I, Typical Mediterranean—TM (CB, CA, ABU, JC); II, Cool Mediterranean—CM (CR, PAR, FUE, HZ); III, Mediterranean with humid and mild winters—MW (ALM); IV, Mediterranean with humid and cool winters—CW (LV); and V, Mediterranean with humid summers—HS (FIG, COL, POT) (for population abbreviations, see Table 1). Climatic group was included as a categorical factor in the variance analyses. Differences in traits among climatic groups indicate genetic differences explained by adaptation to different climates. Likewise, the term population (within climatic group) was included in order to test for genetic differences due to adaptation to other local environmental factors different from climate (e.g., soil properties, nutrients availability, pressures from herbivores, etc.). The interaction term climatic group ×

year was used to evaluate potential differences in plasticity among climatic groups. Both genetic and environmental maternal effects (e.g., acorn mass) appear to exert their greatest effect on cork oak performance during the early years of the seedling growth (Quero et al. 2007, 2008), and their influence rapidly diminishes with age (Ramírez-Valiente et al. 2009b). Nevertheless, in order to avoid possible differences among populations due to differential size as a consequence of maternal effects or other potential factors affecting plant growth in the common garden experiment, we included plant height (measured before the experiment) as a covariate in the models (Shaw and Byers 1998, Casper et al. 2005). The model equation for the analyses of variance was:

$$Y_{ijkn} = \mu + H_n + Y_i + C_j + P(C)_{jk} + YC_{ij} + E_{ijkn}$$

where Y_{ijkn} is the observed value for the variable considered in the n tree of the k population from the j climatic group in the i year, μ is the general mean, H_n is the effect of previous height for individual n , Y_i is the effect of the i year, C_j is the effect of the j climatic group, P_k is the effect of the k population nested within the j climatic group, YC_{ij} is the effect of the interaction between year i and climatic group j and E_{ijkn} is the residual error for Y_{ijkn} .

Additionally, we investigated the potential direct and total (direct + indirect) relationships between functional traits and aboveground growth, as the performance variable under contrasting climatic conditions in order to assess the potential adaptive value of these traits for cork oak under different water availabilities. For this purpose, we constructed the following regression models sensu Lande and Arnold (1983):

$$W = \text{Constant} + S_n X_n + \text{Error (Model I)}$$

$$W = \text{Constant} + S_n X_n + \frac{1}{2} C_n X_n^2 + \text{Error (Model II)}$$

$$W = \text{Constant} + \beta_1 X_1 + \beta_2 X_2 + \dots + \text{Error (Model III)}$$

$$W = \text{Constant} + \beta_1 X_1 + \frac{1}{2} \gamma_{11} X_1^2 + \beta_2 X_2 + \frac{1}{2} \gamma_{22} X_2^2 + \gamma_{12} X_1 X_2 + \dots + \text{Error (Model IV)}$$

where W is the aboveground growth, estimated as the average growth in six spring shoots around the tree crown, X_1, X_2, \dots, X_n represent the trait value in each year for trait 1, 2, ... n , β s are the partial regression coefficients for linear terms in multiple regressions (linear gradients), γ s are the partial regression coefficients for quadratic terms (e.g., $\gamma_{11}, \gamma_{22}, \dots$) and two-way interaction terms (e.g., γ_{12}, \dots) in multiple polynomial regressions (non-linear gradients), S s are the regression coefficients in linear simple regressions (linear differentials) and C s are the partial regression coefficients for quadratic terms in quadratic regressions (non-linear differentials) (for further explanation, see Lande and Arnold 1983). Thus, Models I and II (single-trait regressions) were implemented for each trait and year separately. Models III and IV (multi-trait regressions) were run per year separately but for all traits together. Following Lande and Arnold 1983, growth values were relativized in the regression models by dividing each single growth value by mean growth. Likewise, traits

Table 2. GLM results for the five studied variables. Fixed factors: climatic group, population (climatic group), year, climatic group \times year. Initial height was included in the model as a covariate for all variables. *F*-value, *P*-value and percentage (%) of the variance explained by each factor are shown for each variable.

		Growth	$\Delta^{13}\text{C}$	SLA	LS	N_{mass}
Climatic group	<i>F</i> -value	2.76 ¹	2.89 ¹	16.01 ¹	6.63 ¹	1.35
	<i>P</i> -value	0.027 ¹	0.022 ¹	<0.001 ¹	<0.001 ¹	0.250
	% variance	0.1 ¹	0.4 ¹	11.9 ¹	1.7 ¹	0.5
Population (climatic group)	<i>F</i> -value	1.24	1.91	1.63	1.48	1.08
	<i>P</i> -value	0.275	0.057	0.114	0.161	0.379
	% variance	0.1	0.8	1.0	0.3	0.5
Year	<i>F</i> -value	731.6 ¹	2.16	6.71 ¹	263.7 ¹	0.61
	<i>P</i> -value	<0.001 ¹	0.143	0.009 ¹	<0.001 ¹	0.436
	% variance	30.4 ¹	0.3	2.7 ¹	28.0 ¹	0.5
Climatic group \times year	<i>F</i> -value	2.51 ¹	0.55	0.26	0.42	1.12
	<i>P</i> -value	0.041 ¹	0.701	0.906	0.791	0.347
	% variance	0.2 ¹	0.0	0.0	0.0	0.0
Height	<i>F</i> -value	129.11 ¹	28.63 ¹	7.24 ¹	45.60 ¹	1.92
	<i>P</i> -value	<0.001 ¹	<0.001 ¹	0.007 ¹	<0.001 ¹	0.167
	% variance	61.8 ¹	38.1 ¹	17.8 ¹	52.2 ¹	0.0
	% residual variance	7.5	38.1	66.5	17.8	98.5

¹Statistically significant differences.

were standardized to a mean = 0 and variance = 1 [i.e., standardized value = (value – mean)/SD]. The direct association between phenotypic traits and growth is indicated by phenotypic gradients (β) for linear terms and (γ) for non-linear terms. The total association (direct + indirect) between eco-physiological traits and growth was determined by phenotypic differentials for linear terms (*S*) in Model I and by non-linear differentials for quadratic and two-way interaction terms (*C*) in Model II. The regression coefficients for quadratic terms resulting from the regression analyses of Models II and IV were multiplied by two to obtain the γ and *C* values (see Lande and Arnold 1983, Stinchcombe et al. 2008). We examined for differences between years in standardized differentials and gradients by implementing a test of homogeneity of slopes in an analysis of covariance. Phenotypic relationships between traits were studied by Pearson correlations between all pair traits. Differences between years in the correlations between traits were examined by implementing a test of differences in correlation coefficients. The analyses were performed using STATISTICA 7.0

(Statsoft, Tulsa, OK, USA) and SAS 9.1 (SAS/STAT® Software, SAS Institute).

In order to control the inflation of Type I error derived from repeated testing, the false discovery rate (FDR, the expected proportion of tests erroneously declared as significant) criterion was applied. The FDR was controlled at each *P*-level using a standard step-up procedure (see Benjamini and Hochberg 1995, García 2004).

Results

Population divergence

The results from the analyses of variance showed significant differences between climatic groups in SLA, LS and $\Delta^{13}\text{C}$ (Table 2). There were also significant differences among climatic groups in growth in 2005 (Table 2). Climatic group explained the low (<1% for growth and $\Delta^{13}\text{C}$) to moderate (~12% for SLA) percentage of variance (Table 2).

Table 3. Mean \pm standard error for both years and all climatic groups for the four studied traits.

	$\Delta^{13}\text{C}$ (‰)	SLA (m ² kg ⁻¹)	LS (cm ²)	N_{mass} (mg g ⁻¹)
Year				
2005 (dry)	20.82 \pm 0.06	5.30 \pm 0.04a ¹	1.98 \pm 1.02a ¹	16.60 \pm 1.01
2006 (wet)	20.94 \pm 0.06	5.46 \pm 0.04b ¹	3.23 \pm 1.02b ¹	16.28 \pm 1.01
Climatic group				
I-TM	20.86 \pm 0.06b ¹	5.25 \pm 0.05a ¹	2.54 \pm 1.02b ¹	16.82 \pm 1.01
II-CM	20.73 \pm 0.06a ¹	5.10 \pm 0.05a ¹	2.47 \pm 1.02ab ¹	16.23 \pm 1.01
III-MW	21.05 \pm 0.12b ¹	5.75 \pm 0.09b ¹	3.03 \pm 1.05c ¹	16.54 \pm 1.02
IV-CW	20.75 \pm 0.12ab ¹	5.27 \pm 0.09a ¹	2.29 \pm 1.05a ¹	16.46 \pm 1.02
V-HS	20.99 \pm 0.07b ¹	5.52 \pm 0.05b ¹	2.38 \pm 1.03a ¹	16.15 \pm 1.01

Homogeneous groups at the 95% confidence level are presented by the same letter for traits presenting significant differences among climatic groups.

¹Significant differences (after FDR corrections) among years and among climatic groups.

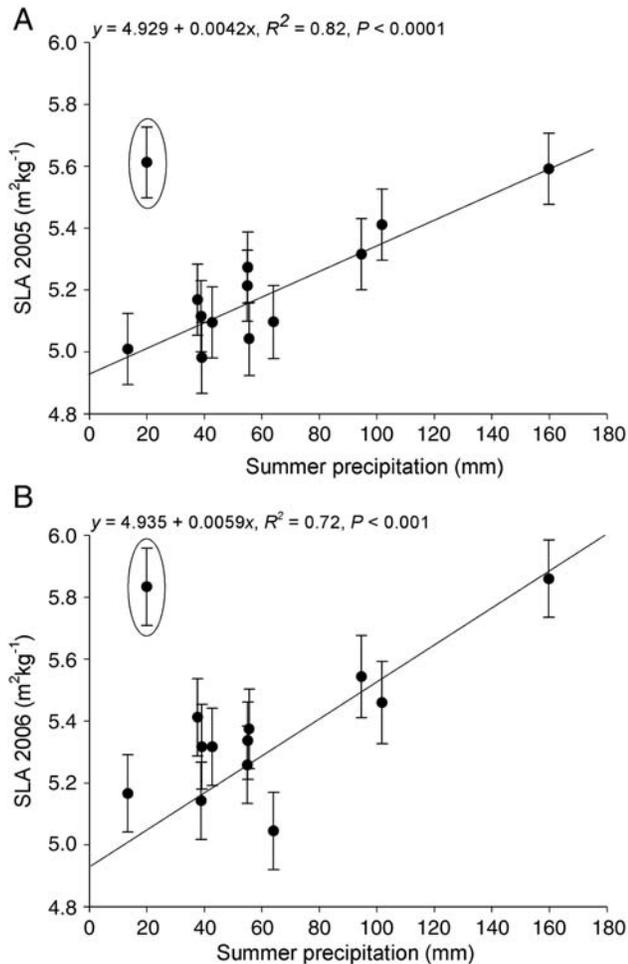


Figure 3. Relationship between shoot summer precipitation at the place of origin and SLA of the 13 cork oak populations in the common garden in Years 2005 (a) and 2006 (b). Equations, linear fits (R^2) and significant levels were obtained when the 'La Almoraima' population was excluded from the analyses (see further explanation in the text).

Cork oak populations originating from rainy summer and winter sites exhibited higher SLA and $\Delta^{13}\text{C}$ (a surrogate of WUE) than the remaining populations (Table 3). In fact, there was a positive association between summer precipitation at the place of origin and SLA (Figure 3) when the 'La Almoraima' population, a population characterized by rainy and mild winters, was excluded from the analyses. Otherwise, there were no significant differences among populations within climatic groups in any variable (Table 2). Population within climatic group explained a low percentage of the variance (0.1–1%) for most traits (Table 2).

Phenotypic plasticity

The analyses of variance revealed that there were differences between the 2 years in growth, SLA and LS (Table 2). The differences between years were particularly high for LS and growth (Table 2). In fact, the environmental factor explained 30.4 and 28.0% of the variation for growth and LS, respec-

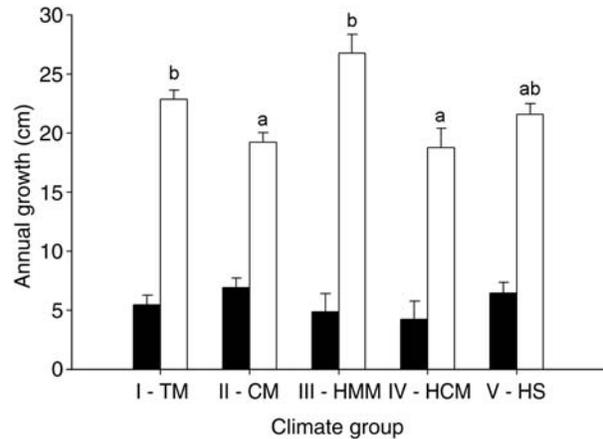


Figure 4. Shoot growth mean and standard errors for climatic groups in both dry year (black) and wet year (white) at the common garden site. Climatic groups: I-TM, II-CM, III-MW, IV-CW and V-HS. Homogeneous groups at the 95% confidence level are represented by the same letter above the bars for the wet year (2006) for which there were significant differences among climatic groups.

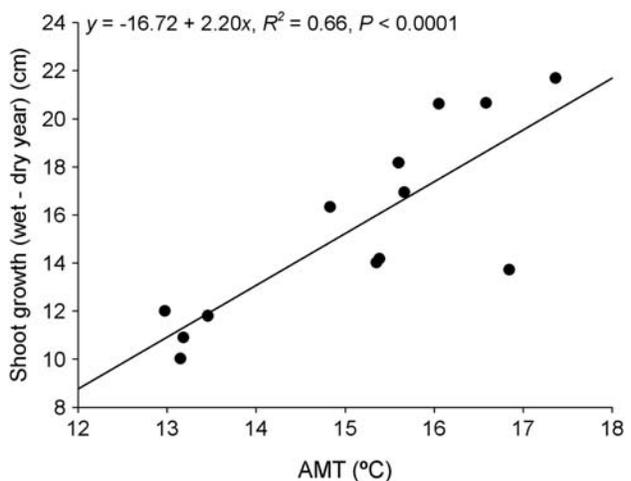


Figure 5. Relationship between shoot growth difference between the wet year (2006) and the dry year (2005) and annual mean temperature (AMT) at the place of origin of the 13 cork oak populations.

Table 4. Phenotypic linear differentials (S) and gradients (β) for four ecophysiological traits (standardized) and relative annual growth for dry (2005) and wet (2006) years.

		2005 (dry)	2006 (wet)
LS	S	0.40***	0.33***
	β	0.37***	0.37***
$\Delta^{13}\text{C}$	S	0.11	0.26***
	β	0.08	0.28***
SLA	S	-0.21** ¹	0.00
	β	-0.11	-0.03
N_{mass}	S	-0.12	-0.04
	β	-0.07	-0.01

Asterisks indicate the significance level. Significance levels were corrected by FDR criterion. See the text for details.

¹Selection gradients and differentials varying statistically between years.

Table 5. Phenotypic correlations between the studied traits for dry (2005; below diagonal) and wet (2006; above diagonal) years.

Trait	LS	SLA	$\Delta^{13}\text{C}$	N_{mass}
LS		0.01	0.11	-0.01
SLA	-0.09		0.16*	0.15*
$\Delta^{13}\text{C}$	0.13*	0.23***		-0.19**
N_{mass}	-0.09	0.27***	-0.20**	

Asterisks indicate the significance level. Significance levels were corrected by FDR criterion. See the text for details.

tively. Plants presented higher SLA and larger LS during 2006 (wet year) than during 2005 (dry year) (Table 3). Initial plant size had a positive effect on growth, SLA and LS and a negative effect on $\Delta^{13}\text{C}$ (Table 2).

The interaction term climatic group \times year was not significant for any trait, indicating the absence of differences in plasticity among climatic groups (Table 2). However, the climatic group \times year term was significant for annual growth. In fact, climatic groups did not vary in growth in the dry year (2005), but they did show differences in growth during the wet year (2006) (Figure 4). Furthermore, there was a positive relationship between AMT and growth in both years, but correlation and slope were much stronger during the wet year (2006) (Growth = $0.485 + 0.311 \times \text{AMT}$, $r = 0.85$, $P < 0.001$) than during the dry year (2005) (Growth = $-15.587 + 2.507 \times \text{AMT}$, $r = 0.62$, $P = 0.024$). As a consequence, a strong and positive relationship between AMT at the place of origin and among-year growth differences was found (Figure 5).

Relationships between ecophysiological traits and aboveground growth

Plants with larger leaves presented larger annual shoot growth under both dry (2005) and wet conditions (2006) (positive S and β in both years; Table 4). SLA showed a significant negative relationship with annual shoot growth ($S = -0.21$, $P = 0.0007$) in the dry year (2005) (Table 4). Thus, plants with more sclerophyllous leaves (lower SLA) grew more during the dry year (2005), but they did not during the wet one (2006). Likewise, plants with higher $\Delta^{13}\text{C}$ (lower WUE) had significantly larger growth in the wet year (2006) but they did not in the dry year (2005) (Table 4). Significant non-linear terms were not detected for any trait and environment (data not shown).

Phenotypic correlations are described in Table 5. Correlations were quite consistent between years and the tests for differences in correlation coefficients showed that none of the correlations varied significantly with environmental conditions (Table 5).

Discussion

Adaptation to environment: population divergence and phenotypic plasticity

Populations differed significantly in all traits associated with plant growth (LS, SLA and $\Delta^{13}\text{C}$). The population diver-

gence in leaf traits was consistent with that expected for populations differing in temperatures and precipitation pattern. Cork oak populations originating from rainy summer and winter sites exhibited higher SLA and $\Delta^{13}\text{C}$ than the remaining populations. SLA and $\Delta^{13}\text{C}$ commonly decreases with decreasing rainfall in forest tree species (e.g., Cunningham et al. 1999, Fonseca et al. 2000, Bussetti et al. 2002, Schulze et al. 2006, Marchin et al. 2008) though such relationship has not been always observed (e.g., Roupsard et al. 1998, Aranda et al. 2010). A decreasing pattern of SLA and $\Delta^{13}\text{C}$ with rainfall has been recently reported in cork oak by Gouveia and Freitas (2009). However, the trait measurements in that study were performed in situ at the places of origin of the trees. Thus, the environment and genetic effect could not be differentiated. In the present study, the large significant differences in SLA among climatic groups, together with their association to the amount of summer precipitation at the place of origin, suggest that among-population differentiation could be at least partly due to an adaptation to different patterns of rainfall.

In contrast, despite the fact that cork oak populations belonging to climatic Groups V (rainy summers) and III (rainy and mild winters) exhibited higher SLA and $\Delta^{13}\text{C}$ than those from other climatic groups, they exhibited significant differences in LS. Trees from rainy summer sites (V), also characterized by cool annual and winter temperatures (Table 1), had much smaller leaves than those trees from the humid winter sites, which present mild/warm annual and winter temperatures (Table 1). This suggests (i) that LS is probably adapted to the different temperature patterns and growth seasons experienced by the different populations and, what is more important, (ii) that LS and SLA can evolve separately despite the strong genetic correlation observed between these two traits in cork oak (Ramírez-Valiente et al. 2009a).

LS and SLA varied across years, evidence of a high phenotypic plasticity in these traits. Besides, both these and the rest of the traits varied, as was expected, for years with different water availabilities. Thus, plants had larger leaves, higher SLA and higher $\Delta^{13}\text{C}$ in the wet year (2006) than in the dry one (2005), which agrees with the results obtained from the phenotypic analyses based on Lande and Arnold (1983) and with the observed pattern of population and climate divergence.

Finally, the genotype \times environment interaction was very weak for all traits, suggesting low population divergence in plasticity for this species as also observed in *Quercus ilex*, a closely related species (Gimeno et al. 2009). However, this term was significant for shoot growth (a performance variable). A plausible explanation for this result could be the potential differences in plasticity among populations in non-measured traits. In fact, studies on other evergreen Mediterranean oaks have reported differences in plasticity in some morphological and physiological traits which have not been studied here (e.g., Balaguer et al. 2001; Gratani et al. 2003). Besides, we observed a positive correlation between population difference in shoot growth between the wet

(2006) and the dry year (2005) with AMT at the place of origin. In fact, populations from warmer sites grew more than populations from cooler sites under wet conditions (Year 2006) but not under dry ones (Year 2005). These results suggest that 'warm' populations take more advantage of favourable conditions than 'cool' ones as observed in other temperate forest tree species (e.g., Lauteri et al. 2004).

Association between ecophysiological traits and growth under dry and wet conditions

There were significant differences in the phenotypic gradient and differentials of SLA across years. Plants with more sclerophyllous leaves (lower SLA) grew more during the dry year (2005), but not during the wet one (2006). Higher SLA is associated with higher leaf area ratio values and higher photosynthetic capacity, and thus, higher potential of carbon gain and growth rate in inter-specific studies (Reich et al. 1997, 1998; Cornelissen et al. 1998; Shipley 2006). However, lower SLA has been reported to provide fitness benefits in dry environments since more sclerophyllous leaves allow the plant to avoid an excess of water use, maintaining photosynthetic activity and carbon gain over a longer time period (Dudley 1996a, 1996b). This strategy fits expectations for drought-tolerant species such as evergreen Mediterranean oaks (Leiva and Fernández-Alés 1998, Bussoti et al. 2002).

There are many selection studies in the literature on SLA and other indices of sclerophylly such as leaf thickness under different irradiance regimes, soil properties, plant densities and herbivore pressures (e.g., Tusic et al. 1998, Steinger et al. 2003, Avramov et al. 2007, Saldaña et al. 2007). However, to our knowledge, there are few studies aimed at analysing the phenotypic gradients and differentials for these traits in response to water availability (e.g., Etterson 2004, Agrawal et al. 2008). (Etterson 2004) showed increasing negative gradients and differentials for SLA along an aridity gradient for *Chamaecrista fasciculata*. In other words, they found that plants presenting more sclerophyllous leaves had increased fitness along an aridity gradient. As a consequence, they suggested that climate alters selection regimens for this trait in their studied species. Although we have not studied the associations of traits with fitness itself, but aboveground growth, the facts that (i) the association between SLA and growth shows differences across years, (ii) SLA decreases with summer and annual rainfall and (iii) populations with higher rainfalls at the places of origin present higher SLA suggest that divergence in water availability causes differential selection for SLA in cork oak (see also Ramírez-Valiente et al. 2009a).

Although there were no significant differences across years in the phenotypic gradients and differentials for the rest of the traits, marginal differences ($P = 0.08$) in partial correlation coefficients of $\Delta^{13}\text{C}$ between the wet year (2006) and the dry year (2005) were observed. In fact, there were both direct or indirect positive associations between higher $\Delta^{13}\text{C}$ (lower WUE) and growth in the wet year (2006), but no di-

rect or indirect positive associations under dry conditions (Year 2005). There is a variety of studies showing different kinds of relationships between water use efficiency (WUE) and aboveground growth/biomass in dry environments, from positive (e.g., Dudley 1996a, 1996b, Ludwig et al. 2004), neutral (e.g., Ludwig et al. 2004) to negative relationships (e.g., Donovan et al. 2007). A similar variety of findings is also reported for wet conditions (e.g., Dudley 1996a, 1996b, Agrawal et al. 2008). Donovan et al. (2007) argued a dehydration strategy as a possible explanation for the negative relationship between WUE and aboveground biomass in the annual *Helianthus anomalous*. Heschel and Riginos (2005) suggested that lower WUE may favour fitness traits when plants experience drought early in the vegetative season. In the present study, the results for $\Delta^{13}\text{C}$ were not completely consistent with those observed for SLA because plants with more sclerophyllous leaves (lower SLA) grew more during the dry year (2005), but not during the wet one (2006). In fact, there was a positive correlation between SLA and $\Delta^{13}\text{C}$ in both years. Thus, we would have expected that lower $\Delta^{13}\text{C}$ had growth benefits in dry conditions (Year 2005) but not in wet ones (Year 2006). Possible explanations for this apparent discrepancy need further research, but they could be related to consequences of SLA on leaf life span (Cavender-Bares et al. 2005), vegetative phenology (Heschel and Riginos 2005, Donovan et al. 2007) and changes in the relationships nitrogen–photosynthesis–WUE due to variation of SLA with water availability (Reich et al. 1998).

Conclusion

In conclusion, our study shows phenotypic differentiation in SLA, LS and $\Delta^{13}\text{C}$ among cork oak populations associated with summer and annual rainfall at the places of origin. SLA and LS responded positively to an increase in summer and rainfalls in the common garden, indicating phenotypic plasticity in these traits. There was also a genotype \times environment interaction for shoot growth and a correlation between the difference in shoot growth among years and annual mean temperature, suggesting a more opportunistic strategy of taking advantage of favourable conditions for the warmer populations. Finally, plants with lower SLA presented larger aboveground growth in a dry year (2005), whereas those with lower water use efficiency showed larger growth under wetter conditions (Year 2006). Finally, we observed that plants with larger leaf sizes had higher growth benefits in both years. Altogether, our results suggest an adaptive value of SLA and LS for cork oak under a Mediterranean climate and their potentially important role for dealing with varying temperature and rainfall regimes through both local adaptation and phenotypic plasticity.

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References

- Agrawal, A.A., A.C. Erwin and S.C. Cook. 2008. Natural selection on and predicted responses of ecophysiological traits of swamp milkweed (*Asclepias incarnata*). *J. Ecol.* 96:536–542.
- Aranda, I., L. Castro, R. Alía, J.A. Pardos and L. Gil. 2005. Low temperature during winter elicits differential responses among populations of the Mediterranean evergreen cork oak (*Quercus suber*). *Tree Physiol.* 25:1085–1090.
- Aranda, I., R. Alía, U. Ortega, A.K. Dantas and J. Majada. 2010. Intra-specific variability in biomass partitioning and carbon isotopic discrimination under moderate drought stress in seedlings from four *Pinus pinaster* populations. *Trees Genet. Genom.* 6:169–178.
- Aronson, J., J.S. Pereira and J.C. Pausas. 2009. Cork oak woodlands on edge: ecology, adaptive management and restoration. Island Press, Washington, DC.
- Avramov, S., D. Pemac and B. Tucic. 2007. Phenotypic plasticity in response to an irradiance gradient in *Iris pumila*: adaptive value and evolutionary constraints. *Plant Ecol.* 190:275–290.
- Balaguer, L., E. Martínez-Ferri, F. Valladares, M.E. Pérez-Corona, F.J. Baquedano, F.J. Castillo and E. Marinque. 2001. Population divergence in the plasticity of the response of *Quercus coccifera* to the light environment. *Funct. Ecol.* 15:124–135.
- Benjamini, Y. and Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc.* 57:289–300.
- Bradshaw, A.D. 1965. Evolutionary significance of phenotypic plasticity in plants. *Adv. Genet.* 13:115–155.
- Busotti, F., D. Bettini, P. Grossoni, S. Mansuino, R. Nibbi, C. Soda and C. Tani. 2002. Structural and functional traits of *Quercus ilex* in response to water availability. *Environ. Exp. Bot.* 47:11–23.
- Casper, B.B., I.N. Forseth and D.A. Wait. 2005. Variation in carbon isotope discrimination in relation to plant performance in a natural population of *Cryptantha flava*. *Oecologia* 145:541–548.
- Cavender-Bares, J., P. Cortes, S. Rambal, R. Joffre, B. Milesa and A. Rocheteau. 2005. Summer and winter sensitivity of leaves and xylem to minimum freezing temperatures: a comparison of co-occurring Mediterranean oaks that differ in leaf lifespan. *New Phytol.* 168:597–612.
- Cornelissen, J.H.C., P. Castro-Díez and A.L. Camelli. 1998. Variation in relative growth rate among woody species. In *Inherent Variation in Plant Growth. Physiological Mechanisms and Ecological Consequences*. Eds. H. Lambers, H. Poorter and M.M.I. Van Vuuren. Backhuys, Leiden, The Netherlands, p 363–392.
- Cunningham, S.A., B. Summerhayes and M. Westoby. 1999. Evolutionary divergences in leaf structure and chemistry, comparing rainfall and soil nutrient gradients. *Ecology* 69:569–588.
- Díaz-Fernández, P.M., P. Jiménez, G. Catalán, S. Martín and L. Gil. 1995. Regiones de procedencia de *Quercus suber* L. ICONA, Madrid, Spain, 49 p.
- Donovan, L.A., S.A. Dudley, D.M. Rosenthal and F. Ludwig. 2007. Phenotypic selection on leaf water use efficiency and related ecophysiological traits for natural populations of desert sunflowers. *Oecologia* 152:13–25.
- Dudley, S.A. 1996a. Differing selection on plant physiological traits in response to environmental water availability: a test of adaptive hypotheses. *Evolution* 50:92–102.
- Dudley, S.A. 1996b. The response to differing selection on plant physiological traits: evidence for local adaptation. *Evolution* 50:103–110.
- Etterson, J.R. 2004. Evolutionary potential of *Chamaecrista fasciculata* in relation to climate change. I. Clinal patterns of selection along an environmental gradient in the Great Plains. *Evolution* 58:1446–1458.
- Farquhar, G.D., J.R. Ehleringer and K.T. Hubick. 1989. Carbon isotope discrimination and photosynthesis. *Annu. Rev. Plant Physiol. Mol. Biol.* 40:503–537.
- Fonseca, C.R., J.M. Overton, B. Collins and M. Westoby. 2000. Shifts in trait-combinations along rainfall and phosphorus gradients. *J. Ecol.* 88:964–977.
- Gandour, M., M.L. Khouja, L. Toumi and S. Triki. 2007. Morphological evaluation of cork oak (*Quercus suber* L.): Mediterranean provenance variability in Tunisia. *Ann. For. Sci.* 64:549–555.
- García, L.V. 2004. Escaping the Bonferroni iron claw in ecological studies. *Oikos* 105:657–663.
- Geber, M.A. and L.R. Griffen. 2003. Inheritance and natural selection on functional traits. *Int. J. Plant Sci.* 164:21–42.
- Gimeno, T.E., B. Pias, J.P. Lemos-Filho and F. Valladares. 2009. Plasticity and stress tolerance override local adaptation in the responses of Mediterranean holm oak seedlings to drought and cold. *Tree Physiol.* 29:87–98.
- Gouveia, A.C. and H. Freitas. 2009. Modulation of leaf attributes and water use efficiency in *Quercus suber* along a rainfall gradient. *Trees* 23:267–275.
- Gratani, L., M. Meneghini, P. Pesoli and M.F. Crescente. 2003. Structural and functional plasticity of *Quercus ilex* seedlings of different provenances in Italy. *Trees Struct. Funct.* 17:515–521.
- Gulias, J., J. Flexas, A. Abadia and H. Medrano. 2002. Photosynthetic responses to water deficit in six Mediterranean sclerophyll species: possible factors explaining the declining distribution of *Rhamnus ludovici-salvatoris*, an endemic Balearic species. *Tree Physiol.* 22:687–697.
- Heschel, M.S. and C. Riginos. 2005. Mechanisms of selection for drought stress tolerance and avoidance in *Impatiens capensis* (Balsaminaceae). *Am. J. Bot.* 92:37–44.
- Heschel, M.S., K. Donohue, N.J. Hausmann and J. Schmitt. 2002. Population differentiation and natural selection for water-use efficiency in *Impatiens capensis* (Balsaminaceae). *Int. J. Plant Sci.* 163:907–912.
- Heschel, M.S., S. Sultan, S. Glover and D. Sloan. 2004. Population differentiation and plastic responses to drought stress in the generalist annual *Polygonum persicaria*. *Int. J. Plant Sci.* 165:817–824.
- Kawecki, T.J. and T. Ebert. 2004. Conceptual issues in local adaptation. *Ecol. Lett.* 7:1225–1241.
- Lande, R. and S.J. Arnold. 1983. The measurement of selection on correlated characters. *Evolution* 37:1210–1226.
- Larcher, W. 2000. Temperature stress and survival ability of Mediterranean sclerophyllous plants. *Plant Biosyst.* 134:279–295.
- Lauteri, M., A. Piura, M.C. Monteverdi, E. Brugnoli, F. Villani and G. Eriksson. 2004. Genetic variation in carbon isotope discrimination in six European populations of *Castanea sativa* Mill. originating from contrasting localities. *J. Evol. Biol.* 17:1286–1296.

- Leiva, M.J. and R. Fernández-Alés. 1998. Variability in seedling water status during drought within a *Quercus ilex* subsp. *ballota* population, and its relation to seedling morphology. *For. Ecol. Manage.* 111:147–156.
- Lortie, C. and L.W. Aarssen. 1996. The specialization hypothesis for phenotypic plasticity in plants. *Int. J. Plant Sci.* 157:484–487.
- Ludwig, F., L.F. Rosenthal, J.A. Johnston, N. Kane, B.L. Gross, C. Lexer, S.A. Dudley, L.H. Riesenberg and L.A. Donovan. 2004. Selection on leaf ecophysiological traits in a desert hybrid *Helianthus* species and early-generation hybrids. *Evolution* 58:2682–2692.
- Marchin, R.M., E.L. Sage and J.K. Ward. 2008. Population-level variation of *Fraxinus americana* (white ash) is influenced by precipitation differences across the native range. *Tree Physiol.* 28:151–159.
- Quero, J.L., R. Villar, T. Marañón, R. Zamora and L. Poorter. 2007. Seed mass effect in four Mediterranean *Quercus* species (Fagaceae) growing in contrasting light environments. *Am. J. Bot.* 94:1795–1803.
- Quero, J.L., R. Villar, T. Marañón, R. Zamora, D. Vega and L. Sack. 2008. Relating leaf photosynthetic rate to whole-plant growth: drought and shade effects on seedlings of four *Quercus* species. *Funct. Plant Biol.* 35:725–737.
- Ramírez-Valiente, J.A., Z.R. Lorenzo, A. Soto, F. Valladares, L. Gil and I. Aranda. 2009a. Elucidating the role of genetic drift and natural selection in cork oak differentiation regarding drought tolerance. *Mol. Ecol.* 18:3803–3815.
- Ramírez-Valiente, J.A., F. Valladares, L. Gil and I. Aranda. 2009b. Population differences in juvenile survival under increasing drought are mediated by seed size in cork oak (*Quercus suber* L.). *For. Ecol. Manage.* 257:1676–1683.
- Reich, P.B., M.B. Waters and D.S. Ellsworth. 1997. From tropics to tundra: global convergence in plant functioning. *Proc. Natl. Acad. Sci. USA* 94:13730–13734.
- Reich, P.B., D.S. Ellsworth and M.B. Walters. 1998. Leaf structure (specific leaf area) modulates photosynthesis–nitrogen relations: evidence from within and across species and functional groups. *Funct. Ecol.* 12:948–958.
- Roupsard, O., H.I. Joly and E. Dreyer. 1998. Variability of initial growth, water-use efficiency and carbon isotope discrimination in seedlings of *Faidherbia albida* (Del.) A. Chev., a multipurpose tree of semi-arid Africa. Provenance and drought effects. *Ann. For. Sci.* 55:329–348.
- Saldaña, A., C.H. Lusk, W.L. Gonzales and E. Gianoli. 2007. Natural selection on ecophysiological traits of a fern species in a temperate rainforest. *Evol. Ecol.* 21:651–662.
- Savolainen, O., T. Pyhajarvi and T. Knurr. 2007. Gene flow and local adaptation in trees. *Annu. Rev. Ecol. Evol. Syst.* 38:595–619.
- Schulze, E.D., N.C. Turner, D. Nicolle and J. Schumacher. 2006. Leaf and wood carbon isotope ratios, specific leaf areas and wood growth of Eucalyptus species across a rainfall gradient in Australia. *Tree Physiol.* 26:479–492.
- Shaw, R.G. and D.L. Byers. 1998. Genetics of maternal and paternal effects. In *Maternal Effects as Adaptations*. Eds. T Mousseau and C Fox. Oxford University Press, New York, p 97–111.
- Shipley, B. 2006. Net assimilation rate, specific leaf area and leaf mass ratio: which is most closely correlated with relative growth rate? A meta-analysis. *Funct. Ecol.* 20:565–574.
- Soto, A, Z.R. Lorenzo and L. Gil. 2007. Differences in fine-scale genetic structure and dispersal in *Quercus ilex* L. and *Q. suber* L.: consequences for regeneration of Mediterranean open woods. *Heredity* 99:601–607.
- Steinger, T., B.A. Roy and M.L. Stanton. 2003. Evolution in stressful environment II: adaptive value and costs of plasticity in response to low light in *Sinapis arvensis*. *J. Evol. Biol.* 16:313–323.
- Stinchcombe, J.R., A.F. Agrawal, P.A. Hohenlohe and S.J. Arnold. 2008. Estimating nonlinear selection gradients using quadratic regression coefficients: double or nothing? *Evolution* 62:2435–2440.
- Sultan, S.E. 1987. Evolutionary implications of phenotypic plasticity in plants. *Evol. Biol.* 21:127–178.
- Tucic, B., V. Tomic, S. Avramov and D. Pemac. 1998. Testing the adaptive plasticity of *Iris pumila* leaf traits to natural light conditions using phenotypic selection analysis. *Acta Oecologica* 19:473–481.
- Valladares, F., S.J. Wright, E. Lasso, K. Kitajima and R.W. Pearcy. 2000. Plastic phenotypic response to light of 16 congeneric shrubs from Panamanian rainforest. *Ecology* 81:1925–1936.
- Valladares, F., E. Gianoli and J.M. Gómez. 2007. *Ecological limits to plant phenotypic plasticity*. *New Phytol.* 146:749–763.
- Wright, I.J., P.B. Reich and M. Westoby. 2003. Least-cost input mixtures of water and nitrogen for photosynthesis. *Am. Nat.* 161:98–111.