

Contrasting patterns of morphological and physiological differentiation across insular environments: phenotypic variation and heritability of light-related traits in *Olea europaea*

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Received: 28 December 2009 / Accepted: 19 May 2010 / Published online: 9 June 2010
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Abstract Phenotypic variation of traits can reflect the ability of plants to adjust to particular environments, but how much of this variation is heritable is not frequently analyzed in natural populations. In the present paper, we investigated the patterns of phenotypic expression in light-related leaf traits of *Olea europaea* subsp. *guanchica*, a woody sclerophyllous species endemic to the Canary Islands. We explored phenotypic differentiation and heritable variation across several island populations differing in light environment. A suite of morpho-functional (leaf size, SLA and leaf angle) and physiological (pigment pools: Chl a/b ratio, xanthophyll cycle and β -carotene) traits was measured in six populations on three islands. In addition, we estimated heritabilities for these traits

following Ritland's method. Variation in morpho-functional, but not in physiological, traits was observed across the islands and was significantly related to the amount of diffuse light experienced by each population. In addition, significant heritabilities were found for morpho-functional traits, whereas expression of similar phenotypes among populations was accompanied by a lack of heritable variation in physiological traits. Most recently established populations did not exhibit lower heritabilities in quantitative traits than older populations, and apparently displayed congruent phenotypes under the local conditions. Our results strongly support the idea that different types of traits show contrasted levels of genetic and phenotypic variation in populations experiencing marked environmental differences.

Communicated by Carlos Ballaré.

Electronic supplementary material The online version of this article (doi:10.1007/s00442-010-1672-7) contains supplementary material, which is available to authorized users.

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Keywords Canary Islands · Diffuse light · Phenotypic differentiation · Pigment pools · Quantitative leaf traits

Introduction

Light environment constitutes a key factor in the performance of photosynthetic organisms, inducing dramatic changes in plant phenotypes. At the leaf-level, phenotypic acclimation to light conditions involves the adjustment of both morpho-functional (e.g., leaf size, leaf angle) and physiological (e.g., photosynthetic rates, pigment pools) traits (Lovelock and Clough 1992; Pearcy 1999). Despite the differences in their functioning, both types of traits are expressed in a coordinated fashion, and allow the expression of phenotypes suitable to particular environmental conditions (Lovelock and Clough 1992; Niinemets et al. 1998).

Given their role in plant performance, physiological and morpho-functional light-related traits are expected to be under selective pressures that determine the proportion of phenotypic variation attributable to a genetic component, i.e., trait heritability (Geber and Griffen 2003). Nevertheless, not only selection is responsible for the levels of heritable variation, and it is often speculated that genetic bottlenecks in populations or genotype–environment interactions may also be important determinants of trait heritability (Maddox and Root 1987; Caruso et al. 2005; Mitchell-Olds et al. 2007). While much attention has been paid to phenotypic responses of plants to contrasting light regimes (Valladares and Pearcy 1998; Pearcy 1999), it is not completely clear how plants respond to particular variations in light (e.g., responses to diffuse light; Reinhardt and Smith 2008), and little is known of the factors associated with the maintenance of genetic variation in these quantitative traits.

In this study, we analyzed the pattern of phenotypic and genetic variation in light-related traits across the populations of a woody plant, *Olea europaea* subsp. *guanchica*. The scenario provided by this species is useful for testing hypotheses relating to phenotypic and genetic change in quantitative traits. First, *O. e. guanchica* occurs on several oceanic islands across the Canarian archipelago, which provides opportunities to explore contrasting light environments (Givnish et al. 2004). Indeed, North Atlantic trade winds strongly determine microclimatic conditions throughout the Canaries, producing remarkable variation in cloudiness and precipitation at both the island and the archipelago scale (García et al. 2001; Marzol 2008). Accordingly, different locations across the archipelago presumably represent a mosaic of environments with conspicuous phenotypic differentiation among populations. Second, insular isolation typically leads some populations to experience genetic bottlenecks, which potentially result in reduced levels of heritability in quantitative traits at the population level (Nei et al. 1975; Whitlock and Fowler 1999; Caruso et al. 2005). A previous study supported the idea that within-population genetic variation in *O. e. guanchica* is related to the age of the substrate where each population occurs (García-Verdugo et al. 2009a), and consequently, recently established populations may show lower heritabilities than older populations.

We studied a set of populations on different islands with different locations within each island, exploring how phenotypic differentiation is related to microenvironmental variation across the archipelago. Our measurements included a suite of morpho-functional and physiological traits, considering that different types of traits could not contribute equally to phenotypic performance. We tested (1) whether different types of traits (morpho-functional and physiological) show similar patterns of phenotypic

variation across insular environments, and (2) whether heritable variation differs between types of traits and is affected by colonization events.

Materials and methods

Study species and population sampling

Olea europaea subsp. *guanchica* Vargas et al. is a sclerophyllous evergreen tree endemic to the Canarian archipelago. Individuals are up to 8 m tall with moderately dense canopies, and constitute small populations with 20–60 individuals in Mediterranean-type woodlands across the archipelago (García-Verdugo et al. 2009b). The present study only considered populations from the western *O. e. guanchica* lineage, as introgression with cultivars on the eastern islands might obscure natural patterns of phenotypic and genetic variation (García-Verdugo et al. 2009a). Climatic conditions among the study islands are similar, with constant, mild temperatures all the year round and a rainy season from November to March, although total precipitation increases from east to west (García et al. 2001). Two of the best preserved populations on each island were selected on the basis of vegetation composition (results not shown), geographical location and substrate age (Fig. 1), with the aim of representing populations with contrasting environmental conditions and different ages since colonization. In each selected population, 12 individuals were chosen for all subsequent analyses along a transect of approximately 100 m. This sample size represented a minimum of 25% of all adult individuals estimated in each population (see Table 1). Juveniles were avoided by including only fruit-bearing individuals.

Microclimatic characterization

The light environment was characterized in each population following two complementary approaches. First, in situ measurements of photosynthetically active radiation (PAR) were recorded in each population every 30 min throughout 1 year (August 2006–August 2007) with an S-LIA-M003 PAR sensor coupled with a U12 datalogger (Onset Computer, MA, USA). In order to ensure total exposure to light and to obtain representative values of the total radiation received by each population, each PAR sensor was placed on top of one of the individuals occurring in the middle of each transect. Second, one hemispheric photograph was taken at the outer canopy layer of each individual with a Nikon Coolpix 4500 digital camera (Nikon Canada, Mississauga, Canada) coupled with a Nikon FC-E8 Fisheye Adapter (Nikon, Tokyo, Japan). All photographs were taken at the same part of the crown

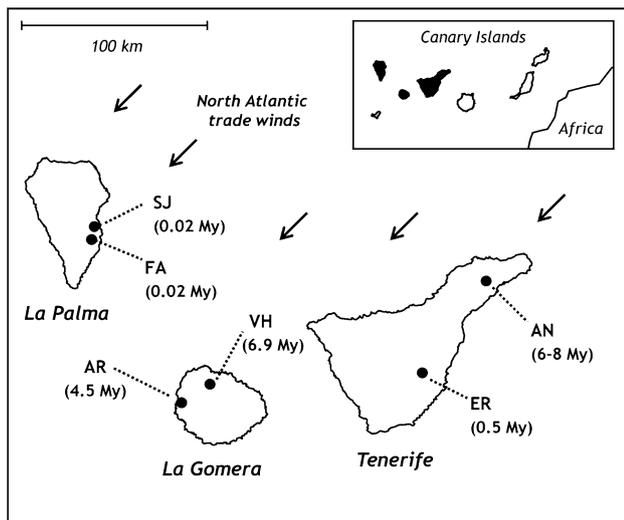


Fig. 1 Study area and location of the six populations considered in the present study. Different locations within each island give rise to contrasting light environments, as they are differently exposed to North Atlantic trade winds. The upper bound limit of population age (in millions of years: My) is given in parentheses below each population code, as described in García-Verdugo et al. (2009a)

where phenotypic measurements were made (see below), before sunset or under uniformly cloudy conditions, following the recommendations described in Hardy et al. (2004) and previous studies (García-Verdugo et al. 2009b). As additional microclimatic variables, we measured soil water content in each population every 30 min during the same year period with an ECH2O EC-10 sensor coupled with the U12 datalogger. One sensor per population was buried at a depth of 25–30 cm. Because volcanic soils were extremely shallow and root uptake in *Olea europaea* is concentrated within 60 cm depth (Fernández et al. 1991),

measurements from these sensors were considered to be an adequate indicator of water soil content. Soil-specific calibrations were obtained from soil samples from each population, as indicated by the manufacturer. Measurements of air temperature and air relative humidity from a previous study (García-Verdugo et al. 2009b) were also considered (see Table 1).

Measurement of light-related traits

In order to characterize the phenotypic variation of plant populations across the islands, three morpho-functional and three physiological leaf traits were measured on the selected individuals. Trait selection was based on the responsiveness of each trait to light conditions, as shown in other Mediterranean plants in previous studies (e.g., Valladares et al. 2000; Rubio de Casas et al. 2007). Sampling was performed in the summer (July–August) to ensure complete development of new leaves during the growing season and the same phenological stage across islands. Leaf size and specific leaf area (SLA) were measured from three current-year, fully-expanded leaves taken from five outer canopy twigs ($n = 15$) per individual, as indicated in García-Verdugo et al. (2009b). Leaf angle was measured with a protractor on 10 current-year leaves of each plant. All morpho-functional measurements were averaged per individual prior to statistical analysis. In addition, three twigs per plant were randomly sampled during the middle of the day from outer canopy layers for pigment analysis, following the protocol described in Jiménez et al. (1997). Once in the laboratory, 24-mm² discs were obtained from the leaves of each collected twig. Leaf discs were ground in a mortar with cool acetone and sodium ascorbate. After filtering through a 0.2- μ m syringe nylon filter, we injected

Table 1 Coordinates, estimated population sizes and microclimatic variables (means \pm SE) measured in the six study populations of *O. e. guanchica*

Population (code)	Coordinates	Island	Estimated population size ^a	Temperature (°C) ^b	Air RH (%) ^b	Soil water content (%/%) ^b	Direct radiation (Mol day ⁻¹) ^c	Diffuse radiation (Mol day ⁻¹) ^c
Anaga (AN)	28°32'N, 16°10'W	Tenerife	50–60	17.7 \pm 0.8 c	76.8 \pm 1.5 bc	18.0 \pm 1.7 ab	14.7 \pm 0.2 c	12.5 \pm 0.2 b
El Río (ER)	28°08'N, 16°32'W	Tenerife	40–50	18.7 \pm 0.8 b	69.0 \pm 1.2 a	17.8 \pm 1.4 b	16.8 \pm 0.3 b	8.6 \pm 0.1 d
Vallehermoso (VH)	28°11'N, 17°15'W	La Gomera	20–30	18.1 \pm 0.7 bc	78.7 \pm 1.5 c	9.4 \pm 0.5 c	13.2 \pm 0.3 d	12.2 \pm 0.3 bc
Arure (AR)	28°08'N, 17°19'W	La Gomera	30–40	16.2 \pm 0.9 d	72.8 \pm 2.5 b	18.7 \pm 1.8 ab	21.8 \pm 0.1 a	11.4 \pm 0.2 c
Finca Amado (FA)	28°38'N, 17°46'W	La Palma	30–40	17.6 \pm 0.8 c	76.2 \pm 1.1 bc	22.1 \pm 1.7 b	14.9 \pm 0.1 c	16.6 \pm 0.2 a
San José (SJ)	28°39'N, 17°46'W	La Palma	20–30	20.0 \pm 0.7 a	72.6 \pm 0.8 b	28.0 \pm 2.6 a	22.4 \pm 0.1 a	15.8 \pm 0.1 a

Different letters next to values denote significant differences among populations for each variable

^a Estimated only for adult individuals in field surveys

^b Means are based on monthly values averaged over a year

^c Means are based on hemispheric photographs obtained from 12 individuals

30 μ L of acetone extract into a Spherisorb ODS2 reverse-phase steel column. Separation of chlorophylls and carotenoids was performed in an HPLC system (Waters, Milford, MA, USA) equipped with a Waters 996 photodiode array detector. For peak identification and quantification, pure commercial standards (VKI, Hørsholm, Denmark) were used. Measurements from three leaves were averaged per individual. Three pigment traits were considered: Chl a/b ratio, xanthophyll-cycle pool (violaxanthin, antheraxanthin and zeaxanthin: VAZ) and β -carotene. β -carotene and VAZ pools were expressed on a total chlorophyll (Chl a + b) basis, following previous studies (Valladares et al. 2000; Rubio de Casas et al. 2007).

Trait heritability estimation

Ritland's (2000) method provides an approach for investigating heritabilities in quantitative traits in long-lived species where crossing experiments are not feasible. This method involves the regression of quantitative trait similarity on marker-estimated relatedness between individuals. Briefly, heritability for each trait and population can be estimated as

$$h^2 = \frac{\text{Cov}(Z_{ij}, r_{ij})}{2 \text{Var}(r_{ij})}$$

where $\text{Cov}(Z_{ij}, r_{ij})$ is the covariance between the phenotypic similarity for a given trait between individuals i and j (Z_{ij}) and their relatedness coefficient (r_{ij}), as inferred from molecular markers. $\text{Var}(r_{ij})$ is the actual variance of relatedness, as described in Ritland (2000).

A critical point of the marker-based heritability method involves the need to measure the so-called "actual variance of relatedness", defined as the variation in relatedness among individuals of a given population (Ritland 1996, 2000). Klaper et al. (2001) found that actual variance was low and declined sharply with physical distance in a *Quercus laevis* population, and consequently, reliable trait heritabilities could not be estimated. In our study system, molecular data suggest that populations were established by multiple founders (García-Verdugo et al. 2010; García-Verdugo et al. in review), which enhances a mixture of relatedness within populations and thus favors significant variance (Ritland 2000). In addition, sampled individuals were on average 15–20 m apart, an optimal distance with regard to maximizing actual variance in other woody species (Klaper et al. 2001; Andrew et al. 2005).

A set of six nuclear microsatellites previously used in *O. e. guanchica* (García-Verdugo et al. 2009a) was amplified in order to calculate pairwise coefficients of relatedness and estimate the actual variance of relatedness for each population. Microsatellites included six variable loci described in other studies: DCA3, DCA4, DCA5, DCA8

(Sefc et al. 2000), oli12 and oli22 (Rallo et al. 2000). PCR-conditions for amplification can be consulted in García-Verdugo et al. (2009a). A total of 77 alleles was obtained, with a mean number of 13 alleles per locus. Additional information can be consulted in the Electronic supplementary material (ESM), online resource 1.

Data analysis

Hemispheric photographs were analyzed with Gap Light Analyzer (GLA) software v2.0 to separate the components of the light (diffuse and direct) reaching each individual (Frazer et al. 1999). Coordinates, elevation and slope were included in the GLA model for each individual, as described in Hardy et al. (2004). Data points from PAR sensors were used to calculate a daily cloudiness index for each population as the proportion of hours throughout the day in which PAR values did not match the typical distribution of completely sunny days. Ideally, this variable ranges from 0 (totally cloudy) to 1 (clear-sky days; Frazer et al. 1999). Daily cloudiness indexes of each population were averaged per month and included in the corresponding GLA model.

To investigate phenotypic differences in trait expression across islands, we performed a nested ANOVA for each trait. Since populations within the same island are closely-related (García-Verdugo et al. 2009a), "Population" was nested as a random factor in "Island". In order to investigate differences in environmental variables, we performed a repeated-measures ANOVA separately for temperature, air relative humidity and soil water content, considering "Population" as within-subject factor. We performed one-way ANOVAs to test differences among populations in diffuse and direct light, considering the measurements obtained for each tree from GLA analysis. Normality and homogeneity of variances were checked for each trait and variable, and leaf size was log-transformed to meet the ANOVA requirements. When significant differences were detected at any spatial scale (traits) or between populations (environmental variables), post-hoc Tukey tests were performed to show homogeneous groups.

To explore the relationship between phenotypic and environmental variation, pairwise correlations were performed. In order to minimize the likelihood of type I error, the number of correlations was limited by considering only those factors and traits that were found to be significant among populations (Curtin and Schulz, 1998).

For heritability estimates, pairwise relatedness coefficients (r_{ij}) and actual variance of relatedness [$\text{Var}(r_{ij})$] were calculated with SPAGeDI (Hardy and Vekemans 2002). To provide a conservative estimate of sampling error, significance of heritability estimates was determined by bootstrapping over individuals (see Andrew et al. 2005

for a comparison of different approaches). Heritability estimates were deemed significant if 95% of the bootstrapped values ($n = 100$) were positive. Negative estimates were assumed to be zero (Falconer 1981) when heritabilities were averaged per population. Lastly, heritability estimates at the population level were correlated with population age in order to test whether colonization events are related to heritable variation in populations.

Results

Microclimatic differences among populations

Analysis of hemispheric photographs revealed substantial differences in PAR across populations. Total PAR values as obtained from hemispheric photographs were similar to those recorded by PAR sensors in each population ($R^2 = 0.84$). As a general trend, total PAR increased from eastern to western populations (Table 1). The most conspicuous variation in light was due to an increase in the diffuse component in the western populations (La Palma), which reached 16.6 and 15.8 Mol day^{-1} in FA and SJ, respectively (Table 1). These values represent an increase of 37–44% in the diffuse component in comparison with the mean value obtained in populations on Tenerife and La Gomera. Differences in direct radiation between islands were less evident and were only significant between sites within each island. Soil water content was also highest in the populations sampled on the island of La Palma (22.1 and 28.0% in FA and SJ vs 15.9% on average in the other populations; Table 1). Air temperature and air relative humidity also showed significant variation across populations, with a mean temperature range of 16.2–20.0°C and a mean relative air humidity range of 69.0–78.7% between populations (Table 1).

Phenotypic differentiation in light-related traits

Significant phenotypic variation was found in all morpho-functional traits throughout the archipelago. Nested

ANOVA showed that these traits were significantly different between islands (SLA and leaf angle) or between populations of the same island (leaf size) (Table 2; Fig. 2). At the island scale, populations on La Palma displayed a higher SLA ($F_{2,3} = 10.64$, $P < 0.05$) and more horizontal leaf angles ($F_{2,3} = 40.47$, $P < 0.001$) than populations on the other islands, whereas leaf size varied within the islands of La Gomera and Tenerife (Fig. 2). In contrast, none of the pigment pools showed significant differences between or within islands (Table 2).

When mean phenotypic values were correlated with radiation estimates, we found that two of the morpho-functional variables were significantly related to diffuse light. Thus, leaf angle and SLA, but not leaf size, were positively correlated with diffuse radiation at the population level (Fig. 3). These results were also supported at the individual level ($n = 72$), and diffuse radiation correlated with SLA ($R = 0.35$, $P < 0.01$) and leaf angle ($R = -0.54$, $P < 0.001$). On the contrary, none of the other microclimatic variables (direct radiation, soil water content, air humidity and air temperature) showed a significant association with any of the morpho-functional traits ($P > 0.05$ for all pairwise comparisons).

Heritability of light-related traits

Significant actual variation of relatedness was found in all the study populations (ESM, online resource 2), allowing estimation of reliable trait heritabilities. The pattern of variation in trait heritability was again markedly different between morpho-functional and physiological traits. Morpho-functional traits displayed significant genetic variation in most of the populations, although heritability estimates for leaf size and leaf angle were not different from zero in some populations (Table 3). In contrast, none of the heritability estimates for pigment pools were significantly greater than zero.

Heritability estimates did not support the hypothesis that time since colonization was related to genetic variation in traits. For instance, the most recently-established populations (FA and SJ, less than 20,000 years; Fig. 1) displayed

Table 2 F ratios from the nested ANOVA used to analyze the differences between islands and between populations of the same island in the six study traits

Source of variation	Leaf size	SLA	Leaf angle	Chl a/b	VAZ/Chl	β carot/Chl
Island ^a	0.86	10.64*	40.47***	1.00	0.32	2.37
Population (Island) ^b	11.03***	1.08	0.54	1.20	0.62	1.21

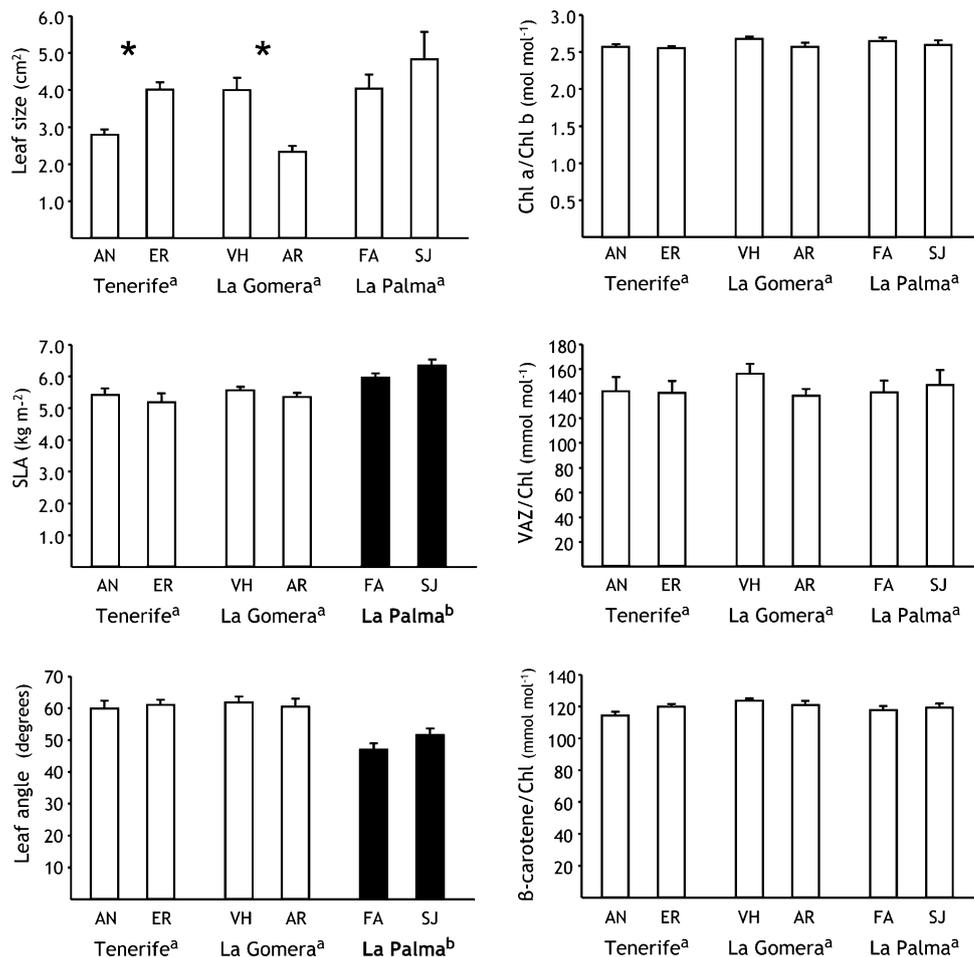
* $P < 0.05$

*** $P < 0.001$

^a Degrees of freedom = 2,3

^b Degrees of freedom = 3,66

Fig. 2 Phenotypic values (mean + 1SE) for morpho-functional (*left panel*) and physiological (*right panel*) leaf traits measured in six populations of *O. e. guanchica*. Significant differences according to Tukey post-hoc tests are represented with a different bar colour and letter (between islands) and an asterisk (between populations within islands). AN Anaga; ER El Río; VH Vallehermoso; AR Arure; FA Finca Amado; SJ San José



the highest mean heritability (0.23 and 0.24; Table 2), while a putatively old population (AN, 6–8 My; Fig. 1) displayed the lowest mean heritability (0.06). Potential time since population colonization and mean heritabilities were not significantly correlated ($n = 6$; $R = -0.58$, $P = 0.23$).

Discussion

Phenotypic variation in contrasting insular environments

We found significant phenotypic differences among *O. e. guanchica* populations occurring on the western Canary Islands. We only detected significant differences, however, in morpho-functional traits (leaf size, SLA and leaf angle) whereas physiological traits (Chl a/b ratio, VAZ/Chl and β -carotene/Chl) were similar among all study populations. Phenotypic differentiation in morpho-functional traits was observed particularly between La Palma and the other two islands, and such variation was in agreement with substantial differences in environmental factors.

Populations on La Palma received a higher amount of diffuse light and presented higher soil water content, which were associated with an increase in SLA and more horizontal leaf angles in the outer canopy layers of plants. We hypothesize that environmental variation across the archipelago may lead to the pattern of phenotypic expression observed in morpho-functional traits. For instance, the positive correlation found between SLA and diffuse light supported the observations of enhanced plant performance under diffuse radiation (Healy et al. 1998; Rocha et al. 2004). Our study, however, could not unambiguously detect the abiotic factors involved in the phenotypic adjustment of populations, as plants respond simultaneously to multi-environmental cues (e.g., Rubio de Casas et al. 2007). Nevertheless, our study strongly supports the idea that different types of traits showed contrasting levels of variation across populations representing marked microclimatic gradients.

With respect to the physiological traits, both Chl a/b ratios and photoprotective pools are expected to increase as plants experience higher irradiance levels (Niinemets et al. 1998; Kitajima and Hogan 2003). In our study, however,

Fig. 3 Bivariate plots (mean \pm 1SE) of leaf traits showing significant differences among populations and the amount of diffuse radiation experienced by each population. *Solid lines* represent the regression line and *dotted lines* the 95% confidence intervals

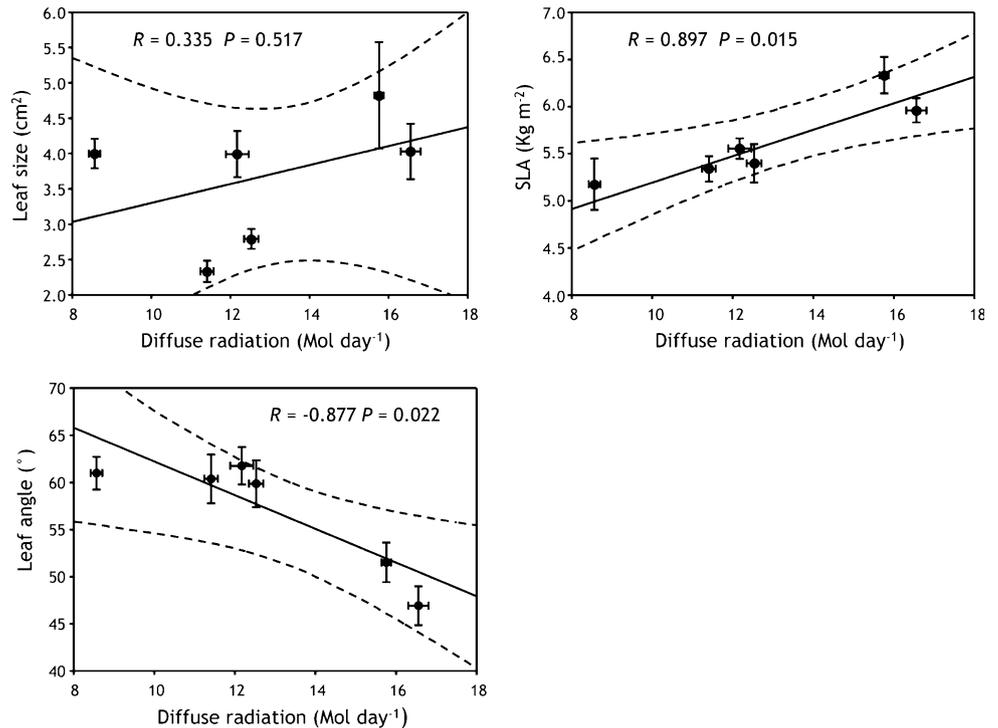


Table 3 Estimates of heritability for each study population and trait, following Ritland’s method (2000)

Population	Leaf size	SLA	Leaf angle	Chl a/b	VAZ/Chl	β carot/Chl	Average h^{2a}
AN	-0.11	0.15*	0.17*	0.03 ns	-0.02	-0.05	0.06
ER	-0.27	0.73*	0.22*	-1.04	-0.47	0.05 ns	0.17
VH	0.73*	0.61*	-0.18	-0.02	-0.11	-0.31	0.22
AR	0.52*	0.38*	-0.20	-0.42	-0.59	-0.06	0.15
FA	0.38*	0.61*	0.37*	-0.76	-0.05	-0.97	0.23
SJ	-0.09	1.06*	0.20*	-0.06	0.05 ns	0.11 ns	0.24

AN Anaga; ER El Río; VH Vallehermoso; AR Arure; FA Finca Amado; SJ San José

* $P < 0.05$; ns traits with positive estimates, but non-significantly different from zero according to the bootstrap procedure

^a Considering negative heritabilities as zero

we did not find significant differences among populations in any of these traits. One possible explanation for this phenotypic similarity in pigment pools is that populations did not experience sufficient variation in light levels to trigger differential responses. Nevertheless, we recorded a variation among populations of approximately 40% in diffuse radiation, and direct radiation was also remarkably variable between some of these (e.g., VH vs SJ; Table 1). Phenotypic similarity in these traits is in agreement with other observations in which sizes in photoprotective pools were conserved among several Mediterranean species (Martínez-Ferri et al. 2000), and those in which plants grown under contrasting sunlight regimes did not show significant differences in certain pools, such as the Chl a/b ratio (Valladares et al. 2000; Mendes et al. 2001). Lack of phenotypic differences between populations in pigment

pools, however, does not imply a fixed response to light in these traits. Substantial variation in pigment pools within the same individual (sun vs shade leaves) is well-documented in Mediterranean plant species, and reflects the potential to light acclimation through high phenotypic plasticity (Valladares et al. 2000; Rubio de Casas et al. 2007; García-Plazaola et al. 2008).

Heritable variation in traits and recent colonization

In agreement with results on phenotypic expression, heritability estimates in our study showed a marked difference between types of traits. Thus, significant heritabilities were detected for all morpho-functional traits in most populations, whereas none of the physiological traits showed significant heritabilities for any population. Phenotypic

plasticity has been advocated as the factor responsible for null or negative heritability estimates in some studies (Maddox and Root 1987; Culley et al. 2006), and leaf-level physiological traits are linked to high plasticity and low heritability values (Geber and Griffen 2003). Another explanation is that *O. e. guanchica* ancestors experienced past episodes of strong selection on pigment pools (Esteban et al. 2009), and consequently, additive variance was depleted (Fisher 1999). Similar sizes in pigment pools have been documented among phylogenetically divergent Mediterranean species (Martínez-Ferri et al. 2000) and may suggest that Mediterranean-type ecosystems have exerted considerable selection on pigment pools.

Heritability estimates at the population level did not support the hypothesis that recent colonization events have led to reduced genetic variability in quantitative traits, as suggested in other empirical (Whitlock and Fowler 1999) and theoretical (Mitchell-Olds et al. 2007) studies. When pigment pools were excluded, for which heritabilities were not significant at any study site, the more recently established populations (SJ, FA, ER) did not show lower heritabilities than presumably older ones. Extensive gene flow among populations in this wind-pollinated, bird-dispersed plant species may have efficiently promoted genetic connectivity across the archipelago (García-Verdugo et al. 2010), allowing the maintenance of heritable variation even in recent populations.

Contrasting patterns of variation in morpho-functional versus physiological traits

One of the most remarkable results of this study was that those traits showing significant phenotypic variation among populations also presented significant heritabilities (morpho-functional traits), whereas similar phenotypic expression under contrasting field conditions was accompanied by a lack of heritable variation (pigment traits). Because Ritland's method for heritability estimation is based on phenotypic variance among individuals (Ritland 2000), one suspects that the lack of heritability is the consequence of negligible within-population variation in those traits. However, variation among individuals was similar or even higher in some pigment pools than that of morpho-functional traits (within-population range of CVs: Chl a/b = 5–8%, VAZ/Chl = 13–25%, β -carotene/Chl = 4–8%, leaf size = 16–52%, SLA = 6–17%, leaf angle = 9–15%). Rather than a computational artifact, this result appears to relate the patterns of phenotypic expression and genetic variance in light-related traits (Whitlock and Fowler 1999). Estimates of heritability obtained with this method should, however, be interpreted with caution. One of the advantages of Ritland's method is that individuals can be measured under field conditions which, however, implies a higher

variability in trait expression, increasing experimental error. Large sample sizes for individual characterization may solve this problem, although other sources of variation (e.g., seasonality; Klaper et al. 2001) should be considered when interpreting these estimates in highly plastic traits.

Conclusions

Plant populations of *O. e. guanchica* showed contrasting patterns of both phenotypic and genetic variation between morpho-functional and physiological traits. The present study cannot identify the causes behind these patterns, but our data suggest that variation in morpho-functional traits might be related to environmental variation in light levels across islands. Further research is needed to address whether a lack of heritable variation in pigment traits is linked to high phenotypic plasticity and strong patterns of selection in Mediterranean environments, and to investigate the potential consequences of null heritabilities in these physiological traits.

Acknowledgments We wish to thank A Justo, MI Blanch Matute, R Domínguez, C Granado, R Rubio, S Valea, GM García, E Manrique, E Torres, K Carrillo, P Vargas and E Pérez-Corona for their valuable assistance during the experimental design, field work and sample processing. We are also indebted to Prof. MS Jiménez and D. Morales (University of La Laguna, Tenerife, Spain) for providing equipment and continued support for this research. We sincerely acknowledge the numerous comments of three anonymous reviewers that significantly improved the manuscript. Special thanks to Mr. Cormac de Brun for revision of the English. This research was funded by the Spanish Ministerio de Educación y Ciencia (project CGL2005-03062/BOS: Procesos y mecanismos evolutivos en *Olea europaea* L.), by the Comunidad de Madrid (project REMEDINAL, S-0505/AMB/0335) and by an FPU grant to C García-Verdugo (AP-2004-3448).

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