New Phytologist

Research

Plasticity, instability and canalization: is the phenotypic variation in seedlings of sclerophyll oaks consistent with the environmental unpredictability of Mediterranean ecosystems?

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Received: *12 June 2002*
Accepted: *27 August 2002*

Summary

- Evergreen oaks from the Mediterranean basin exhibit a conservative resource-use strategy based on a reduced expression of phenotypic variation (i.e. canalization). We hypothesized that genetic variation across closely related species is more canalized than the response to environmental variation.
- Seedlings of *Quercus ilex* and *Q. coccifera*, two important oak species from the Mediterranean basin that belong to the same subgenus and section, were grown in contrasted light and nutrient environments following a factorial design. Phenotypic variation was explored in a total of 75 variables including photosynthetic capacity, nutrient allocation, allometric relationships and crown architecture.
- Path analysis showed that phenotypic variation was not significantly affected by differences between species but by those between and within environments, which are argued to be primarily linked to phenotypic plasticity and developmental instability, respectively. This finding is interpreted as evidence of genetic canalization across species.
- The similar importance of plasticity and instability as sources of phenotypic variation and the high degree of genetic canalization are consistent with the expected role of the environmental unpredictability of Mediterranean ecosystems in shaping the developmental patterns of these two species.

Key words: developmental instability, genetic canalization, Mediterranean Basin, nutrient availability, phenotypic plasticity, *Quercus coccifera* (kermes oak), *Quercus ilex* (holm oak), sun and shade.

Introduction

The Mediterranean climate is very unpredictable, which can be seen in its high interannual variability (Cowling *et al.*, 1996; Rodó & Comín, 2001; Valladares & Pearcy, 2002). Vegetation patterns in the Mediterranean basin exhibit a high spatiotemporal heterogeneity from small (less than 1 m^2, Lavorel *et al.*, 1994) to local and regional scales (Shoshany, 2000), which is experienced by plants as a high temporal and spatial unpredictability of environmental conditions (Baldocchi & Collineau, 1994). Besides, ecosystems in the Mediterranean basin are prone to experience a concatenation of stochastic disturbances, including fire, clearing, grazing and land use change (Walter, 1973; Terradas, 2001). These disturbances, particularly fire, have long been recognised as determinant ecological and evolutionary factors (Naveh, 1975; Keeley, 1991; Terradas, 2001). Mediterranean plants are thus exposed to unpredictable changes in light, water and nutrient availability, but we are still far from understanding the extent to which these unpredictable environmental cues can elicit an adaptive response.

Recent revisions of evolution under fluctuating selection have raised a challenging hypothesis on the significance of phenotypic variation in environments prone to change unpredictably. Rapidly fluctuating selection would favour a reduced phenotypic expression of genetic variance, that is a genetic canalization (Kawecki, 2000). Genetic canalization is thought to ensure phenotypic constancy within and across populations (Debat & David, 2001) and across closely related species (Badyaev & Foresman, 2000). However, genetic canalization does not exclude the capacity of a given genotype to produce different phenotypes in response to the environment (i.e. phenotypic plasticity). In fact, phenotypic plasticity enables different genotypes to assume a single phenotype (Bradshaw, 1965). Simultaneous promotion of both plasticity and genetic canalization would result in canalized reaction norms (Debat & David, 2001).

Under these predictions, a phenotypic homeostasis based on genetic canalization and phenotypic plasticity would be adaptive in the unpredictable environments experienced by Mediterranean plants. This hypothesis is consistent with recent pieces of evidence on the ecology and evolution of Mediterranean plants. The apparent lack of selection on genetic variation observed in some Mediterranean species (Farley & McNeilly, 2000) could be explained by a high genetic canalization that would reduce the heritable phenotypic variation available to natural selection (Badyaev & Foresman, 2000). It would also be explained by a high degree of phenotypic plasticity that would obscure selective differences among genotypes (Sultan, 1996; Balaguer *et al.*, 2001). This phenotypic homeostasis is also consistent with the conservative resource-use strategy reported for Mediterranean evergreen oaks (Valladares *et al.*, 2000a). This strategy was argued to involve a lower degree of plasticity than that observed in evergreen shrubs from tropical habitats (Valladares *et al.*, 2000a, 2000b). However, this comparison between habitats did not contribute to determine the relative importance of genetic canalization and phenotypic plasticity in Mediterranean plants.

This paper tackles the question of whether the patterns of phenotypic variation in Mediterranean plants are compatible with a combination of genetic canalization and phenotypic plasticity. An affirmative answer to this question is a necessary, but not sufficient, condition to validate the proposed role of environmental unpredictability in Mediterranean ecosystems. Thus, we hypothesize that the phenotypic variability due to genetic variation across closely related species is insignificant compared with that elicited by environmental cues, such as light and nutrient availability. The species chosen are two important evergreen oaks from the Mediterranean basin, *Quercus coccifera* L. and *Q. ilex* ssp. *ballota* (Desf.) Samp., which provide a good system to validate these hypotheses experimentally. First, they belong to the same subgenus (*Quercus*) and the same section (*Cerris*), and their genetic and ecological proximity frequently allow hybridization and introgression between them (Nixon, 1993). Second, their longevity (well above 100 yr) and tolerance to stress and disturbance (Martínez-Ferri *et al.*, 2000; Peñuelas *et al.*, 2001) suggest an adaptation to variable environments primarily by means of physiological and morphological plasticity (Sultan, 1987). The results of Valladares *et al.* (2000a) for 37 variables are re-appraised and combined with data on 38 new variables to gain robustness in the dissection of the factors affecting phenotypic variation in these two Mediterranean oaks. Leaf vs whole-plant responses were compared with test whether phenotypic plasticity is independent from the level of organisation as proposed by Robinson & Rorison (1988) and Balaguer *et al.* (2001).

Materials and Methods

Plant material and experimental design

Acorns of *Quercus ilex* ssp. *ballota* (Desf.) Samp. and *Quercus coccifera* L. were planted in February 1996 in a nursery in the vicinities of Torremocha del Jarama (Madrid, Spain). The acorns were collected in autumn 1995; those from *Q. ilex* were collected in Valle del Tietar (Toledo, Spain) and those of *Q. coccifera* in Enguera (Valencia, Spain). The areas of origin of the acorns and the location of the nursery share a Mediterranean-type climate with a dry and hot summer and a cold winter; precipitation is mostly in autumn and spring. Following germination and initial growth, seedlings were placed in 15-l pots filled with washed river sand. A factorial experiment of three factors (species, light and nutrient availability) of two levels each was designed to test for main effects and interactions on 36 morphological and physiological variables. Four to six plants from each species-light-nutrient treatment combination were selected at random for the different measurements. A metal frame with several layers of neutral shade cloth was placed over half of the plants to produce a low light environment (shade); the other half of the plants were kept outdoors (sun environment). Plants in the shade enclosure had five times less daily photosynthetic photon flux density (PFD) available than plants in the sun (e.g. 9.1 vs 47.4 mol m^2 d^{-1} PFD during the period of measurements in August 1998). Plants were watered daily and air temperature did not significantly differ between the two light treatments. The influence of nutrient availability was studied by means of slow release nutrient pellets provided to half of either sun and shade plants (nutrient rich treatment) while the other half of the sun and shade plants was grown in the sandy soil (nutrient poor treatment). 3.1 kg of Plantacote Mix 4 M (15/17/15 N P^{-1} K^{-1}) plus 4.4 kg of Guanumus Angibaud (3/35/2 N P^{-1} K^{-1}) per m^3 of sand was provided to the plants of the nutrient rich treatment. At the end of the experiment (August 1998), plants were harvested and separated into leaves, stems and roots for the different structural and nutrient analyses. Leaves, stems and roots were

Table 1 Morphological variables for the two evergreen oaks: shoot height (cm), shoot volume (cm^3), shoot weight (g), root weight (g), total leaf area (cm^2), leaf area ratio (LAR, $m^2\ kg^{-1}$), leaf weight ratio (LWR, $g\ g^{-1}$), internode length (mm), shoot height per supporting biomass ($mm\ g^{-1}$), root-shoot ratio, and supporting biomass (% of shoot weight). Data are mean of four plants. Letter code indicates significant differences (ANOVA Tukey test, $P < 0.01$) between the levels of each treatment (lower case for light treatment, upper case for nutrient treatment). Significant interaction ($P < 0.05$) between species and nutrient treatment was found for root weight. NP = nutrient poor, NR = nutrient rich. Numbers within parentheses are the code used in Fig. 1.

	Q. ilex		*Q. coccifera*		*Q. ilex*		*Q. coccifera*	
Variable	Sun	Shade	Sun	Shade	NR	NP	NR	NP
(1) Shoot height	32.9^{a}	28.1^{a}	19.9^{b}	19.6^{b}	$34.4^{A.}$	$26.6^{B.}$	$22.8^{C.}$	$16.7^{D.}$
(2) Shoot volume	3606^{a}	$2300^{a,b}$	1566^{b}	973^{c}	$4243^{A.}$	$1664^{B.}$	$1857^{B.}$	$681^{C.}$
(3) Shoot height per supporting biomass	73.8^{a}	136.1^{b}	99.0^{a}	190.7^{b}	$74.3^{A.}$	$135.6^{A.}$	$136.6^{A.}$	$153.0^{A.}$
(4) Supporting biomass	0.31^{a}	0.29^{a}	0.31^{a}	0.27^{a}	$0.31^{A.}$	$0.29^{A.}$	$0.30^{A.}$	$0.28^{A.}$
(5) Shoot weight	18.9^{a}	8.6^{b}	8.2^{b}	4.1^{c}	$19.4^{A.}$	$8.2^{B.}$	$8.4^{B.}$	$4.0^{C.}$
(6) Root weight	15.5^{a}	9.8^{b}	8.7^{b}	5.6^{c}	$14.4^{A.}$	$10.9^{B.}$	$7.3^{C.}$	$6.9^{C.}$
(7) Root-shoot ratio	0.89^{a}	1.07^{a}	1.09^{a}	1.20^{a}	$0.76^{A.}$	$1.34^{B.}$	$0.90^{A.}$	$1.58^{B.}$
(8) Total leaf area	542.3^{a}	429.1^{a}	295.7^{a}	319.8^{a}	$693.6^{A.}$	$277.8^{B.}$	$415.6^{B.}$	$199.8^{C.}$
(9) LAR	1.43^{a}	2.36^{b}	$1.61^{a,b}$	3.47^{c}	$2.23^{A.}$	$1.55^{B.}$	$3.15^{C.}$	$1.93^{A.,B.}$
(10) LWR	0.35^{a}	0.33^{a}	0.31^{a}	0.32^{a}	$0.38^{A.}$	$0.30^{A.}$	$0.36^{A.}$	$0.27^{A.}$
(11) Internode length	4.0^{a}	9.2^{b}	3.1^{c}	5.4^{d}	$7.0^{A.}$	$6.3^{A.}$	$5.2^{A.}$	$3.3^{A.}$

finely ground and dried for 48 h at 65°C. Nutrient analyses (N, P, K content) were carried out at the Unit of Analysis of the Centre of Environmental Sciences (CSIC) Spain. More details of the experimental conditions and on leaf-level variables can be found in Valladares *et al.* (2000a).

Crown light capture and gas exchange

Photosynthetic response to irradiance was measured in one fully expanded current-year leaf of three plants per species per treatment during August 1998 with a portable open gas exchange system (ADC3, Analytical Development Co., Hoddesdon, UK) as described in Valladares *et al.* (2000a). Photosynthetic and respiration rates plus all the parameters of the photosynthetic response of single leaves to irradiance were used to scale up to the whole plant by means of the 3-D plant architecture model Y-plant (Pearcy & Yang, 1996). Y-plant was used to simulate light interception, carbon gain, and transpiration by the crown of the two evergreen oaks. Y-plant was shown to predict accurately the measured frequency distribution of PFD on the leaves of the simulated plants, and it provides simulations for selected individual leaves as well as for the whole shoot (Valladares & Pearcy, 1998). Y-plant has been revised to include energy balance simulations for each leaf, which then gives the transpiration rates and leaf temperatures (Pearcy & Valladares, 1999). Whole crown carbon gain was used in the calculations of photosynthetic nitrogen use efficiency (NUE, daily carbon gain divided by total plant nitrogen) and water use efficiency (WUE, daily carbon gain divided by daily transpiration). The crown of three individual plants per species and treatment (a total of $3 \times 2 \times 2 \times 2 = 24$ plants chosen at random) were measured and reconstructed in 3-D with Y-plant.

Estimators of phenotypic variation

Phenotypic variation for each of the 38 variables studied (Tables 1–3) was estimated by the coefficient of variation, which has been frequently used for this purpose (e.g. Hamdford, 1980). Phenotypic variation includes between-environment, between-species and within-environment variation. An index of between-environment variation ranging from 0 to 1 was calculated for each variable and species as the difference between the minimum and the maximum mean values among the two levels of each treatment divided by the maximum mean value. Its standardized form allows comparisons across variables expressed in different units and with contrasting variation ranges. The index was calculated for plant response to PFD and to nutrients independently. This index has been previously called plasticity index (Valladares *et al.*, 2000a, 2000b; Balaguer *et al.*, 2001) and was used to assess phenotypic variation induced by environmental factors in the same fashion as other similar indices (Robinson & Rorison, 1988; Schmid, 1992; Pigliucci *et al.*, 1999; Richardson *et al.*, 2001). Whether this index is a good estimator of phenotypic plasticity is left for the discussion of the results, and for this reason the index is referred to in the text, tables and figures simply as an index of between-environment variation. The same reasoning was followed to calculate indices of phenotypic variation within environments and between species, but in these cases the maximum and minimum values were taken among individuals of the same species within the same environment, and among mean values of different species within the same environment, respectively. Values for these latter indices were obtained independently for the sun and the shade, and the nutrient rich and nutrient poor environments.

Table 2 Nitrogen, phosphorus and potassium content of leaves, stems and roots (expressed as a fraction of the total), and of the whole plant (in mg g^{-1}) and daily nitrogen use efficiency per plant (NUE, mmol CO_2 g^{-1} leaf N day^{-1}) of the two evergreen oaks in the different treatments. Data are mean of six independent samples. Letter code indicates significant differences (ANOVA Tukey test, $P < 0.01$) between the levels of each treatment (lower case for light treatment, upper case for nutrient treatment). Significant interactions ($P < 0.05$) between species and light treatment were found for NUE, and between species and nutrient treatment were found for P-plant. NP = nutrient poor, NR = nutrient rich. Numbers within parentheses are the code used in Fig. 1.

	Q. ilex		*Q. coccifera*		*Q. ilex*		*Q. coccifera*	
Variable	Sun	Shade	Sun	Shade	NR	NP	NR	NP
(12) N-leaves	0.42^{a}	0.45^{a}	0.45^{a}	0.44^{a}	$0.45^{A.}$	$0.42^{A.}$	$0.4^{A.}$	$0.43^{A.}$
(13) N-stems	0.14^{a}	0.12^{a}	0.12^{a}	0.10^{a}	$0.15^{A.}$	$0.11^{A.}$	$0.12^{A.}$	$0.11^{A.}$
(14) N-roots	0.44^{a}	0.42^{a}	0.47^{a}	0.46^{a}	$0.40^{A.}$	$0.46^{A.}$	$0.48^{A.}$	$0.46^{A.}$
(15) N-plant	9.9^{a}	8.6^{a}	8.3^{a}	8.4^{a}	$11.0^{A.}$	$7.5^{B.}$	$10.2^{A.}$	$6.5^{B.}$
(16) P-leaves	0.30^{a}	0.29^{a}	0.21^{a}	0.25^{a}	$0.33^{A.}$	$0.27^{A.,B.}$	$0.26^{A.,B.}$	$0.21^{B.}$
(17) P-stems	0.17^{a}	0.15^{a}	0.12^{a}	0.12^{a}	$0.18^{A.}$	$0.14^{B.}$	$0.15^{B.}$	$0.09^{C.}$
(18) P-roots	0.53^{a}	0.55^{a}	0.67^{b}	$0.64^{a,b}$	$0.49^{A.}$	$0.59^{B.}$	$0.60^{B.}$	$0.71^{C.}$
(19) P-plant	0.79^{a}	1.05^{a}	1.79^{b}	2.59^{c}	$0.95^{A.}$	$0.93^{A.}$	$2.56^{B.}$	$1.82^{C.}$
(20) K-leaves	0.36^{a}	0.36^{a}	0.31^{a}	0.29^{a}	$0.40^{A.}$	$0.32^{A.}$	$0.33^{A.}$	$0.27^{A.}$
(21) K-stems	0.2^{a}	0.14^{a}	0.16^{a}	0.16^{a}	$0.21^{A.}$	$0.13^{B.}$	$0.19^{A.}$	$0.13^{B.}$
(22) K-roots	0.44^{a}	0.5^{a}	0.53^{a}	0.55^{a}	$0.39^{A.}$	$0.55^{B.,C.}$	$0.48^{B.,A.}$	$0.6^{C.}$
(23) K-plants	4.83^{a}	4.58^{a}	5.28^{a}	5.00^{a}	$5.15^{A.}$	$4.27^{B.}$	$5.5^{A.}$	$4.78^{B.}$
(24) NUE	22.9^{a}	17.7^{b}	25.9^{a}	33.1^{c}	$18.5^{A.}$	$25.4^{B.}$	$27.5^{B.}$	$31.1^{C.}$

Table 3 Displayed and self-shaded area (DA and SSA, respectively, expressed as fraction of foliage area) for high and low elevation angles of the sun, daily PFD absorption (both in mol photons m^{-2} d^{-1} and as percentage of a horizontal surface), carbon gain (in mmol CO_2 m^{-2} d^{-1}, in mmol CO_2 $plant^{-1}$ day^{-1}, and as percentage of an equivalent horizontal surface), transpiration (both in mol H_2O m^{-2} d^{-1} and as percentage of an equivalent horizontal surface), water use efficiency (both in mmol CO_2 mol^{-1} H_2O and as percentage of an equivalent horizontal surface) and nitrogen use efficiency at the whole shoot level for the two evergreen oaks. Variation among individual leaves within the shoot in daily assimilation is presented as the coefficient of variation (standard deviation divided by the mean of all leaves of the crown). Data are mean of four plants. Letter code indicates significant differences (ANOVA Tukey test, $P < 0.01$) between the levels of each treatment (lower case for light treatment, upper case for nutrient treatment). A significant light × nutrient–species interaction ($P < 0.05$) was found for DA and SS for high angles. NP = nutrient poor, NR = nutrient rich. Numbers within parentheses are the code used in Fig. 1.

	Q. ilex		*Q. coccifera*		*Q. ilex*		*Q. coccifera*	
Variable	Sun	Shade	Sun	Shade	NR	NP	NR	NP
(25) DA for high angles	0.31^{a}	0.44^{b}	0.35^{a}	0.44^{b}	$0.35^{A.}$	$0.39^{A.}$	$0.41^{A.}$	$0.38^{A.}$
(26) DA for low angles	0.25^{a}	0.30^{b}	0.25^{a}	0.30^{b}	$0.26^{A.}$	$0.28^{A.}$	$0.27^{A.}$	$0.28^{A.}$
(27) SSA for high angles	0.37^{a}	0.34^{a}	0.40^{a}	0.35^{a}	$0.35^{A.}$	$0.35^{A.}$	$0.36^{A.}$	$0.39^{A.}$
(28) SSA for low angles	0.20^{a}	0.12^{b}	0.19^{a}	0.11^{b}	$0.17^{A.}$	$0.16^{A.}$	$0.15^{A.}$	$0.15^{A.}$
(29) PFD absorbed	21.3^{a}	6.4^{b}	22.7^{a}	6.2^{b}	$13.4^{A.}$	$14.2^{B.}$	$14.4^{B.}$	$14.9^{C.}$
(30) PFD absorbed (%)	49^{a}	62^{b}	48^{a}	63^{b}	$54^{A.}$	$57^{A.}$	$55^{A.}$	$57^{A.}$
(31) Carbon gain-area	132.6^{a}	58.3^{b}	104.7^{c}	57.2^{b}	$86.3^{A.}$	$105.4^{B.}$	$71.5^{C.}$	$92.3^{D.}$
(32) Carbon gain-plant	7.19^{a}	2.50^{b}	3.09^{b}	1.83^{c}	$6.00^{A.}$	$2.93^{B.}$	$2.97^{B.}$	$1.84^{C.}$
(33) Carbon gain (%)	58^{a}	44^{b}	72^{c}	54^{a}	$50^{A.}$	$51^{A.}$	$60^{B.}$	$63^{B.}$
(34) Transpiration-area	42.2^{a}	13.1^{b}	32.8^{c}	12.7^{b}	$27.3^{A.}$	$28.0^{A.}$	$22.4^{B.}$	$23.1^{B.}$
(35) Transpiration (%)	58^{a}	53^{b}	63^{c}	57^{a}	$56^{A.}$	$55^{A.}$	$60^{B.}$	$61^{B.}$
(36) WUE-area	2.9^{a}	3.7^{b}	2.7^{a}	3.5^{b}	$3.4^{A.}$	$3.2^{A.}$	$3.1^{A.}$	$3.1^{A.}$
(37) WUE (%)	105^{a}	71^{b}	112^{c}	72^{b}	$89^{A.}$	$86^{A.}$	$92^{B.}$	$92^{B.}$
(38) Variation in assim	0.53^{a}	1.29^{b}	0.44^{c}	0.98^{d}	$0.86^{A.}$	$0.95^{A.}$	$0.75^{B.}$	$0.68^{B.}$

Statistics

Three-way analysis of variance (ANOVA, Tukey test, SYSTAT 6.0 Windows version 1996, SPSS Inc. Chicago IL, USA) was used to test for differences among species, PFD and nutrient treatments, and interactions. Number of plants pooled together for the analysis of the effect of each treatment was eight (for variables in Tables 1 and 3) or 12 (for variables in Table 2) per species. In all cases, the data met the assumptions of normality and homoscedasticity. A simplified format to present the results (i.e. only means and significant differences are shown, see Tables 1–3) was chosen due to the

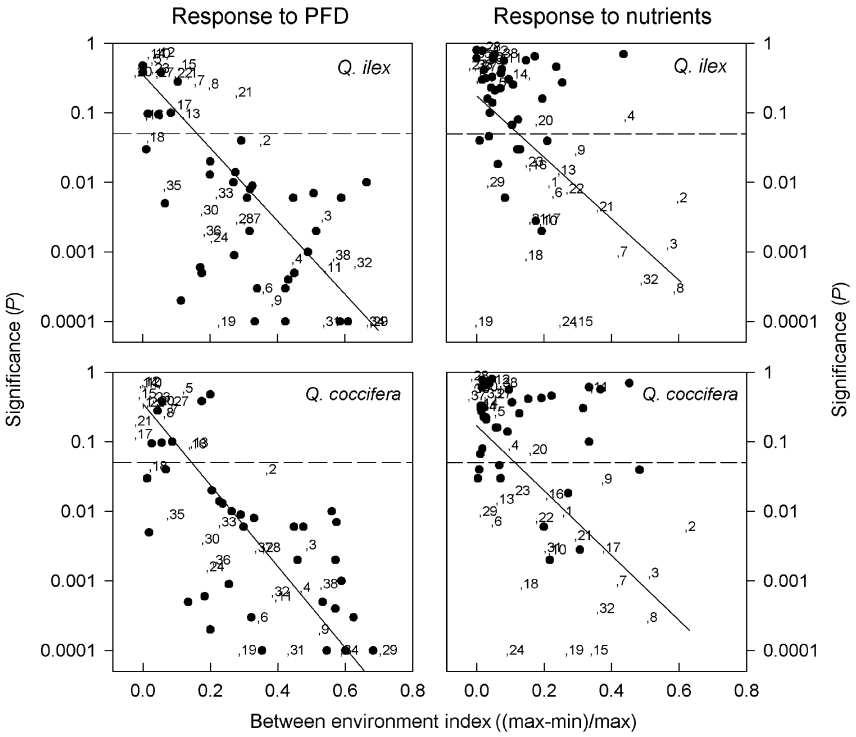

Fig. 1 Between-environment index ((max-min)/max) against the significance of the treatment term (P from the ANOVA) in *Quercus ilex* (upper graphs) and *Q. coccifera* (lower graphs) in response to PFD (left hand graphs) and nutrient availability (right hand graphs) for the 38 variables examined in the present study (numbers, which are given in Tables 1–3) plus the 37 variables examined in Valladares *et al*. (2000a) (dots). Dashed lines indicate the significance threshold of $P = 0.05$. The coefficient of determination (the square of the correlation coefficient) values are as follows: *Q. ilex* response to PFD $r^2 = 0.66$ ($P < 0.001$), response to nutrients $r^2 = 0.37$ ($P < 0.01$), *Q. coccifera* response to PFD $r^2 = 0.76$ ($P < 0.0001$), response to nutrients $r^2 = 0.39$ ($P < 0.001$).

large number of variables and factors. Since many of the variables studied are size dependent and differences among the plants in different treatments can be due to ontogenetic differences at the time of comparison and not to the treatment itself (McConnaughay & Coleman, 1999), plant size (= biomass) was used as a covariate in the analyses. Linear regression was used to explore the relationship between plasticity index and significance of the mean differences (Fig. 1).

Following the procedures of path analysis as described by Mitchell (1993), we analysed the dependence of phenotypic variation on between-environment, between-species and within-environment variation. Path analysis is a more general form of multiple regression that allows consideration of complicated causal schemes and that can be used when independent variables are not truly independent or are correlated. We deliberately selected a simplified approach to the path diagram process based on basic principles on phenotypic plasticity (Schlichting & Pigliucci, 1998). Since our path diagram had five variables our sample size should be 50–100 (Mitchell, 1993). In our case the observations were each of the morphological and physiological variables examined so our sample size was 75 (38 variables of the present study plus 37 variables of Valladares *et al.*, 2000a). In path analysis, the thickness of the arrow in the diagram is proportional to the path value and represents the relative strength of a given relationship. Path values are derived from standardized partial regression coefficients so path values can be quantitatively compared. While other paths may also be feasible, our intent was not to explore the relative goodness-of-fit of different models but to quantitatively compare the relative influence of between-environment, between-species and within-environment variation in the phenotypic variation observed. In addition to direct effects, we used path analysis to calculate the strengths of the indirect influences of a given factor on another as described by Mitchell (1993). In our path diagram, species differences had both direct and indirect effects on total phenotypic variation since species could differ either in phenotypic plasticity (between environment) and developmental stability (within environment), both of which in turn affect total phenotypic variation.

Results

Plant architecture

Significant ($P < 0.01$) differences between the two oak species were found in shoot height, volume (estimated as an ellipsoid) and weight, root weight and internode length at the end of the experiment, which were larger in *Quercus ilex* than in *Q. coccifera* (Table 1). Shoots were taller, larger (in volume), and heavier in nutrient rich than in nutrient poor plants. Sun plants were larger and heavier but not taller than shade plants. Shoot height per supporting biomass (stem plus branches and petioles) was larger in the shade due to the longer internodes of shade plants in comparison with sun plants. Roots were heavier in the sun than in the shade. Root : shoot ratio was higher in the nutrient poor than in the nutrient rich

treatment, but was not affected by light. Total leaf area was not affected by light, but leaf area ratio (LAR) was larger in the shade than in the sun. Both total leaf area and LAR were larger in enriched than in nonenriched plants. Leaf weight ratio (LWR) and the fraction of the crown biomass invested in supporting structures were not affected by either light or nutrients (Table 1). Thus, allocation of above ground biomass to leaves and stems was rather constant across treatments and species, while the architecture of this biomass and the relative investment in shoots vs. roots varied significantly across treatments and species.

Nutrient content and allocation

Nitrogen (N) was mainly allocated to leaves and roots, while N allocation to stems was only 10–15% of the total plant N (Table 2). Half of the total phosphorus (P) and potassium (K) of the plant was allocated to roots, while P and K of the leaves represented one third of the total. While light treatment had no direct effect on nutrient content and allocation in the two oak species, both content and allocation were affected by nutrient availability. N allocation was constant across the nutrient treatments, but N content of the plant was the highest in nutrient rich plants (Table 2). Nutrient limitations increased allocation of P and K to roots, and decreased P and K allocation to stems and leaves in the two species. Enriched plants had more total K than nonenriched plants in the two species, but total P increased with increasing nutrient availability only in *Q. coccifera*. *Q. coccifera* had higher plant P content and allocated a larger fraction of P to roots than *Q. ilex*. Total N and K were similar in the two species. NUE was higher in *Q. coccifera* than in *Q. ilex*, but a species–light treatment interaction was found: NUE of *Q. coccifera* increased in the shade, while the reverse was true for *Q. ilex*. NUE increased with decreasing nutrient availability in the two species.

Shoot light capture and gas exchange

10–45% of the foliage was self-shaded for the different solar elevations (Table 3). Leaf elevation angle was not affected by nutrient treatment but it increased with light availability (Valladares *et al.*, 2000a), which affected light capture by the whole shoot. Significant differences among treatments were found for the fraction of the foliage that was self-shaded (SSA) and displayed (displayed area, DA = projected area – self-shaded area). Sun plants exhibited lower DAs for both high (> 45°) and low (< 45°) solar elevation angles (Table 3). The differences in DA for low elevation angles were due to increased SSA in sun vs. shade plants, while the differences in DA for high elevation angles were due to decreased projected area in sun plants. Nutrient treatment had no effect on DA or SSA for low solar elevation angles, but a significant nutrient × species–light interaction was found for DA and SSA at high solar elevation angles: nutrient rich plants of *Q. ilex* exhibited lower DA and higher SSA than their nutrient rich counterparts in the sun, while the reverse was true in the shade, and no effect was observed in *Q. coccifera*.

Sun plants absorbed more PFD per day on a leaf area basis than shade plants (Table 3). This was due to the dominant effect of the higher PFD available in the sun than in the shade. When daily absorbed PFD was expressed as a fraction of that absorbed by a horizontal surface on the same light environment, shade plants harvested 12% more total daily PFD than sun plants (Table 3). Nutrient poor plants harvested more daily PFD on an area basis than nutrient rich plants, but no effect of the nutrient treatment was observed when absorbed PFD was expressed as a fraction of that absorbed by a horizontal surface. Daily shoot carbon gain on a leaf area basis exhibited the same trend as the absorbed PFD: higher in the sun than in the shade, and higher in nutrient poor than in nutrient rich plants. However, when expressed as a fraction of the daily carbon gain that would exhibit an equivalent horizontal photosynthetic surface, the effect of light treatment was the opposite (14–18% higher carbon gain in the sun), and nutrient treatment had no effect (Table 3). Sun plants were larger than shade plants, and enriched plants were larger than nonenriched plants, so carbon gain was larger in the sun than in the shade and in enriched than in nonenriched plants when it was expressed on a per plant basis. *Q. ilex* exhibited larger carbon gain than *Q. coccifera* on a leaf area and a per plant bases, but the situation was reversed when it was expressed as a fraction of a horizontal photosynthetic surface.

Transpiration of the whole crown throughout the day was higher in the sun than in the shade (Table 3); it was higher in *Q. ilex* than in *Q. coccifera* on a leaf area basis while this difference was reversed when expressed as a fraction of an equivalent horizontal surface. Water use efficiency (WUE = carbon gain divided by transpiration) was higher in the shade than in the sun and no differences were found between the two species when expressed on a leaf area basis. However, differences in self shading and available PFD for individual leaves among different light treatments and species translated into a higher WUE in the sun than in the shade and a higher WUE in *Q. coccifera* than in *Q. ilex* when expressed as a fraction of an equivalent horizontal surface. No effect of nutrient availability was found for either transpiration or WUE.

Leaves were more evenly illuminated in the sun than in the shade, and in *Q. coccifera* than in *Q. ilex*, which translated into lower variability in daily carbon gain among leaves in the sun than in the shade, and in *Q. coccifera* than in *Q. ilex* (Table 3).

Plasticity and significance of the response to light and nutrients

In both species, the between-environment index was correlated with the significance of the light and nutrient effect

Table 4 Mean between-environment index (BEI = [max-min]/max mean values within species) of the two oak species to photosynthetic photon flux density (PFD) and nutrient treatment and percentage of the variables with significant differences between the two levels of each treatment (ANOVA, $P < 0.01$) for the 38 variables of Tables 1–3.

	Q. ilex				*Q. coccifera*			
	Response to PFD		Response to nutrients		Response to PFD		Response to nutrients	
Group of variables	BEI	Significant	BEI	Significant	BEI	Significant	BEI	Significant
Morphological variables (total of 11, Table 1)	0.30[a]	45%	0.35[a]	63%	0.28[a]	54%	0.33[a]	54%
Nutrient variables (total of 13, Table 2)	0.12[a]	8%	0.21[b]	54%	0.08[a]	15%	0.20[b]	61%
Light capture and as exchange variables (total of 14, Table 3)	0.38[a]	92%	0.09[b]	25%	0.36[a]	92%	0.08[b]	25%
Total	0.26[a]	48%	0.22[a]	47%	0.24[a]	54%	0.21[a]	47%

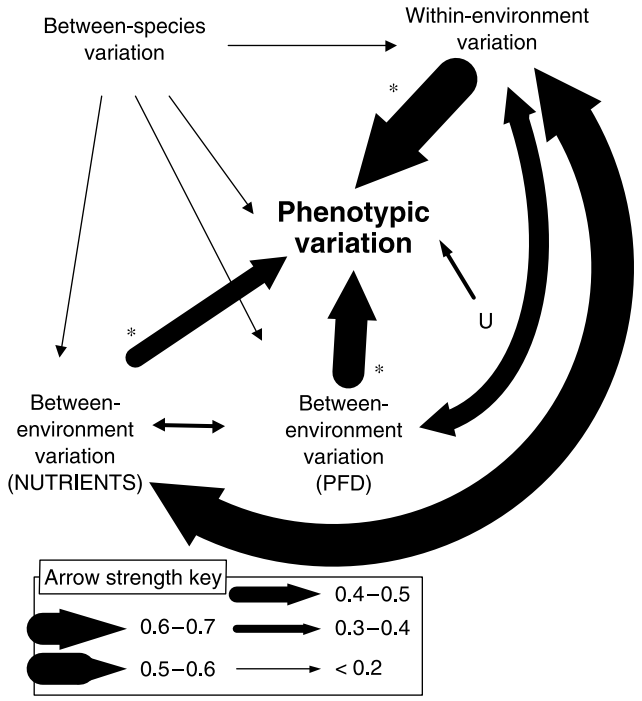

Fig. 2 Path diagram for describing the dependence of phenotypic variation (estimated by the coefficient of variation) of seedlings of *Quercus ilex* and *Q. coccifera* on differences between species (estimated by (max-min)/max mean values within environments), between nutrient and PFD environments (estimated by (max-min)/max mean values within species), and within-environment (estimated by (max-min)/max individual values within species and environments). One-headed arrows indicate dependence (paths) while two-headed arrows indicate correlation. The strength of the dependence or correlation is indicated by the arrow thickness according to the key provided. Asterisks indicate significant paths ($P < 0.05$). U represents the residual term. Total number of observations is 75 (38 variables of the present study plus 37 of Valladares *et al*. (2000a)).

from the corresponding ANOVA (Fig. 1). Treatment effects were not attributable to variations in plant size. The coefficient of determination and its significance was lower in the correlation for the response to nutrients than for that to light (Fig. 1), despite the lack of differences between the mean response to these environmental factors (Table 4). This fact was due in part to the low responsiveness of leaf-level variables (see dots in Fig. 1). Both species exhibited a similar response except in the case of the morphological response to nutrients, which was higher in *Q. ilex* than in *Q. coccifera*. In total, half of the studied variables exhibited significant differences between the two levels of both treatments (Table 4). Structural variables were equally responsive to light and nutrients, while nutrient variables exhibited a larger responsiveness to the nutrient treatment than to the light treatment, and light capture and gas exchange variables exhibited a larger responsiveness to the light treatment than to the nutrient treatment.

Phenotypic variation

Between-environment variation determined to a large extent the phenotypic variation observed among individuals from the different treatments and species, but its influence was of less importance than that of within-environment variation as indicated by the corresponding path strengths (Fig. 2). The response to nutrient availability had less influence on phenotypic variability than that to PFD. Between- and within-environment variations were positively correlated, while the response to nutrients and to PFD were only weakly correlated (Fig. 2). The similar phenotypes of the two species under the different treatments translated into a nonsignificant influence of species differences on phenotypic variation either directly or via its influence on between- and within-environment variation.

Discussion

Relative contributions of different sources of phenotypic variation

Phenotypic expression of the expected genetic differences between the two oak species did not contribute significantly to the phenotypic variation across 75 traits. These traits ranged from those at the subcellular level, such as PSII quantum yield and photosynthetic pigment composition, to those at the plant level, such as root/shoot partitioning and light absorption efficiency of the crown. This result, based on such a wide variety of traits, suggests that the two species share similar developmental patterns of individuals. This finding supports the existence of a high degree of genetic canalization in these two species (cf. Stearns *et al.*, 1995), and agrees with other studies of saplings showing a consistent plastic response to light across species (Lei & Lechowicz, 1998). Previous reports on Mediterranean woody species have also stressed the phenotypic convergence across genera and families in functional and morphological traits within a given environment (Gratani, 1997). Indirect contributions of interspecific genetic differences to phenotypic variation were not significant either, suggesting that both species share a similar degree of phenotypic plasticity and developmental stability. Genetic divergence in these species seems to result in significantly different degrees of phenotypic plasticity only when populations from extremely contrasted environments are compared (Balaguer *et al.*, 2001).

At the population level, a trait can be canalized against genetic and/or environmental variations (Debat & David, 2001). Contrary to the observed genetic canalization, the significant effects of the PFD and nutrient treatments evidence that environmental responses in the oak species studied are not environmentally canalized. In our study, phenotypic variation between environments can be attributed to intraspecific genetic variation, environmentally inducible developmental instability and phenotypic plasticity. However, considering that plant material came from a single population per species, a more significant contribution from the intrapopulation than from the interspecific genetic variation seems highly unlikely. Moreover, increasing values of the between-environment index were correlated with increasing significance of the treatment term (Fig. 1). This kind of correlation has been interpreted as a plastic response to the environment (cf. Schlichting, 1986). Therefore, the significant contribution of the between-treatment variation seems to be mainly due to phenotypic plasticity and not to a noisy bias caused by genetic heterogeneity or by an increased frequency of phenodeviants resulting from developmental instability. Thus, the index of between-treatment variation can be used as a reliable estimator of phenotypic plasticity. This index (Valladares *et al.*, 2000a, 2000b) and similar ones (Robinson & Rorison, 1988; Richardson *et al.*, 2001) have been used in this way in previous studies although the factors affecting total phenotypic variation were not explored and the relative importance of phenotypic plasticity could not be well established. Plasticity in the current study was assessed in response to contrasted but homogeneous environments, which might have underestimated the plasticity expressible in response to the temporal heterogeneity of natural habitats (Wayne & Bazzaz, 1993). However, plasticity was not overestimated due to ontogenetic drifts, since variations in plant size did not account for the effect of the light or nutrient treatment.

Unexpectedly, the greatest source of phenotypic variation was the within-environment variation. This contribution could be attributed to developmental instability and phenotypic plasticity in response to environmental heterogeneity within each treatment, assuming that the genetic source of phenotypic variation is negligible according to the above reasoning. However, plasticity could not have been a major component of within-environment variation considering that environmental variation is expected to have been much lower within than between treatments. The within-environment index was calculated using differences not between means but between individual values, what would have magnified the effects of phenodeviants. Since developmental instability has been viewed as deviations from intended phenotypes (Møller & Swaddle, 1997), the index is expected to reflect to a greater extent this source of variation. No previous report has assessed the contribution of developmental instability to local phenotypic variation in Mediterranean plant populations, although its relevance has been outlined in Mediterranean woody species in response to edaphic heterogeneity and stress (e.g. Alados *et al.*, 1999). In our study, within-environment variation was correlated with the between-environment variation which contrasts with the increasing evidence of independence between phenotypic plasticity and developmental instability as components of phenotypic variation (van Kleunen *et al.*, 2000). This correlation can reflect the expression of phenotypic plasticity in response to environmental variation within each treatment as well as the reported correlation between leaf size and leaf developmental instability (Evans & Marshall, 1996; Møller & Shykoff, 1999). However, both possibilities would have been consistent with a higher correlation between the within-environment index and the light treatment contribution, since this treatment induced a more significant response than the nutrient treatment. The higher correlation with the nutrient treatment seems to be due to the larger fraction of the variation induced but not significantly explained by this treatment (Fig. 1), which suggests a higher frequency of phenodeviants in response to this environmental factor. Alternatively, these correlations may indicate a developmental link between plasticity and developmental stability as previously reported for leaf traits in other species (Perfectti & Camacho, 1999).

Whole-plant vs leaf-level processes

The low leaf responsiveness of *Q. ilex* and *Q. coccifera* to nutrient availability found in a companion study (Valladares *et al.*, 2000a) was not paralleled by a similarly low whole plant responsiveness. When nutrient content and allocation was considered at the whole plant level, mean responsiveness to nutrient availability increased to equal responsiveness to light (Table 2). These results translated into no effect of nutrient availability on the nitrogen use efficiency (NUE) at the leaf level (Valladares *et al.*, 2000a) but a significant decrease of NUE with nutrient availability at the whole plant level (Table 2). This result contrasts with the proposed independence of phenotypic plasticity from the level of organization (Robinson & Rorison, 1988; Balaguer *et al.*, 2001), and emphasizes the importance of phenotypic plasticity at the whole-plant level as a concerted response of different traits (Ryser & Eek, 2000). For instance, the high maximum leaf photosynthetic rate (A_{max}) of *Quercus ilex* was neutralized by the high efficiency of light distribution within the crown of *Q. coccifera*. While A_{max} of the former species was 60% higher than that of the latter (Valladares *et al.*, 2000a), crown carbon gain on an area basis was only 15% higher (Table 3).

At the leaf level, xanthophyll cycle pool increased with high light only at low nutrient availability (Valladares *et al.*, 2000a) as found in other studies (e.g. Skillman & Osmond, 1998), but light and nutrient availability did not interact at the crown level. Further studies are necessary to elucidate whether this lack of enhanced architectural photoprotection at high light and low nutrient availability is either due to the fact that leaf-level mechanisms provide enough photoprotection under these circumstances or to the fact that foliage area, which directly translates into avoidance of excessive light by increasing self-shading, is reduced under nutrient limitations.

Ecological and evolutionary consequences

The results of the current investigation suggest that phenotypic variation in *Q. ilex* and *Q. coccifera* is similarly caused by developmental instability and phenotypic plasticity, and limited by a high degree of genetic canalization. Although each of these components separately might be adaptive in extremely different scenarios, the combination is consistent with the selective pressures imposed by a highly heterogeneous and unpredictable environment. Firstly, genetic canalization, traditionally considered as an evolutionary result of stabilizing selection, seems to be also favoured by weak to moderately strong fluctuating selection (Kawecki, 2000; Debat & David, 2001). Secondly, developmental instability, traditionally viewed as the inability of a genotype to direct its development, has been alternatively interpreted as a bet-hedging strategy in unpredictable environments (Simons & Johnston, 1997). Finally, phenotypic plasticity, reported to be adaptive only when the environmental change is predictable (Scheiner, 1993; Valladares *et al.*, 2000b, 2002; Pigliucci, 2001), could also be adaptive in habitats where environmental variability is unpredictable, such as the horizontal heterogeneity experienced by stoloniferous plants (Stuefer *et al.*, 1998) or the amphibious environments of wetland systems (Robe & Griffiths, 2000). The influence of environmental unpredictability on the adaptive value of phenotypic plasticity is scale-dependent. In a temporal scale, phenotypic plasticity seems to be adaptive when changes between generations are predictable (Scheiner, 1998), but also when those that occur within a life span are unpredictable (Sultan, 1987; Winn, 1996). In a spatial scale, the phenotypic plasticity that allows individuals to respond to the unpredictability of their immediate environment may differ in its genetic basis from that promoted by predictable changes across large areas (Wu, 1998). It must also be taken into account, however, that due to the pre-Mediterranean origin of the two oak species studied (Herrera, 1992), the exaptive nature of their traits can not be rule out (after terminology of Gould & Vrba, 1982).

Unpredictability characterises the ecosystems of the Mediterranean Basin (Blondel & Aronson, 1999) and seems to condition local adaptation in Mediterranean communities (Imbert *et al.*, 1999). Its effect on phenotypic variation is, however, not manifested in annual plants in response to macroenvironmental gradients (Volis *et al.*, 2002). In contrast, our findings are consistent with a determinant role of environmental unpredictability in shaping the phenotypic variation in seedlings of long-lived woody plants. This effect might be differentially marked at this early stage of plant development. Our study has a number of limitations, for example the low number of replicates (4–6), which does not provide enough statistical power to rule out differences in the phenotypic variability between the two species, the fact that plasticity may become expressed in an enhanced manner latter in the ontogeny of these long-lived species, and genetic canalization would have been better tested in a comparison of different populations of these oaks. However, our results confirmed the expected pattern under environmental unpredictability and suggest trends that deserve further exploration. The species studied often behave as late-successionals (Costa *et al.*, 1997), and their seedlings are naturally exposed to the high spatio-temporal heterogeneity imposed by evergreen canopies, whose unpredictability has been reported in other habitats (Pearcy, 1983; Valladares *et al.*, 1997). Patterns of phenotypic variation may change throughout the development of individual plants, but the species studied have to cope with remarkable environmental changes and frequent but unpredictable perturbations during their whole life spans. Further studies are needed to assess the long-term influence of these events on the sources of phenotypic variation throughout the ontogeny of these long-lived Mediterranean species.

Acknowledgements

Thanks to Hans Cornelissen for constructive criticisms and to Rosa Colomer and the staff of the nursery Viveros Barbol (Madrid) for their qualified help with the plants. Financial support was provided by Spanish CICYT (grant CLI97-0735-CO3-01) and MCYT (grant REN2000-0163-P4, ECOFIARB).

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