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Population divergence in the plasticity of the response of *Quercus coccifera* to the light environment

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Summary

1. *Quercus coccifera*, a slow-growing, evergreen oak, grows in contrasting environments in the Mediterranean Basin. Habitat-based selection may have promoted divergence between populations with respect to phenotypic plasticity and genetic variability.

2. We tested the hypothesis that populations of the *Q. coccifera* originating from a rock outcrop, a continental garrigue formation and an oceanic forest would differ in their plastic response to light intensity. Plants from these populations were grown from acorns in a common garden at 100% and 20% full sunlight. Light response analysis was based on photochemical efficiency, xanthophyll pool, nutrient allocation, growth, crown architecture and light absorption.

3. Light-responsive characters ranged from the subcellular to the whole-plant level. The greatest divergences between sun and shade phenotypes were observed in leaf size, leaf angle and leaf area ratio. However, plasticity in these traits depended on plant provenance.

4. Regardless of the level of organization, populations were invariably ranked in the same order of plasticity when averaged over light-responsive features, with plants originating from the rock outcrop showing the least plasticity and those from the forest the largest. The forest population also had the greatest genetic variability with respect to the isoperoxidase polymorphism.

5. Among populations, plants originating from the phosphorus-deficient rock outcrop contained 30% more P per unit dry weight. Plants from the forest population had 5% more photoprotective xanthophylls, 30% larger total leaf area, with less lobed and larger leaves and a differential plasticity in leaf azimuth.

6. Differences among populations suggested ecotypic differentiation towards less phenotypic plasticity in the most homogeneous light environments. The ecological breadth of the species seemed to be derived not only from its tolerance of Mediterranean conditions but also from the specialization of its populations in contrasting habitats.

Key-words: ecotypes, genetic variability, leaf azimuth, phenotypic plasticity, photochemical efficiency

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Introduction

Of all the environmental factors affecting plant performance, light is perhaps the most spatially and temporally heterogeneous (Pearcy 1999). Under evergreen canopies, long periods of diffuse light are interrupted unpredictably by bright sunflecks and the opening of small gaps or clearings (Pearcy 1983; Valladares, Allen & Pearcy 1997; Lei & Lechowicz 1998). In contrast, the light environment is more homogeneous in open habitats (Ögren & Sundin 1996), such as on cliffs and rock outcrops, where competition among plants for light is weak or non-existent (Matthes-Sears & Larson 1999).

Contrasted light environments create different selective pressures that might drive evolutionary divergence of plant populations (Linhart & Grant 1996). Such divergence is expected to promote the dominance of specialized genotypes (Sultan 1992; Pigliucci 1996). However, it has been proposed that selection in

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heterogeneous environments leads to the coexistence of genotypes with greater phenotypic plasticity – i.e. a greater ability to express alternative phenotypes in response to environmental variations (Sultan 1987; Lortie & Aarssen 1996).

In the Mediterranean Basin, the sclerophyll Kermes oak, *Quercus coccifera* L., experiences light environments of contrasted heterogeneity. In remnant patches of relict oceanic forests, *Q. coccifera* reaches a height of 15 m (Catarino, Correia & Correia 1982; Costa, Morla & Sainz 1997). As the dominant woody species at these sites, it casts a deeper shadow than other Mediterranean sclerophylls and semideciduals due to its complex, multilayered canopy (Caldwell *et al.* 1986; Werner, Correia & Beyschlag 1999). Over wide regions of the Mediterranean Basin, *Q. coccifera* dominates the garrigue landscape, characterized by dense shrub formations (Terradas 1999). In contrast, on exposed rock outcrops, it forms a stunted and widely spaced scrub reaching a height of less than 2 m (Martínez-Ferri *et al.* 2000). Along this gradient, the seasonal scarcity of water in Mediterranean ecosystems exacerbates the influence of irradiance on plant physiology and growth (Joffre, Rambal & Damesin 1999).

The occurrence of *Q. coccifera* in these contrasted light environments could be accounted for by its wide tolerance of Mediterranean conditions, such as summer drought (Tenhunen *et al.* 1985; Rambal & Leterme 1987) and saturating light intensities (Martínez-Ferri *et al.* 2000). However, populations across this environmental gradient might have experienced different adaptive constraints for phenotypic plasticity and environmental specialization, as reported for other taxa (Ryser & Eek 2000; Weinig 2000).

Assuming the existence of a variation in the potentially adaptive value of phenotypic plasticity, we hypothesized that divergence in the plasticity of the response to light availability should have occurred in populations of *Q. coccifera* from a forest, a garrigue and a rock outcrop. Plants raised from acorns were grown in a common garden experiment at two different light treatments (100% and 20% full sunlight). Physiological and structural light use efficiency of each population in both experimental light environments were analysed from leaf to crown level and interpreted with respect to biomass and nutrient allocation patterns in whole plants. Intrapopulation genetic variability and interpopulation similarities were estimated on the basis of the electrophoretic patterns of leaf peroxidases.

Table 1. Means (± 1 SE) of pH measured in water, organic matter, total nitrogen (N), available phosphorus (P) and available potassium (K) in samples of shallow soil (n = 3/4; 5 cm depth) and climatic characteristics at the seed provenance sites (rock, garrigue and forest)

	Rock*	Garrigue*	Forest†
pH	7·8 ± 0·2	7·8 ± 0·1	6·8 ± 0·2
Organic matter (%)	1·37 ± 0·30	1·34 ± 0·86	10·32 ± 1·09
N (%)	0·97 ± 0·05	0·48 ± 0·01	0·35 ± 0·04
P (μg g^{-1})	3·0 ± 0·2	5·3 ± 1·3	21·0 ± 5·0
K (μg g^{-1})	154 ± 88	527 ± 133	363 ± 133
Annual rainfall (mm)	403	350	747
Mean annual air temperature (°C)	13·9	15·2	15·9

Soil data kindly provided by Mr A. Jordán* and Dr M. A. S. Clemente†.

Materials and methods

SEED ORIGINS AND NURSERY

In November 1996, we collected acorns of *Q. coccifera* L. from 1 ha plots in three natural populations growing in contrasting environments on the Iberian Peninsula: a continental rock outcrop ('rock') at Cañada de Verich (40°52′ N 00°07′ W, Teruel, eastern central Spain); a continental garrigue-type formation ('garrigue') at Mata de Olmos (40°51′ N 00°32′ W, Teruel, Spain); and a patch of evergreen forest in an oceanic location ('forest') at Serra da Arrábida (38°28′ N 09°00′ W, Setúbal, central Portugal). Distances between the sites ranged from ≈ 40 to 800 km. All sampling sites exhibited a Mediterranean climate with a summer drought longer than 2·9 months per year. Mean annual rainfall and air temperature at these sites are shown in Table 1. Mean annual air temperatures oscillated around monthly means of ≈ 20 °C at the continental stands and about half that at the oceanic site. Edaphic variables measured at the sampling sites are summarized in Table 1. Soil samples collected from fissures and cracks at the rock site and from shallow soils developed on a hard limestone bedrock at the garrigue site had higher pH values than samples collected in the forest site, which has a brown humic-rich soil (Catarino *et al.* 1982). The first two sampling sites were poor in organic matter and available phosphorus – the soil was particularly deficient in P at the rock site.

Immediately after collection, acorns were transported and sown in a nursery at Torremocha del Jarama (40°51′ N 03°29′ W, Madrid, Spain), in 5 L plastic pots, in a mixture of peat (Epogron PP1A, Griendtsveen, Netherlands) and washed river sand (4 : 1, $pH_{(water)}$ = 6·5; total N = 0·35%; total P = 0·21 mg g^{-1}; total K = 1·34 mg g^{-1}). Seedlings were drip-irrigated for 6 h per day every 2 or 3 days.

ISOZYME ANALYSES OF LEAF PEROXIDASES

In November 1997, 0·5 g samples of fully expanded, current-year leaves from 9–10 different individuals of each population, grown in a common environment, were ground in liquid N_2 with a Mikrodismembrator (Braun Meilsungen GmbH, München, Germany) with insoluble polyvinylpolypyrrolidone (1 g g^{-1} tissue) in 66 mM Na-K phosphate, pH 7·0. The extracts were filtered and centrifuged at 10 000 *g* for 10 min. The resulting supernatant constituted the soluble fraction used for the separation of peroxidase isoenzymes (Castillo, Penel & Greppin 1984). Thin-layer isoelectric

focusing was performed in Ampholine-polyacrylamide gels (Pharmacia Biotech GmbH, Uppsala, Sweden) in a 3·5–10 pH gradient. Enzyme extracts (20 μL) were applied to the gel layer. Focusing was carried out at constant power (30 W) at a maximum of 1500 V for 2 h. Isoperoxidase bands were stained with 2 mM tetramethyl-benzidine and 3 mM H_2O_2 in Na-acetate buffer, pH 4·5. The isoperoxidase bands were scanned and analysed. The presence or absence of each band was recorded. Patterns were identified and grouped by frequencies. The degree of similarity between populations was estimated by means of the Rogers and Tanimoto Coefficient (S_{rt}, Sneath & Sokal 1973).

EXPERIMENTAL DESIGN

In December 1997, 20 seedlings from each place of origin were assigned randomly to two light treatments. All seedlings in each treatment were placed in four rows of five plants, occupying a $1·70 \times 1·30$ m area. Seedlings were grown outdoors in either full sunlight ('sun') or neutral shade ('shade'), provided by a white standard raffia cloth which decreased daily photosynthetic photon flux density (PPFD) by 80%. The range of PPFDs used corresponds to the measured reduction in light intensity in the understorey of an oak woodland (Valladares & Pearcy 1998). During the summer, air temperature (T), PPFD and relative humidity (RH) were measured every 2 min in each treatment with 2 temperature sensors (thermistor; Grant Instruments Ltd, Cambridge, UK), a quantum sensor (SKP210; Skye Instruments Ltd, Powys, UK) and a relative humidity probe (HMP 35 A; Vaisala Oyj, Helsinki, Finland), connected to a Squirrel 1200 data logger (Grant Instruments). Maximum and minimum daily temperatures never differed by more than 5 °C between treatments, and midday vapour pressure deficit decreased by less than 2 kPa in the shade treatment.

All measurements were taken at the end of the experiment in August 1998, outside of the vegetative growth period (cf. Castro-Díez & Montserrat-Martí 1998).

CHLOROPHYLL FLUORESCENCE

In vivo chlorophyll *a* fluorescence signals of attached, fully expanded and current-year leaves at the top crown layer were recorded for five plants per population provenance and light treatment. Recordings were made with a portable fluorometer (PAM-2000; Heinz Walz GmbH, Effeltrich, Germany), equipped with a leaf-clip holder to monitor incident solar radiation ($PPFD_{leaf}$) and leaf temperature (T_{leaf}). Chlorophyll fluorescence was measured twice on each leaf, before dawn and at midday. Predawn maximum (F_m) and minimum fluorescence (F_o) were measured to calculate the maximum photochemical efficiency of PSII ($[F_m - F_o] / F_m$ [= F_v / F_m]). F_m, diurnal maximum fluorescence induced by saturating light pulses (F'_m), and diurnal minimal fluorescence observed in leaves immediately darkened and exposed to far-red light for 5·5 s after every saturating light pulse (F'_o) were used to calculate diurnal non-photochemical quenching ($qN = [F_m - F'_m] / [F_m - F'_o]$; cf. Buschmann 1995). Quantum yield of non-cyclic electron transport (ΦPSII), photochemical quenching (qP) and photochemical efficiency of the open reaction centres of PSII ($[F'_m - F'_o] / F'_m$ [= F'_v / F'_m]) were calculated as described by Genty, Briantais & Baker (1989).

DETERMINATION OF PHOTOSYNTHETIC PIGMENTS

Leaf samples from five plants per provenance and light treatment were collected before dawn and at noon, and immediately stored in liquid N_2 for pigment extraction (Martínez-Ferri *et al.* 2000). Leaf samples (100 mg) were extracted in 5 mL cool acetone in the presence of sodium ascorbate. After filtering, 30 μL of acetone extract were injected into a Spherisorb ODS2 reverse-phase steel column (Waters Corp., Milford, MA, USA; 25 cm length, 4·6 mm inner diameter and 5 μm particle diameter). Chlorophylls and carotenoids were separated by HPLC (Waters Corp.) equipped with a Waters 996 photodiode array detector. Solvents for HPLC analyses (LabScan Ltd, Dublin, Ireland) were degassed before use by bubbling helium. For peak identification and quantification, pure commercial standards (VKI, Hørsholm, Denmark) were used. Leaf contents of violaxanthin, antheraxanthin and zeaxanthin as well as the total pool (VAZ) were expressed with respect to total chlorophyll content (Chl_{a+b}). The de-epoxidation state of the xanthophyll cycle (DPS) was calculated as the ratio of antheraxanthin and zeaxanthin to the total xanthophyll cycle pool (Adams *et al.* 1995).

BIOMASS AND NUTRIENT ANALYSES

Four plants of each provenance–treatment combination were harvested at the end of the experiment. Leaves, stems and roots were separated, dried at 65 °C for 48 h and weighed. Root/shoot ratio (R/S; root dry weight per shoot dry weight), root weight ratio (RWR; root dry weight per plant dry weight), leaf weight ratio (LWR; leaf dry weight per plant dry weight), specific leaf area (SLA; leaf area per leaf dry weight), leaf area ratio (LAR; leaf area per plant dry weight), degree of leaf lobulation (ILB; leaf perimeter per leaf area), mean leaf area (MLS) and total leaf area per plant (TLA) were calculated.

Plant nitrogen (N), phosphorus (P) and potassium (K) concentrations were determined at the Centro de Ciencias Medioambientales (CSIC, Madrid, Spain). Finely ground samples were digested in HNO_3 and $HClO_4$ in a warm sand bath at atmospheric pressure. Phosphorus and K concentrations were determined by emission spectrometry in an inductively coupled

plasma mass spectrometer (ICP5500; Perkin Elmer, Norwalk, CT, USA). Total N concentration was determined in a Kjeltec-auto 1030 analyser (Tecator, Höganäs, Sweden) after digestion in H_2SO_4 with K_2SO_4 by the Kjeldahl procedure, using $SeSO_4$ as a catalyst.

CROWN ARCHITECTURE

Diurnal cycles of light absorption of four independent seedlings from each provenance–treatment combination were assessed using the computer model YPLANT (Pearcy & Yang 1996), whose predictive accuracy has been verified in contrasting environments (Pearcy & Yang 1998; Valladares & Pearcy 1998; Valladares & Pugnaire 1999). The model assumes leaf orientation adjustments during a diel course to be negligible, and reconstructs crown architecture node-by-node from direct measurements of azimuths, angles from the horizontal and internode, petiole and leaf lengths. For each node, the model inserts branches, petioles and/or leaves as three-dimensional vectors (Pearcy & Yang 1996). Angles from the horizontal and azimuths of all current-year leaves (1126 leaves in total) were measured in 24 plants. The model can handle up to 20 different leaf contours. Two different contours (corresponding to the sun and shade patterns) and the leaf length at each particular node were used to mimic all leaves of the sampled plants. Diurnal evolution of direct light absorption was simulated by rotating the modelled plant crown to specific orientations corresponding to the angles and azimuths of the solar disc along its path at a given latitude. At these discrete intervals, crown absorption of diffuse light was also estimated on the basis of vectors from 160 sky sectors (eight azimuth and 20 solar angles). Basic variables derived form this simulation were the projected leaf area normal to incident PPFD, calculated as the actual leaf area reduced by the cosine of incidence, and the displayed area, calculated as the projected area reduced by leaf overlap (self-shading). The efficiency of the canopy performance was assessed (Valladares & Pugnaire 1999) from three main variables: (i) projection efficiency (E_p, the ratio of the projected to the actual leaf area); (ii) display efficiency (E_d, defined as the ratio of the displayed to the actual leaf area); and (iii) fraction of self-shaded leaf area, calculated as E_p minus E_d (SS).

PHENOTYPIC PLASTICITY INDEX AND STATISTICAL ANALYSIS

Unlike regulatory responses to environmental changes, differences in maxima, minima and time constants derived from growth in different environments are expressions of phenotypic plasticity (Pearcy 1999). Therefore, physiological traits, such as characters based on chlorophyll fluorescence signal and pigment composition, were measured within each light treatment in exposed leaves at predawn and midday to obtain either maximum or minimum values (cf. Martínez-Ferri *et al*. 2000). Among the measured traits, a character was only considered to be plastic when the light treatment effect was significant ($P < 0{\cdot}05$) in a one-way ANOVA in at least one of the provenances. Phenotypic plasticity in those characters was taken to be the positive difference between the means obtained in the two light treatments for each provenance, relative to the maximum mean (Valladares *et al*. 2000b).

Differences between populations in the similarity index (S_{rt}) were tested for by one-way ANOVA followed by an LSD (least significant difference) test. Differences and interactions among provenances and treatments were tested by a two-way ANOVA that, in the case of R/S, RWR, LWR and LAR, incorporated plant biomass as a covariate. Single values of leaf angle and E_p, E_d and SS for each solar angle were averaged for each plant. Differences in the diurnal course of pigment composition among provenances and light treatments were evaluated with a two-way repeated measure ANOVA (ANOVAR), using 'time of day' as a within-subject variable. Assumptions of normality and homoscedasticity were tested using the Kolmogorov–Smirnov test and Cochran's *C*-test, respectively. In the event of heteroscedasticity, variables were either log-transformed or analysed with the non-parametric Kruskal–Wallis test. Pairwise comparisons were performed only after a significant ANOVA using the LSD test. An χ^2 test was used to test for divergence from the expected uniform distribution of the relative frequencies of leaf azimuths among four categories (north [315–44°], east [45–134°], south [135–224°], and west [225–314°]) within each provenance and light treatment combination. Differences in ranking between populations, based on the phenotypic plasticity index across light-responsive traits within each organizational level, were tested for by a Friedman ANOVA by ranks.

Results

PEROXIDASE ELECTROPHORETIC PATTERNS

Electrophoretic separation of isoperoxidases yielded nine different patterns (A–I) with a variable number of bands (6–11, Fig. 1). Within provenances, most polymorphism occurred in samples from the forest population, with five different electrophoretic patterns (B, F, G, H, I), whereas samples from the rock and garrigue populations exhibited only three different patterns (A, B, C and C, D, E, respectively). Dominant patterns were observed only in the rock and garrigue populations, where patterns B and C accounted for 70% and 55% of the variation, respectively. The electrophoretic patterns displayed by samples originating from the forest provenance were found to occur at similar frequencies (10–30%). Between provenances, the forest and rock populations were the least similar ($S_{rt} = 0{\cdot}55$) and the garrigue population was nearly equidistant from the rock ($S_{rt} = 0{\cdot}58$) and forest ($S_{rt} = 0{\cdot}60$)

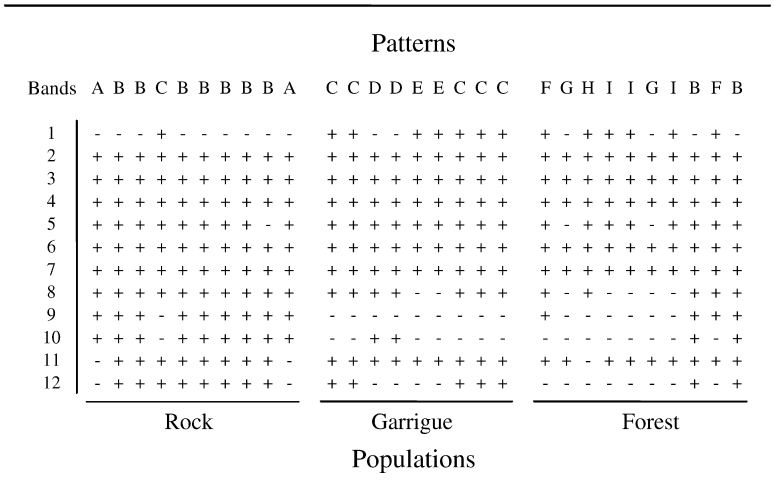

Fig. 1. Composition of the isoenzymatic peroxidase patterns (A–I) of *Q. coccifera* seedlings grown in a common garden experiment from acorns originating from a continental rock outcrop (rock), a continental garrigue-type formation (garrigue) and a patch of evergreen forest in an oceanic location (forest). Within each isozyme pattern, the presence (+) or absence (–) of each band is indicated.

populations. The similarity between the forest and rock populations was significantly less than that between the forest and garrigue populations ($F_{2,277} = 3{\cdot}24$, $P < 0{\cdot}05$, $n = 90$ or 100).

CHLOROPHYLL FLUORESCENCE AND PHOTOSYNTHETIC PIGMENT CONTENT

Neither seed provenance nor light treatment had a significant effect on predawn F_v/F_m (Table 2). The photochemical efficiency of PSII did not revert to its optimum before dawn in any provenance–treatment combination, exhibiting a suboptimal mean of F_v/F_m across treatments ($0{\cdot}797 \pm 0{\cdot}003$, $n = 30$). Despite this lack of difference in F_v/F_m, predawn DPS was significantly less in plants grown in full sunlight ($F_{1,24} = 83{\cdot}1$, $P < 0{\cdot}001$; Table 2). However, this smaller proportion of de-epoxidated xanthophylls in 'sun' plants was observed in the context of a significantly larger pool of total xanthophylls (VAZ_{pd}; $F_{1,24} = 33{\cdot}4$, $P < 0{\cdot}001$; Table 2). Seedlings of different provenances had the same DPS_{pd} and there was no interaction between provenances and light treatments.

At midday, fluorescence measurements were taken when $PPFD_{leaf}$ ranged between 1432 and 1623 μmol m^{-2} s^{-1}, and 78 and 188 μmol m^{-2} s^{-1} for the sun and shade treatments, respectively. In parallel, T_{leaf} ranged between 31·8 and 33·1 °C, and 28·6 and 29·0 °C, for the sun and shade treatments, respectively. Within light treatments, no significant difference was observed among provenances in any of the chlorophyll fluorescence variables (Table 2). Differences between sun and shade plants at midday were explained by the different values of $PPFD_{leaf}$. There was no significant interaction between provenances and treatments.

Total chlorophyll per leaf surface area did not differ between predawn and midday, light treatments or populations (Table 2). Midday DPS values were, on average, 28% larger in sun plants than in shade plants ($F_{1,24} = 18{\cdot}9$, $P < 0{\cdot}001$), but within provenances, this difference was significant only in the forest and garrigue populations ($F_{1,8} > 10{\cdot}4$, $P < 0{\cdot}05$). As was the case before dawn, the VAZ value, approximately 20% larger in sun plants than in shade plants, was maintained at midday ($F_{1,24} = 29{\cdot}9$, $P < 0{\cdot}001$). Across light treatments and sampling times, forest plants had a larger VAZ pool ($F_{2,24} = 8{\cdot}4$, $P < 0{\cdot}002$). However, there was a significant interaction between light treatments and plant provenances ($F_{2,24} = 5{\cdot}8$, $P < 0{\cdot}01$). This was due to the particularly marked differences between sun and shade plants in the forest provenance

Table 2. Means (± 1 SE; $n = 5$) of maximum photochemical efficiency of PSII (F_v/F_m), quantum yield of non-cyclic electron transport (ΦPSII), photochemical efficiency of the open reaction centres of PSII (F'_v/F'_m), photochemical quenching (qP), non-photochemical quenching (qN), total chlorophyll content (Chl_{a+b}; μmol m^{-2}), predawn and midday de-epoxidation state of the xanthophyll cycle (DPS_{pd} and DPS_{md}, respectively), and predawn and midday xanthophyll cycle pool (VAZ_{pd} and VAZ_{md}, respectively; mmol (mol chl)$^{-1}$) of seedlings originating from rock, garrigue and forest populations and grown at 100% (sun) or 20% (shade) full sunlight. Bold lettering indicates significant differences between treatments within sites (ANOVA and LSD; $P < 0{\cdot}05$)

	Rock		Garrigue		Forest	
	Sun	Shade	Sun	Shade	Sun	Shade
F_v/F_m	0·800 ± 0·007	0·806 ± 0·005	0·793 ± 0·009	0·792 ± 0·006	0·787 ± 0·005	0·800 ± 0·005
PSII	**0·317 ± 0·035**	**0·617 ± 0·027**	**0·318 ± 0·035**	**0·674 ± 0·016**	**0·305 ± 0·036**	**0·630 ± 0·020**
F'_v/F'_m	**0·573 ± 0·002**	**0·752 ± 0·008**	**0·629 ± 0·027**	**0·732 ± 0·014**	**0·535 ± 0·043**	**0·752 ± 0·003**
qP	**0·560 ± 0·076**	**0·821 ± 0·037**	**0·503 ± 0·065**	**0·921 ± 0·026**	**0·570 ± 0·041**	**0·838 ± 0·028**
qN	**0·901 ± 0·010**	**0·745 ± 0·019**	**0·848 ± 0·031**	**0·726 ± 0·037**	**0·889 ± 0·032**	**0·711 ± 0·020**
Chl_{a+b}	540 ± 92	540 ± 49	545 ± 143	608 ± 125	548 ± 134	456 ± 134
DPS_{pd}	**0·14 ± 0·03**	**0·35 ± 0·06**	**0·14 ± 0·07**	**0·36 ± 0·06**	**0·14 ± 0·01**	**0·32 ± 0·09**
DPS_{md}	0·46 ± 0·01	0·41 ± 0·07	**0·56 ± 0·11**	**0·36 ± 0·09**	**0·59 ± 0·09**	**0·40 ± 0·03**
VAZ_{pd}	**163 ± 16**	**120 ± 9**	**147 ± 24**	**116 ± 8**	**176 ± 36**	**118 ± 18**
VAZ_{md}	**124 ± 15**	**100 ± 15**	**119 ± 14**	**98 ± 10**	**139 ± 6**	**102 ± 16**

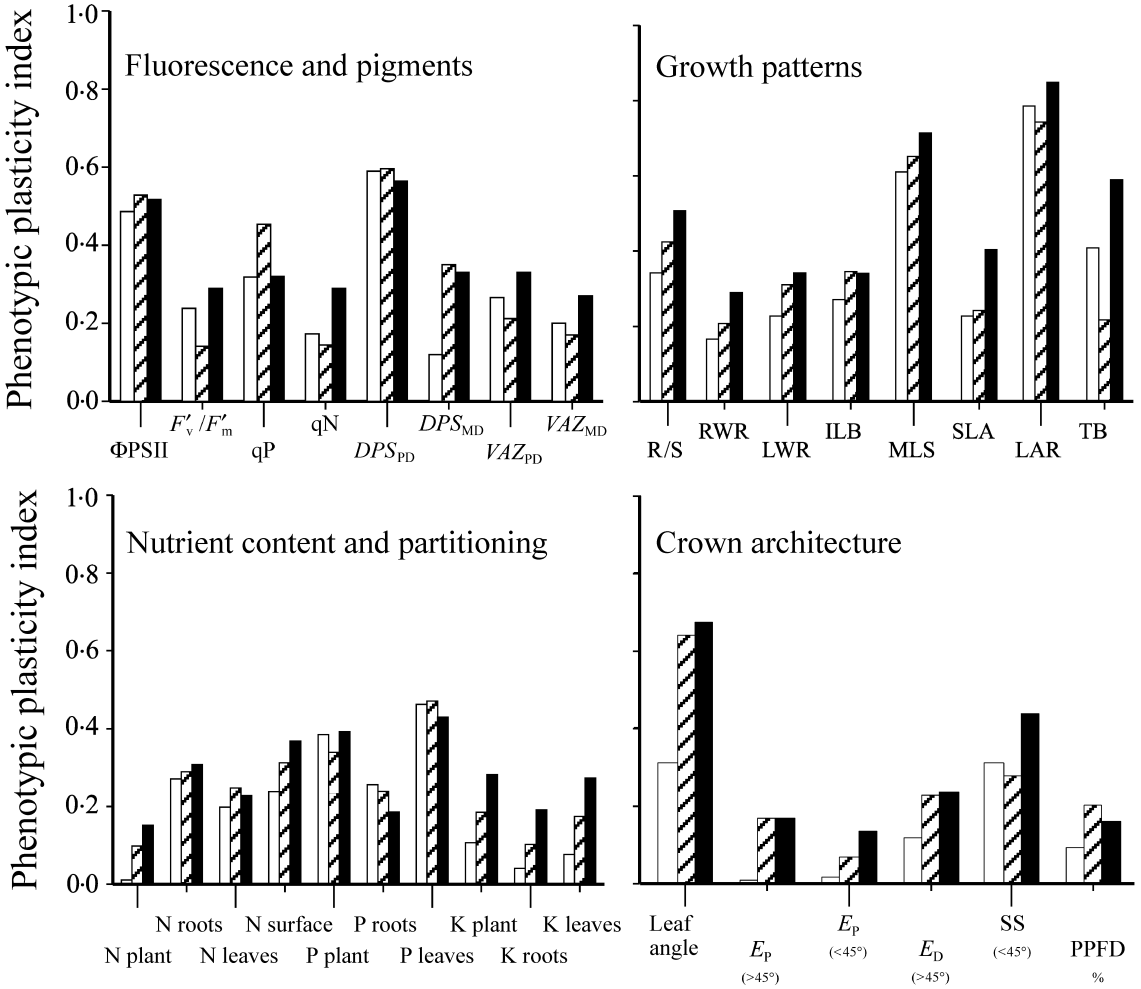

Fig. 2. Phenotypic plasticity index ([maximum – minimum] / maximum) in traits that responded significantly to 80% reduction in light intensity of plants originating from the rock (open bars), garrigue (hatched bars), and forest populations (solid bars). Variables grouped under 'Fluorescence and pigments', 'Growth patterns', 'Nutrient content and partitioning' and 'Crown architecture' are described in Tables 2, 3, 4 and 5, respectively.

in comparison with those in plants from the other populations, which resulted in a higher plasticity index of this character in the forest population (Fig. 2).

In all chlorophyll fluorescence and pigment characters except F_v/F_m and Chl_{a+b}, plants had a plastic response to the light treatment (i.e. a significant effect of treatment in at least one of the populations revealed by a one-way ANOVA). When averaged over all plastic traits, the populations were ranked in order of decreasing plasticity index: forest (0·35) > garrigue (0·33) > rock (0·30). Non-parametric analysis indicated significant differences in ranking between the forest and the rock population ($\chi^2_{8,1} = 4{\cdot}5$, $P < 0{\cdot}03$). The forest provenance was more plastic than the rock population in seven of eight characters. Only DPS_{pd} had a slightly smaller plasticity in the forest provenance (Fig. 2). The greatest differences between both populations were observed in the plasticity of qN and DPS_{md} (40% and 65% less, respectively, in the rock population). Seedlings of garrigue provenance had the largest phenotypic plasticity index of the studied populations in ΦPSII, qP, DPS_{pd} and DPS_{md}, but the smallest F'_v/F'_m, qN, VAZ_{pd} and VAZ_{md}.

BIOMASS AND NUTRIENT ALLOCATION PATTERNS

Table 3 summarizes the effects of the sun and shade treatments on plant growth. Regardless of plant provenance, growth in the shade treatment was characterized by a larger above-ground biomass allocation (40% in R/S; $F_{1,18} = 51{\cdot}9$, $P < 0{\cdot}001$), increasing the relative partitioning to leaf biomass (29% LWR; $F_{1,18} = 50{\cdot}5$, $P < 0{\cdot}001$) at the expense of root growth (21% RWR; $F_{1,18} = 51{\cdot}5$, $P < 0{\cdot}001$). Plants grown in the shade not only had a larger fraction of their biomass in the leaves, but also their leaves were less lobed, larger and had a greater surface area per unit dry weight ($F_{1,18} > 35{\cdot}5$, $P < 0{\cdot}001$). These effects resulted in 78% more leaf surface area per unit plant weight (LAR; $F_{1,18} = 32{\cdot}1$, $P < 0{\cdot}001$). Thus, sun and shade plants did not differ in their total leaf areas despite the significantly smaller plant biomass (33% TB; $F_{1,18} = 15{\cdot}9$, $P < 0{\cdot}001$) of the shade plants. The treatment effect on R/S, RWR, LWR and LAR remained significant even when the two-way ANOVA accounted for plant biomass as a covariate ($F_{1,17} > 30{\cdot}9$, $P < 0{\cdot}0001$).

Table 3. Means (± 1 SE; $n = 4$) of root/shoot ratio (R/S), root weight ratio (RWR), leaf weight ratio (LWR), leaf lobulation (ILB), mean leaf size (MLS), specific leaf area (SLA), leaf area ratio (LAR), total plant biomass (TB) and total leaf area per plant (TLA) of seedlings originating from the rock, garrigue and forest populations and grown at 100% (sun) or 20% (shade) full sunlight. Bold lettering indicates significant differences between treatments within sites (ANOVA and LSD; $P < 0.05$)

	Rock		Garrigue		Forest	
	Sun	Shade	Sun	Shade	Sun	Shade
R/S ($g\ g^{-1}$)	**1·51 ± 0·17**	**1·01 ± 0·12**	**1·79 ± 0·05**	**1·05 ± 0·07**	**1·48 ± 0·12**	**0·76 ± 0·09**
RWR ($g\ g^{-1}$)	**0·60 ± 0·03**	**0·50 ± 0·03**	**0·64 ± 0·01**	**0·51 ± 0·02**	**0·59 ± 0·02**	**0·43 ± 0·03**
LWR ($g\ g^{-1}$)	**0·30 ± 0·02**	**0·38 ± 0·02**	**0·26 ± 0·02**	**0·37 ± 0·02**	**0·30 ± 0·02**	**0·45 ± 0·03**
ILB ($cm\ cm^{-2}$)	**4·97 ± 0·36**	**3·66 ± 0·25**	**5·02 ± 0·42**	**3·35 ± 0·32**	**3·88 ± 0·14**	**2·61 ± 0·14**
MLS (cm^2)	**1·39 ± 0·13**	**3·38 ± 0·49**	**1·58 ± 0·26**	**4·24 ± 0·82**	**2·38 ± 0·20**	**7·41 ± 0·49**
SLA ($m^2\ kg^{-1}$)	**5·06 ± 0·32**	**6·46 ± 0·51**	**4·97 ± 0·25**	**6·49 ± 0·69**	**4·88 ± 0·27**	**7·96 ± 0·19**
LAR ($m^2\ kg^{-1}$)	**0·21 ± 0·03**	**0·89 ± 0·18**	**0·25 ± 0·04**	**0·89 ± 0·20**	**0·25 ± 0·03**	**1·41 ± 0·34**
TB (g)	7·14 ± 1·35	4·32 ± 0·98	6·24 ± 0·40	4·92 ± 0·26	**10·2 ± 1·69**	**4·44 ± 0·49**
TLA (cm^2)	107 ± 22	102 ± 19	80 ± 6	116 ± 8	145 ± 12	162 ± 19

Although seedlings from different provenances did not differ in total biomass, there were significant differences in biomass allocation. Across light treatments, plants originating from the forest partitioned relatively more biomass to the shoots (13% smaller R/S than rock and 27% significantly [$F_{2,18} = 3·7$, $P < 0·05$] smaller R/S than garrigue). As a consequence, there was a larger LWR and TLA associated with a smaller RWR ($F_{2,18} > 4·3$, $P < 0·05$). Leaves of plants from the forest population were less lobed and larger ($F_{2,18} > 8·1$, $P < 0·01$). However, leaf size was particularly responsive to the light treatment in this population in comparison with plants of other provenances. This indicated that only this interaction between plant provenance and light treatment was significant among growth variables ($F_{2,18} = 6·0$, $P < 0·01$). Plants from the rock population had mean values between those of the forest and garrigue plants in TB, TLA and all variables related to biomass partitioning (i.e. R/S, RWR, LWR and SLA).

All growth characters except TLA responded to the light treatment in at least one of the populations. Across growth traits, populations were ranked in the same order of decreasing mean plasticity as described for chlorophyll fluorescence and pigment characters, i.e. forest (0·48) > garrigue (0·38) > rock (0·37). The rock population displayed the least phenotypic plasticity in all variables except LAR and TB (Fig. 2). The forest population was the most plastic population in R/S, RWR, LWR, MLS, SLA, LAR, TB, yielding phenotypic plasticity indices of up to 0·82 in LAR and 0·69 in MLS. Leaf lobulation of plants from the forest population was as plastic as that of garrigue plants (Fig. 2). Friedman ANOVA showed that plasticity of the forest population was significantly greater than those of the rock ($\chi^2_{8,1} = 8·0$, $P < 0·005$) and garrigue populations ($\chi^2_{8,1} = 4·5$, $P < 0·03$).

Shading increased the whole-plant concentration of N, P and K ($F_{1,18} > 8·2$, $P < 0·01$). The increased biomass allocation to leaves of shade plants was paralleled by an increased partitioning of total N, P and K to leaves ($F_{1,18} > 6·8$, $P < 0·05$). Thus, leaf nutrient concentrations per unit leaf dry weight did not differ between light treatments. This shift in nutrient partitioning to the leaves occurred at the expense of a decreased allocation of N, P and K to roots ($F_{1,18} > 6·3$, $P < 0·05$; Table 4). However, this shift in N allocation was not proportional to the increase in leaf area and, as a result, N concentration with respect to leaf area was less in shade plants than in sun plants ($F_{1,18} = 11·0$, $P < 0·01$). Shade plants also displayed an increased allocation of N to the stems ($F_{1,18} = 7·0$, $P < 0·05$).

Seedlings originating from the rock population contained 31% more P per unit plant dry weight than those from the garrigue and forest populations ($F_{2,18} = 3·4$, $P < 0·05$). Leaf K concentrations, expressed either with respect to surface area or dry weight, were smaller in the forest plants than in those from garrigue ($F_{2,18} > 3·6$, $P < 0·05$). No significant interaction between provenance and light treatment was found in either nutrient concentrations or allocation patterns.

Populations ranked in order of decreasing mean phenotypic plasticity in nutrient characters repeated the previously observed pattern: forest (0·28) > garrigue (0·25) > rock (0·20). Only in the P partitioning between leaves and roots was this relationship reversed. However, across traits, the rock plants were significantly less plastic than those from the garrigue and forest populations ($\chi^2_{10,1} = 3·6$, $P < 0·05$).

PLANT ARCHITECTURE

Leaf angle in the shade plants was half that in the sun plants ($F_{1,18} = 43·8$, $P < 0·001$; Table 5). However, this difference was not significant between rock plants. The small plasticity of this character in this population accounted for the significant interaction between treatments and provenances ($F_{2,18} = 6·4$, $P < 0·01$). In the sun plants, the smallest leaf angles were observed in the rock population but in the shade, the rock plants had the largest angles. The largest phenotypic plasticity index of leaf angle was in the forest population, yielding one of the highest plasticity indices in the study (0·68; Fig. 2).

Table 4. Means (± 1 SE; $n = 4$) of whole-plant concentrations of nitrogen (N), phosphorus (P) and potassium (K), and their partitioning into the root, stem and leaf fractions for seedlings originating from rock, garrigue and forest populations and grown at 100% (sun) or 20% (shade) full sunlight. Bold lettering indicates significant differences between treatments within sites (ANOVA and LSD; $P < 0.05$)

	Rock		Garrigue		Forest	
	Sun	Shade	Sun	Shade	Sun	Shade
N (mg g^{-1})	7·86 ± 0·12	7·77 ± 0·27	7·62 ± 0·17	8·45 ± 0·52	**7·16 ± 0·34**	**8·44 ± 0·28**
Root (%)	**47·2 ± 4·14**	**34·5 ± 2·78**	**51·0 ± 1·36**	**36·5 ± 2·84**	**50·1 ± 1·84**	**34·7 ± 1·81**
Stem (%)	9·42 ± 1·51	11·5 ± 0·82	10·2 ± 0·63	12·1 ± 1·22	8·07 ± 0·85	11·2 ± 1·47
Leaf:						
(%)	**43·3 ± 2·76**	**54·1 ± 2·84**	**38·7 ± 1·17**	**51·5 ± 1·41**	**41·8 ± 1·42**	**54·1 ± 2·68**
(mg m^{-2})	2·31 ± 0·16	1·76 ± 0·19	2·31 ± 0·11	1·57 ± 0·53	**2·04 ± 0·13**	**1·29 ± 0·09**
P (mg g^{-1})	**1·15 ± 0·12**	**1·87 ± 0·36**	0·82 ± 0·18	1·24 ± 0·26	0·79 ± 0·05	1·30 ± 0·16
Root (%)	**71·7 ± 2·96**	**53·3 ± 3·66**	**73·5 ± 0·60**	**55·9 ± 4·64**	**73·0 ± 3·82**	**59·4 ± 3·57**
Stem (%)	8·07 ± 2·72	9·12 ± 1·33	6·71 ± 1·09	6·71 ± 2·57	8·04 ± 0·99	7·33 ± 1·02
Leaf:						
(%)	**20·2 ± 1·45**	**37·6 ± 4·41**	**19·8 ± 1·24**	**37·4 ± 6·38**	**19·0 ± 3·32**	**33·2 ± 3·81**
(mg m^{-2})	0·16 ± 0·03	0·30 ± 0·08	0·13 ± 0·04	0·20 ± 0·06	0·11 ± 0·03	0·12 ± 0·02
K (mg g^{-1})	4·41 ± 0·43	4·94 ± 0·11	4·44 ± 0·22	5·45 ± 0·40	**3·71 ± 0·20**	**5·17 ± 0·52**
Root (%)	55·1 ± 3·05	52·8 ± 3·40	56·5 ± 1·58	50·0 ± 3·46	**59·6 ± 3·39**	**48·2 ± 4·17**
Stem (%)	13·4 ± 2·25	13·0 ± 0·66	13·8 ± 1·24	14·1 ± 1·36	12·9 ± 0·62	14·0 ± 1·65
Leaf:						
(%)	31·6 ± 1·96	34·2 ± 3·15	29·7 ± 2·14	36·0 ± 2·94	**27·5 ± 3·31**	**37·8 ± 4·03**
(mg m^{-2})	0·94 ± 0·11	0·70 ± 0·07	1·03 ± 0·07	0·87 ± 0·18	0·71 ± 0·11	0·55 ± 0·09

Table 5. Average values (± 1 SE; $n = 4$) of leaf angle, projected, displayed and self-shaded fractions of the total leaf area (E_p, E_d and SS, respectively) for solar elevation angles greater or less than 45°, daily integrated PPFD absorption (both in mol photons m^{-2} day^{-1} and as percentage of a horizontal surface) in seedlings originating from rock, garrigue and forest populations and grown at 100% (sun) or 20% (shade) full sunlight. Bold lettering indicates significant differences between treatments within sites (ANOVA or Kruskal–Wallis followed by LSD; $P < 0.05$)

	Rock		Garrigue		Forest	
	Sun	Shade	Sun	Shade	Sun	Shade
Leaf angle (°)	24·5 ± 1·6	**20·3 ± 1·2**	**30·9 ± 5·6**	**11·2 ± 2·0**	**36·6 ± 3·4**	**11·9 ± 1·7**
E_p (> 45°)	0·80 ± 0·01	0·81 ± 0·02	**0·74 ± 0·04**	**0·89 ± 0·01**	**0·72 ± 0·03**	**0·87 ± 0·01**
E_p (< 45°)	0·41 ± 0·01	0·40 ± 0·01	0·43 ± 0·01	0·40 ± 0·02	**0·44 ± 0·02**	**0·38 ± 0·01**
E_d (> 45°)	**0·36 ± 0·05**	**0·41 ± 0·02**	**0·35 ± 0·03**	**0·45 ± 0·01**	**0·36 ± 0·03**	**0·47 ± 0·04**
E_d (< 45°)	0·25 ± 0·02	0·29 ± 0·01	0·27 ± 0·01	0·29 ± 0·01	0·29 ± 0·01	0·30 ± 0·01
SS (> 45°)	0·44 ± 0·04	0·40 ± 0·02	0·39 ± 0·02	0·44 ± 0·01	0·37 ± 0·05	0·41 ± 0·04
SS (< 45°)	**0·16 ± 0·02**	**0·11 ± 0·01**	0·16 ± 0·02	0·11 ± 0·03	**0·16 ± 0·02**	**0·09 ± 0·01**
PPFD	**22·9 ± 2·2**	**5·8 ± 0·2**	**22·8 ± 1·6**	**6·5 ± 0·3**	**23·3 ± 1·4**	**6·4 ± 0·3**
PPFD percentage	0·51 ± 0·05	**0·56 ± 0·02**	**0·51 ± 0·04**	**0·64 ± 0·03**	**0·52 ± 0·03**	**0·62 ± 0·03**

Although foliage orientation in response to the light treatment was not mediated through changes in azimuth orientation of the leaves in rock and garrigue plants, shade plants from the forest population had significantly more leaves orientated to the south ($\chi^2_2 = 9·5$, $P < 0·01$; Fig. 3).

Regardless of plant provenance, the steeper leaf angle in the sun plants increased the fraction of the total photosynthetic surface area projected to the sun at low solar elevations (E_p; Fig. 4). However, E_p decreased from a sun elevation angle of 35° to the zenith with respect to that projected by shade plants (Fig. 4). Differences between light treatments were significant only within the forest population at low solar elevations ($\chi^2_1 = 8·0$, $P < 0·01$) and across populations at high solar elevations ($F_{1,18} = 29·3$, $P < 0·001$). Diurnal evolution of E_p in shade plants was closer to that depicted by a theoretical horizontal surface (Fig. 4). Self-shading was maximal at the solar zenith, when it affected nearly half of the total projected area (Table 5). This fraction of self-shaded total leaf area in sun plants exceeded that in shade plants, but only when the solar elevation angle was < 45° ($F_{1,18} = 11·2$, $P < 0·01$; Table 5). A smaller fraction of the leaf area was exposed to incident PPFD (E_d) in sun plants compared to shade plants at low ($F_{1,18} = 5·7$, $P < 0·03$) and high ($F_{1,18} = 11·0$, $P < 0·004$) solar elevation angles. The diurnal course of E_d favoured light interception in the early morning, while the reverse was true at midday in comparison with a horizontal surface (Fig. 4).

Although no significant plant-provenance effect was found in any of the plant architecture variables, there

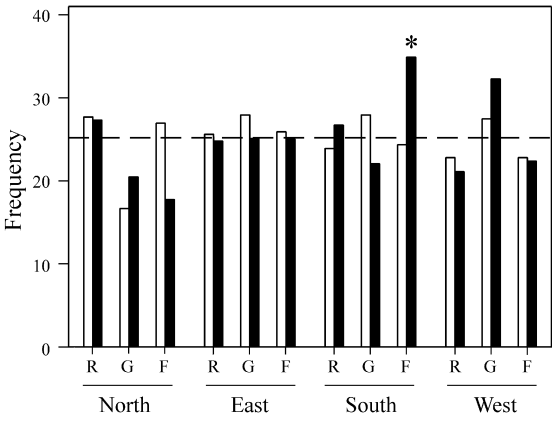

Fig. 3. Frequency distribution of leaf azimuth orientations ('North', 315–44°; 'East', 45–134°; 'South', 135–224°; 'West', 225–314°) in plants grown at 100% (open bars) and 20% full sunlight (solid bars) originating from the rock (R), garrigue (G) and forest populations (F). Significant ($P < 0{\cdot}01$) divergence from a uniform distribution (represented by — — —) is indicated by * (n = 127–290).

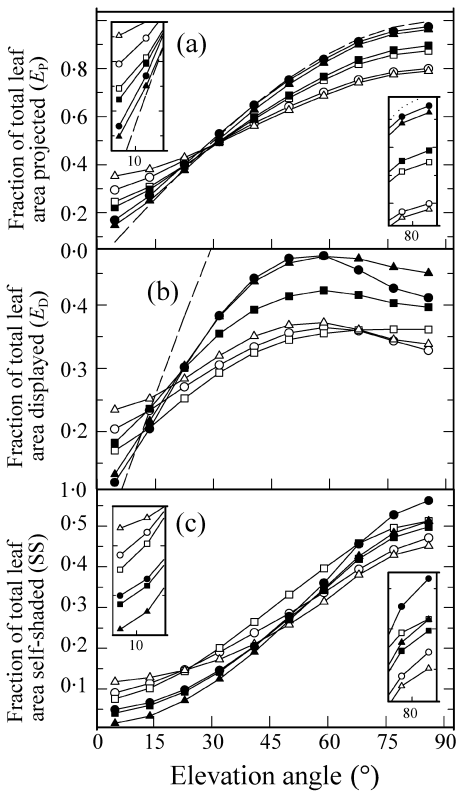

Fig. 4. Fraction of total leaf area projected to the incident PPFD (a), projected and not shaded (b) and both projected and shaded (c) as a function of solar elevation angle in plants from the rock (□, ■), garrigue (○, ●) and forest (▲, △) populations, grown at 100% (open symbols) and 20% (closed symbols) full sunlight compared to an equivalent horizontal surface (— — —).

was a significant interaction between provenances and treatments in E_p ($F_{2,18} = 5{\cdot}2$, $P < 0{\cdot}05$). This interaction was due to the close diurnal evolution of these variables in sun and shade plants of rock provenance in contrast with those of plants from the other populations (Fig. 4). The similarity between the responses of the rock plants to the sun and shade treatments was reflected in the small plasticity of this population in E_p at low and high solar angles, and in E_d at high solar angles (Fig. 2). In E_p, E_d and SS, the forest population was either equally or more plastic than any other population (Fig. 2).

Obviously, plants absorbed more PPFD per day in the sun than in the shade treatment, but when light absorption was expressed as a percentage of the PPFD theoretically absorbed by a horizontal surface, plants absorbed a proportionally larger PPFD in the shade than in the sun ($F_{1,18} = 11{\cdot}5$, $P < 0{\cdot}003$). However, this difference in the light absorption efficiency was not significant in plants from the rock population (Table 5), consistent with the small plasticity they exhibited for this character (Fig. 2). As for the previous sets of characters, the populations repeated the same pattern when ranked in order of decreasing plasticity of plant architecture features: forest (0·30) > garrigue (0·26) > rock (0·15), and differences in ranking between the forest and the rock population were significant ($\chi^2_{6,1} = 6{\cdot}0$, $P < 0{\cdot}01$).

Discussion

DEGREE OF PLASTICITY IN LIGHT-RESPONSIVE FEATURES

Consistent with the expected developmental flexibility of long-lived organisms in responding to environmental variations (Sultan 1987), *Q. coccifera* seedlings expressed different phenotypes in response to differences in light intensity. Among 40 traits tested in plants of the same age, 33 traits responded significantly to a quantitative variation in the light environment. These characters were plastic according to Schlichting's (1986) definition, although a potential contribution of developmental instability to phenotypic variation cannot be excluded totally (Alados *et al.* 1998). Plastic features 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. Plasticity was also detected in features at the organ level, such as nutrient allocation or specific leaf area. Variations in plant biomass did not account for the effect of the light treatment on biomass partitioning, indicating that its plasticity did not result from ontogenetic drift in biomass allocation patterns (Coleman, McConnaughay & Ackerly 1994; McConnaughay & Coleman 1999).

The most plastic features in response to the light treatment were leaf size, leaf angle and leaf area ratio. Variation in these features allows structural acclimation in *Q. coccifera* to spatial variations in the natural light environment, where light quantity and quality covary (Caldwell *et al.* 1986; Meister *et al.* 1987; Rambal *et al.* 1996), but it is of only limited value in modulating

light interception throughout diurnal cycles (Werner *et al*. 1999). Consequently, leaves of the outer crown must, periodically, withstand photoinhibitory irradiances at midday in exposed environments (Martínez-Ferri *et al*. 2000). In the present study, the light treatment had no effect on F_v/F_m. However, ratios were sub-optimal even in shaded plants. These ratios coincide with those recorded in summer, at the same provenance sites, in previous studies (cf. Martínez-Ferri *et al*. 2000; Werner *et al*. 1999), which suggests there is site-independent seasonal variation in this characteristic (Werner *et al*. 1999 found optimal ratios only in autumn).

DIVERGENCE IN THE PLASTICITY OF LIGHT-RESPONSIVE TRAITS AS A SIGN OF ECOTYPIC DIFFERENTIATION

Previous studies have concluded that the degree of plasticity of a phenotypic feature is dependent on the species, but not on the level of organization of the trait (Robinson & Rorison 1988). Independently of the level of analysis, the *Q. coccifera* populations were invariably ranked in the same order of decreasing mean plasticity when averaged over light-responsive features: forest > garrigue > rock. Our findings indicate taxon dependency, implying ecotypic differentiation within *Q. coccifera.* In this species, a change in plasticity may be a consequence of habitat-based selection, as proposed by Lortie & Aarssen (1996). Our estimate of genetic variation suggests that where ecotypic differentiation gave rise to greater phenotypic plasticity, greater genetic variation at the population level was also promoted. This is consistent with other reports (Sultan 1987; Sultan 1996), which concluded that phenotypic plasticity tended to obscure selective differences among genotypes, maintaining genetic variation largely unavailable to selection. We cannot completely rule out the possibility that the phenotypic plasticity in the forest population was overestimated due to a biased distribution of genotypes between light treatments, but this seems unlikely given the experimental design.

Ecotypic differentiation was assessed in a common garden experiment to avoid confounding effects derived from wide-range comparisons across environments and genotypes, as recommended in previous studies (Dudley & Schmitt 1995; Oleksyn *et al*. 1998). Nevertheless, since plants were less than two years old, maternal effects associated with the parental environment cannot be excluded. However, parents in unfavourable environments modulate their commitment of resources to offspring to maintain offspring size and quality, mainly at the expense of reducing the number of offspring (Sultan 1996). Even if parental plants had been unable to buffer their offspring fully from their own light, moisture and nutrient limitations, the lack of differences between *Q. coccifera* provenances in traits that are prone to influence by maternal effects, such as total seedling biomass (Leiva & Fernández-Alés 1998) or total N content (Sultan 1996), suggests that maternal effects had only limited influence on the differential response of the populations.

Evergreen Quercus species in the Mediterranean Basin are descendants of lineages that did not evolve under contemporary Mediterranean climates (Palamarev 1989). Their character syndrome might have persisted unchanged since they were subject to the pre-Mediterranean conditions of the pre-Pliocene periods (Herrera 1992). However, populations of evergreen Quercus at two of the provenance sites of the present study (i.e. rock and garrigue) only date back around 5000 years (Jalut *et al*. 1997). Consequently, the agreement between the populations' differences in the plasticity of the response to light intensity and the expected heterogeneity of the light environment in their current habitats suggests a relatively recent ecotypic differentiation in *Q. coccifera*.

For plasticity in response to the light environment to be interpreted as a product of adaptive processes, the phenotype evoked by the environment must have a greater fitness compared to its alternatives (Dudley & Schmitt 1996). Although the difference in root/shoot partitioning between forest and garrigue plants might ultimately be related to fitness (Pigliucci 1997), it cannot be attributed to a greater plasticity in the forest population. Plants from this population did not differ in root/shoot partitioning from those from the least plastic rock population. Phenotypes arising under uniform light regimes do not reflect the selective advantage of plasticity in variable light environments (Sultan *et al*. 1998). In heterogeneous light environments, a requirement for dynamic acclimation of the photosynthetic apparatus (Anderson, Park & Chow 1997) would also imply a selective advantage of the larger pool of photoprotective xanthophylls and the differential plasticity in leaf azimuth in plants from the forest population.

ECOLOGICAL IMPLICATIONS OF PLASTICITY IN *QUERCUS COCCIFERA*

Phenotypic plasticity tends to be positively associated with a broader ecological distribution (Cordell *et al*. 1998; Sultan *et al*. 1998; Bell & Sultan 1999; Valladares *et al*. 2000a). However, our results suggest that the adaptive value of phenotypic plasticity varies across the geographical range of *Q. coccifera*. The smaller phenotypic plasticity in the light response of most characters in the rock population can be interpreted as a sign of specialization in a homogeneous light environment (Sultan 1987). Generalizations about phenotypic plasticity based solely on the response to light intensity have to be considered with caution, but the intraspecific differences observed in our experiment are also consistent with variations in the adaptive value of plasticity along a gradient from more favourable to less favourable environments (Lortie & Aarssen 1996), or from wet to dry sites (Schlichting 1986).

Evidence of ecotypic differentiation in plants from the rock outcrop does not support the interpretation of this population being the result of a poorly competitive character syndrome that prevents individuals from inhabiting less harsh environments, as reported for other taxa (Walck, Baskin & Baskin 1999). On the contrary, the observed differences among populations suggest that, besides the well-known stress tolerance of *Q. coccifera*, ecotypic differentiation in this species could also account for its occurrence in contrasting habitats.

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