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ORIGINAL ARTICLE

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Within- and among-tree variation in leaf morphology of *Quercus petraea* (Matt.) Liebl. natural populations

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Abstract Variation in leaf morphology of *Quercus petraea* in response to several ecological conditions has been studied extensively, although not explicitly in the context of within- and among-tree variation. This study examined leaf morphology and anatomy of *Q. petraea*, growing in five natural Italian populations adapted to different ecological environments, to understand the pattern of within- and among-tree variation in this species. We used an ANOVA model with both crossed and nested effects. All levels contributed significant components of variation. Within-tree variation due to branch position was large, particularly in thickness and productivity (40%). For 19 of 32 variables, the variation among trees was surprisingly lower than the within-tree variation explained by branch position. Trends in leaf morphology and anatomy with branch position exhibited the sun-shade dichotomy. Patterns of crown plasticity showed lower values in the two xeric populations. Results suggest the need for taxonomic studies to consider variation as a quantitative attribute of individual trees.

Keywords *Quercus petraea* · Morphological traits · Variation · Position effect · Plasticity

Introduction

Leaves are the most important organs for plant production. Leaf structure is a compromise between what is required for photosynthetic gain and what is necessary for transpirational loss.

It is well known that arrangement, size, shape and anatomy of leaves differ greatly in plants growing in different environments. On the other hand, few studies have addressed the within-tree variation in leaf morphology. The great leaf variability existing among taxa and among individuals within taxa may overwhelm our perceptions of variability within individual plants. However, a high degree of variation among vegetative characters within trees is evident in studies such as those by Sokal et al. (1986), Blue and Jensen (1988) and Ashton et al. (1998).

Within-tree variation in oak leaf morphology has been widely recognized (De Rivas 1972; Baranski 1975; Olsson 1975; Blue and Jensen 1988). The wide range of morphological variation can often confound interpretation of comparisons among trees of the same species. For this reason, Blue and Jensen (1988) recommend that intraspecific comparisons should be based "on leaves collected at approximately the same height and location and either (1) on the same date or (2) after leaf growth has stopped".

To our knowledge, no such study has examined foliar structure at this scale for *Quercus petraea* (Matt.) Liebl. (sessile oak), a shade-tolerant species with a widespread range across Europe.

Though much work has been done determining the differences in leaf morphology and anatomy between *Q. petraea* populations (Dupouey and Badeau 1993; Bruschi et al., unpublished data), little has been done to examine these relationships at intra-population and intra-individual level. The sessile oak is interesting for this kind of study because it shows extensive variation both in leaf morphology and in tree architecture (Dupouey and Badeau 1993; Bacilieri et al. 1995; Buck-Sorlin and Bell 2000).

Italian populations are at the southern end of the European distribution range and their study is of great interest to understand the evolutionary history of the species and to reconstruct the recolonization routes in the post-glacial period (Dumolin-Lapegue et al. 1998).

Italian sessile oaks show great levels of phenotypic and genetic diversity that are evidence of this species' marked capacity to adapt to several and difficult ecological conditions. However, particularly in central and southern Italy, the occurrence of *Q. petraea* can be described as sporadic, because many forest habitats have been transformed into agricultural fields.

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In this paper, we quantify patterns of variation in leaf characteristics within and among *Q. petraea* trees in populations adapted to different ecological conditions. The level of intra- and inter-population diversity of these populations of *Q. petraea* was studied in a previous work by using morphological markers and hypervariable molecular markers such as microsatellites (Bruschi et al. 2000, unpublished data).

Materials and methods

A total of 100 even-aged trees of *Q. petraea* were sampled in 1997 from five stands in Italy. Three of these stands (Monte Corona, Tatti and Monterufoli) are located in central Italy, one (Carrega) in northern Italy and one (Piano Costantino) in southern Italy (Table 1). In spite of its sporadic occurrence, sessile oak can be found over the whole Italian range, with the exception of Sardinia. Only one natural *Q. petraea* population is known to grow in Sicily on the Madonie mountain chain (Piano Costantino). The sampled populations encompass the Italian peninsular range of the species and are isolated from each other except Tatti and Monterufoli, which are neighbouring.

The sampling design and methods were similar for each population. We first selected 20 mature trees from a relatively small area (0.5–1 ha) of apparently homogeneous open canopy sessile oak forest. Trees were 8–10 m tall. From the crown of each tree we randomly selected a total of four outermost branches (light subsample) and four innermost branches (shade subsample). Branches were collected from the four cardinal compass directions, avoiding lammas and epicormic shoots. Forty fully expanded leaves and eight twigs were randomly selected from each subsample after the elimination of broken, incomplete or damaged units. The leaves were practically of the same age, although there is a small variation in budburst both among trees and within trees (personal observations). In our experimental design, we considered only branch position because, as shown by Baranski (1975) and Blue and Jensen (1988), the most important factor in within-plant variation is position (inner vs outer) regardless of compass direction or height.

The list of traits analysed is reported in Table 2 (Bruschi et al. 2000). Macromorphological characteristics were determined for 20 leaves of each subsample. Assessment of pubescence was carried out on five leaves and four twigs for each subsample, which were assigned to one of six classes following Bruschi et al. (2000).

Portions from the central region of five leaves on each of 100 plants were fixed in FAA. Three 30 µm cross-sections of each portion were cut with a freezing microtome and viewed using light microscopy. Leaf thickness was measured in different places within each section, but avoiding the region around the mid-rib, using an ocular micrometer at ×100 magnification.

The total surface area was measured with a LICOR LI–3100 Leaf Area Meter on ten leaves stripped of the petiole for each subsample. Afterwards these leaves were dried at 70°C for 72 h and leaf mass per area was calculated as the dry weight (mg) divided by lamina area (cm^2).

Micromorphological characteristics were determined by analysing images of four leaves for each subsample using a scanning electron microscope (Bruschi et al. 2000).

Table 2 List of morphological and anatomical characters examined

Macromorphological measures	
LP (cm)	Length of petiole
MWL (cm)	Maximal width of lamina
HMW (cm)	Height of maximal width (length of lamina from terminal lobe to widest part)
MDS (cm)	Maximal depth of sinus
WHL (cm)	Width of the widest lobe
DVL (cm)	Distance of principal vein to top of the widest lobe
DS (cm)	Distance of the principal vein to the sinus (placed below the widest lobe)
WTL (cm)	Width of the terminal lobe
LL (cm)	Length of lamina
Thickness measures	
TTL (µm)	Total thickness of lamina
TUE (µm)	Thickness of upper epidermal cells
TP (µm)	Thickness of palisade cells
TS (µm)	Thickness of spongy cells
TLE (µm)	Thickness of lower epidermal cells
Productivity measures	
AREA (cm^2)	Leaf area
LMA (mg cm^2)	Leaf mass per unit area
Micromorphological measure	
SD (no. cm^2)	Stomatal density
LS (µm)	Length of stoma
WS (µm)	Width of stoma
FR (µm)	Freedom of rim (width of stomatal opening uncovered from waxes)
NST (no. cm^2)	Number of stellate trichomes
NGT (no. cm^2)	Number of glandular trichomes
NR (no.)	Number of rays in stellate trichomes
LRS (µm)	Length of rays of stellate trichomes
SAI	Stomatal area index (stomatal density × stomatal length)
SA (μm^2)	Stomatal area
Pubescence measures	
DOR (%)	Midrib on abaxial surface (dorsal)
AXI (%)	Midrib on abaxial surface (axillar)
PET (%)	Petiole
TSH (%)	Terminal shoot
TW (%)	Twig

Assumptions of normality were checked with Shapiro-Wilk's test. Normality of distribution of characters was assessed for all variables, except DVL, DS, Area, LMA and SD, which showed a high right asymmetry. Normality for these traits was obtained after logarithmic transformation (Sokal and Rohlf 1995). An analysis of variance (ANOVA) with both nested and crossed effects was performed following the model:

$$Y = P + B + PB + T(P) + \varepsilon \quad (1)$$

to test the main effects of population (P) and branch position (sun/shade) (B), their interaction, and the nested effects of tree

Table 1 Characteristics of the study areas

Label	Locality	Coordinates	Altitude (m)	Precipitation[a] (mm)	Temperature[a] (°C)	Parent-rock
CR	Carrega	44°43′N, 10°80′E	200	860	12.3	Clay
TT	Tatti	43°21′N, 10°56′E	550	870	13.0	Sandstone
MR	Monterufoli	43°16′N, 10°45′E	400	880	13.5	Serpentine
MC	Monte Corona	43°13′N, 12°17′E	600	825	13.5	Sandstone
MAD	Madonie	37°53′N, 14°40′E	1417	687	18.8	Schist

[a] Mean annual value

Table 3 *F*-statistics for the analysed traits from a nested-crossed analysis of variance. (*n.s.* not significant at $P > 0.05$; * $0.01 < P < 0.05$; ** $0.01 < P < 0.001$; *** $P < 0.001$)

Macromorphological measures										Thickness measures				
Variance component	LP	MWL	HMW	WHL	MDS	DVL	DS	WTL	LL	TTL	TUE	TP	TS	TLE
Population	3.1*	2.0 n.s.	1.2 n.s.	3.2*	1.72 n.s.	1.10 n.s.	0.47 n.s.	2.73 n.s.	6.67**	12.1***	1.20	7.82**	6.58**	2.42*
Position	21.8***	8.9**	0.40 n.s.	1.15 n.s.	0.17 n.s.	2.85 n.s.	8.56**	0.10 n.s.	7.83**	8.54**	35.22***	12.23**	21.97***	0.45 n.s.
Trees within population	1.7 n.s.	2.4**	3.1***	1.43 n.s.	0.84 n.s.	2.18*	3.70***	0.72 n.s.	1.56 n.s.	2.77**	1.44 n.s.	1.76 n.s.	1.07 n.s.	0.84 n.s.
Population × position interaction	1.7 n.s.	1.1 n.s.	0.57 n.s.	5.2***	0.63 n.s.	0.48 n.s.	0.48 n.s.	0.86 n.s.	3.12*	0.54 n.s.	0.15 n.s.	1.29 n.s.	0.77 n.s.	2.96 n.s.

Productivity measures			Pubescence measures				
Variance component	AREA	LMA	DOR	AXI	PET	TSH	TW
Population	7.65**	3.25*	1.12 n.s.	9.23***	1.92 n.s.	4.32*	8.65***
Position	16.92***	10.0**	0.02 n.s.	0.13 n.s.	0.35 n.s.	0.39 n.s.	2.62 n.s.
Trees within population	2.33**	2.18*	0.97 n.s.	0.81 n.s.	1.62 n.s.	2.85**	1.14 n.s.
Population × position interaction	1.33 n.s.	1.63 n.s.	0.42 n.s.	1.65 n.s.	0.98 n.s.	1.07 n.s.	1.39 n.s.

Micromorphological measures										
Variance component	SD	LS	WS	FR	NST	NGT	NR	LRS	SAI	SA
Population	10.89**	7.69**	4.35*	3.3*	3.42**	5.0**	3.07 n.s.	4.57**	0.88 n.s.	3.85*
Position	8.47**	0.14 n.s.	4.64**	16.97***	17.97***	0.93 n.s.	0.29 n.s.	12.55**	13.5***	8.43**
Trees within population	5.72***	2.07*	2.01 *	4.93***	1.62 n.s.	4.37***	1.02 n.s.	1.54 n.s.	1.4 n.s.	1.6 n.s.
Population × position interaction	0.98 n.s.	0.2 n.s.	0.42 n.s.	1.14 n.s.	0.59 n.s.	1.53 n.s.	0.69 n.s.	0.86 n.s.	0.45 n.s.	0.97 n.s.

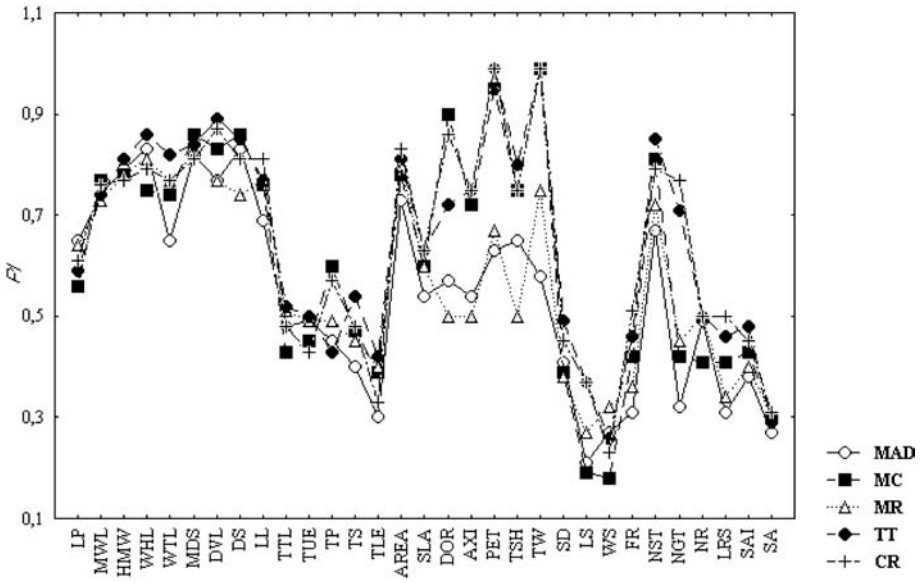

Fig. 1 Plot of plasticity (*Pl*) for the five analysed populations

within population *T* (*P*) on all measured traits. The population by branch position interaction was calculated to determine if the effect of position varied significantly among populations. This model had two fixed factors (*P* and *B*) and one random effect (*T*). Populations cannot be considered a random sample of the variation within the species: they were selected to represent the Italian geographical range, but the choice was limited by the low availability of sessile oak provenances in central and southern Italy. The *F* statistic for *B* was calculated using the mean square from *PB*; all other *F* statistics were calculated with mean square error. Homogeneity of variances were tested at all levels with the Bartlett test (Sokal and Rohlf 1995). Patterns of variation were evaluated by comparing percent of total variance of the different hierarchical levels. Total within-population plasticity (Pl) was calculated by population for each measure using the smallest and the greatest mean values Pl=1–*(x/X)*, where *x* is the smallest value and *X* is the largest value for any given leaf measure (Ashton et al. 1998). To estimate measurement error, we measured each leaf twice using a small subsample of 100 leaves chosen across all samples. The percentage of measurement errors was 1.2–1.5% for macromorphological traits, 3.7–5.2% for thickness and productivity traits, 5.5–7% for pubescence and 0.7–1.3% for micromorphological traits. All these measurement errors were sufficiently small to allow reliable determination of the variables of interest. To investigate the multivariate relationships among characters and how they change with branch position in relation to environment, a discriminant analysis was carried out on the total data set. The scatterplot of the discriminant scores corresponding to each case of each population/position combination in the multivariate space defined by the first two functions was obtained to help visualize multivariate phenotypic variations. To verify the correct attribution of sampled trees to *Q. petraea* we also included in the analysis ten trees classified as *Q. pubescens* Willd, based on Bruschi et al. (2000). Statistica for Windows computer software was used for all statistical analyses.

Results

Variance analysis

All hierarchical levels (populations, trees within population, branch position and interaction between branch position and population) contributed significantly to variation in *Q. petraea* foliage (Table 3). Variation due to populations was significant in 20 of 31 traits ($P<0.05$); variation due to trees was significant in 13 of 31 traits ($P<0.05$); variation due to branch position was significant in 17 of 31 traits; and variation due to population × branch position was significant in only 2 of 31 traits ($P<0.05$) (Table 3).

Comparisons of variance components across leaf characteristics

Table 4 contains values for the percentage of the total variance within each morphological trait that was accounted for at each level of the hierarchy. Populations accounted for 14% of the variance in macromorphological traits, 18.5% in thickness and productivity traits, 25% in pubescence traits and 18% in micromorphological traits. Position of branches accounted for 21% of the variance in macromorphological traits, 40% in thickness and productivity traits, 7% in pubescence traits and 25% in anatomical traits. Trees within populations accounted for 19.5% of the variance in macromorphological traits, 12% in thickness and productivity traits, 19% and 16% in pubescence and micromorphological traits, respectively. Interaction between population and branch position accounted for 6–16% of the variance. Collectively, the different hierarchical levels accounted for 60–76% of the total variation in the leaf characteristics (most in morphology, pubescence and productivity traits, least in thickness and in micromorphology). Thus, variation among leaves within branch, plus variation within leaves, plus measurement error always constituted less than half of the total random variation, and as little as 24%.

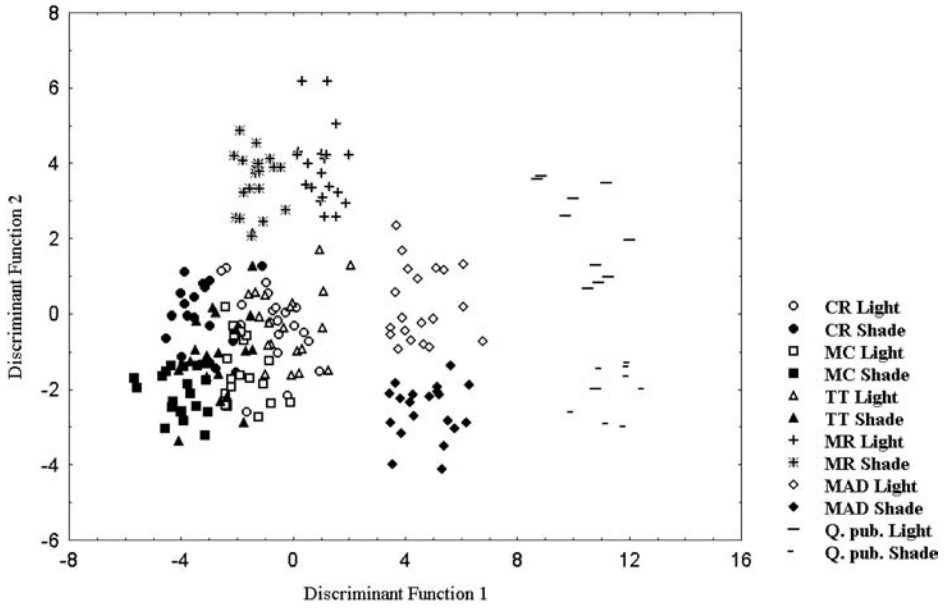

Fig. 2 Scatterplot of the discriminant analysis: discriminant scores for the first two functions of the means of considered parameters for the different populations are shown on the axes

Table 4 Partitioning of variation by hierarchical component in all traits of *Quercus petraea* foliage

Trait	Percentage of variation				
	P	B	T(P)	PxB	Error
LP	7.2	45.6	3.4	5.7	38.0
MWL	6.5	27.8	15.4	7.3	33.0
HMW	15.2	4.6	48.7	7.3	24.2
WHL	21.3	9.5	6.4	39.8	23.0
MDS	17.5	8.2	13.7	15.2	45.4
DVL	15.4	31.2	28.9	7.5	17.0
DS	5.0	54.3	21.2	6.3	13.2
WTL	20.5	7.6	10.3	12.2	49.4
LTL	16.2	2.3	27.3	42.4	11.8
TTL	28.3	31.2	15.5	2.4	22.6
TUE	7.3	53.9	12.2	4.8	21.8
TP	23.3	37.8	6.7	5.9	26.3
TS	21.1	58.3	4.1	3.2	13.3
TLE	28.5	11.3	15.4	16.5	28.3
AREA	18.5	45.4	16.6	2.5	17.0
LMA	14.3	40.8	13.5	7.9	23.5
DOR	23.5	5.6	18.9	7.5	44.5
AXI	29.8	6.8	20.5	17.6	25.3
PET	16.5	6.3	18.3	15.7	43.2
TSH	27.3	7.6	19.3	15.2	30.6
TW	36.5	9.8	16.7	20.4	16.6
SD	22.5	32.5	17.3	4.0	23.7
LS	18.5	4.6	15.4	10.7	50.8
WS	16.3	17.1	12.4	8.9	45.3
FR	20.2	43.5	15.7	8.9	11.7
NPS	19.4	53.4	6.7	2.5	18.0
NGT	20.3	4.6	24.5	11.7	38.9
NR	8.5	7.5	23.3	11.7	49
LRS	21.7	45.7	8.9	6.3	17.4
SAI	12.0	28.5	17.6	9.5	32.4
SA	17.4	15.4	13.9	8.7	44.6
Means	19.5	23.5	16.5	11.5	28.5

Differences in leaf attributes among branch position

Trends in leaf morphology and anatomy with branch position exhibited the sun-shade dichotomy. Greatest palisade (TP) and spongy layer (TS) thickness and overall leaf thickness (TTL), as well as the highest stomatal density (SD) and stomatal area index (SAI) were measured at the outermost part of the crowns (Table 5). Thinnest leaves, lowest stomatal density and SAI were measured at the innermost part of the crowns. Length of stomata (LS) was constant but width (WS) and freedom of rim (FR) was greater in sun than in shade leaves. Among the macromorphological traits, length of petiole (LP) was greater at the outer crown position while maximum width of lamina (MWL) was greatest at the inner crown position. DS values were higher in sun leaves. Leaf area (Area) was smallest at the outer crown position while leaf mass per unit area (LMA) was smallest at the inner crown position. Significant interaction between sun-shade dichotomy and population was observed only in two macromorphological traits (WHL = height of maximum width and LL = leaf length) (Table 3).

Leaf plasticity

Of all characters measured, greatest plasticity was found for pubescence traits (0.75) and macromorphology (0.77) (Table 5). Particularly, pubescence of petiole (PET) and pubescence of twig (TW) showed very high values: 0.85 and 0.86, respectively (Table 5). The differences among populations were high, with the two more xeric (Madonie and Monterufoli) showing the lower values for pubescence traits (Table 5, Fig. 1). Measures of plasticity for macromorphology were consistently about the same among populations. Plasticity of leaf thickness (0.46) and micromorphology (0.42) was relatively low. Total

Table 5 Measures of *Q. petraea* plasticity (*Pl*) in the five analysed populations for the various measurements of leaf structure. We report also the mean values for sun and shade leaves in each population. *Pl**= means of *Pl* for each trait. Means followed by the same letter in the same row are not significantly different at $P<0.001$ according to LSD test

Traits	Population															
	MAD			MC			MR			TT			CR			Pl*
	Sun	Shade	Pl	Sun	Shade	Pl	Sun	Shade	Pl	Sun	Shade	Pl	Sun	Shade	Pl	
LP	1.66d	1.45b	0.65	1.85f	1.71d	0.56	1.58c	1.37a	0.64	1.70d	1.57c	0.59	1.75e	1.63cd	0.61	0.61
MWL	4.42a	4.55b	0.74	5.54g	6.43i	0.77	4.75c	5.41f	0.73	4.88d	5.15e	0.74	5.21e	5.69h	0.76	0.75
HMW	4.45d	4.49d	0.79	5.98e	6.01e	0.78	3.72b	3.74b	0.78	3.55a	3.54a	0.81	3.83c	3.85c	0.77	0.79
WHL	0.87a	0.88a	0.83	0.98b	1.01b	0.75	1.15c	1.13c	0.81	1.10c	1.08c	0.86	1.03b	1.02b	0.79	0.81
WTL	0.45a	0.43a	0.65	0.67b	0.67b	0.74	0.65b	0.67b	0.77	0.58b	0.60b	0.82	0.60b	0.61b	0.77	0.75
MDS	1.44b	1.42b	0.82	1.38b	1.41b	0.86	1.29a	1.29a	0.82	1.31a	1.29a	0.84	1.30a	1.30a	0.81	0.83
DVL	2.65a	2.64a	0.77	3.0b	2.98b	0.83	2.75ab	2.79ab	0.77	2.63a	2.65a	0.89	2.78ab	2.80ab	0.87	0.83
DS	1.56a	1.81b	0.83	1.54a	1.80b	0.86	1.52a	1.81b	0.74	1.54a	1.78b	0.85	1.57a	1.79b	0.81	0.82
LL	7.86a	7.91a	0.69	9.85d	9.88d	0.76	9.71d	9.75d	0.77	9.49c	9.53c	0.77	9.21b	9.18b	0.81	0.76
Means			0.75a			0.76a			0.76a			0.80b			0.77a	0.77
TTL	206.80e	185.70d	0.48	167.60bc	149.40a	0.43	193.2de	162.70b	0.51	189.40d	172.0c	0.52	173.80c	150.70a	0.48	0.48
TUE	25.70b	21.50a	0.49	23.50b	19.60a	0.45	24.60b	21.10a	0.49	23.50b	20.10a	0.50	23.20b	19.40a	0.43	0.47
TP	87.70d	78.50c	0.45	82.30 cd	74.20c	0.60	79.60c	65.40b	0.49	81.10 cd	74.90c	0.43	67.70b	56.80°	0.57	0.50
TS	77.70a	69.80c	0.40	47.90b	41.60a	0.47	73.80d	61.20c	0.45	70.60d	63.10c	0.54	69.80d	61.20c	0.48	0.47
TLE	15.7b	15.9b	0.30	13.9a	14.0a	0.39	15.2b	15.0b	0.40	14.2a	13.9a	0.42	13.1a	13.3a	0.33	0.37
Means			0.42a			0.47b			0.47b			0.48b			0.46b	0.46
AREA	19.70a	24.90b	0.73	25.20b	33.40c	0.78	25.30b	30.76c	0.80	24.67b	34.10c	0.81	28.40bc	35.30c	0.83	0.79
LMA	15.95d	11.04c	0.54	10.51c	7.25ab	0.60	12.62c	8.97b	0.60	10.53c	6.88a	0.63	8.62b	6.35a	0.63	0.60
Means			0.63a			0.69bc			0.70b			0.72c			0.77d	0.69
DOR	2.23c	2.22c	0.57	1.30a	1.32a	0.90	2.19c	2.20c	0.50	1.51b	1.50b	0.72	1.29a	1.30a	0.86	0.71
AXI	3.05e	3.03e	0.54	1.10a	1.12a	0.72	2.06a	2.08d	0.50	1.35c	1.37c	0.75	1.25b	1.24a	0.75	0.65
PET	1.84b	1.86b	0.63	1.23a	1.19a	0.95	1.17a	1.19a	0.67	1.24a	1.20a	0.99	1.18a	1.21a	0.99	0.85
TSH	2.55c	2.53c	0.65	2.10b	2.13b	0.75	2.31c	2.35c	0.50	1.83a	1.85a	0.80	1.71a	1.73a	0.75	0.69
TW	2.07d	2.05d	0.58	0.84c	0.81c	0.99	0.99c	0.97c	0.75	1.05c	1.01c	0.99	0.59a	0.58a	0.99	0.86
Means			0.59a			0.86b			0.58a			0.85b			0.87b	0.75
SD	398.2d	371.8 cd	0.41	351.4c	334.8b	0.39	498.9f	436.7e	0.38	341.3b	302.6 g	0.49	382.3d	329.7b	0.45	0.42
LS	23.10a	23.40a	0.21	26.06b	26.0b	0.19	22.75a	23.07a	0.27	25.12ab	25.87ab	0.37	24.35a	24.51a	0.37	0.28
WS	17.47a	17.85a	0.27	18.87b	18.65b	0.18	18.35b	18.26b	0.32	22.82c	21.97c	0.26	19.75b	19.93b	0.23	0.25
FR	4.37a	6.45c	0.31	6.09c	8.25e	0.42	5.24b	7.02d	0.36	9.64f	11.08 h	0.46	8.02e	9.58f	0.51	0.41
NPS	79.64d	76.49d	0.67	42.78b	37.51a	0.81	46.09c	43.32b	0.72	45.87c	42.03c	0.85	42.65b	39.76a	0.79	0.77
NGT	67.98c	67.35c	0.32	124.87d	125.12d	0.42	61.54b	62.03b	0.45	62.78b	62.67d	0.71	56.57a	56.74a	0.77	0.53
NR	3.30a	3.30a	0.49	3.01a	3.30a	0.40	4.00b	3.70ab	0.50	3.30a	4.00b	0.50	3.00a	3.30a	0.50	0.48
LRS	110.87c	107.23c	0.31	114.98d	110.32c	0.40	93.65b	91.22b	0.34	115.76d	111.57a	0.46	83.59a	80.77a	0.50	0.40
SAI	9,198c	8,700b	0.38	9,157c	8,700b	0.43	11,343d	10,074d	0.40	8,573f	7,828b	0.48	9,366c	8,080d	0.45	0.43
SA	214.89a	222.41b	0.27	261.85c	258.20c	0.30	222.29b	224.31b	0.30	305.24d	302.65d	0.29	256.08c	260.11c	0.31	0.29
Means			0.36a			0.39b			0.40b			0.48c			0.49c	0.43
Total			0.55a			0.63b			0.58a			0.67c			0.65c	0.62

pattern of variation shows that Madonie and Monterufoli were the least plastic, while Tatti and Carrega are the most plastic. Montecorona is intermediate between these two groups.

Multivariate analysis

To investigate multivariate associations among the characters measured, we performed a discriminant analysis on data for all populations in both branch positions. Three of the seven roots had a significant associated eigenvalue. Traits highly correlated with each of the three functions are reported in Table 6. We plotted the population cases on the root 1/root 2 plane (Fig. 2). Function 1 accounts for 52% of the total variance and separates Madonie (MAD) population from the others and light subsamples of Monterufoli (MR), Carrega (CR), Montecorona (MC) and Tatti (TT) from the respective shade subsample. This function separates clearly all populations of *Q. petraea* from *Q. pubescens*. Function 2 represents another 28% of the total variance and separates Monterufoli from the other populations and Madonie light subsamples from Madonie shade subsamples. The matrix of cases correctly classified (Table 7) shows that Madonie and Monterufoli subsamples were the ones with higher discriminating power (100% of cases correctly classified).

Table 6 Standardized coefficients for the statistical multivariate analysis

	Root 1	Root 2	Root 3
TTL		–1.04	–0.48
AXI	0.35		–0.37
TS	–0.45	0.58	–0.48
NGT	–0.39		
MWL		–1.33	
TSH	0.84		
TP		0.52	
LRS	0.92		
TW	–0.85		
DS	0.42	0.35	0.53
LMA		–0.50	
FR	0.82	0.43	
LL	–1.62	0.40	–0.36
PET	0.74	–0.35	
TUE		0.30	
DOR	–0.67		
Eigenvalue	10.53	5.63	2.13
% of explained variance	52.0	28.0	10.0

Discussion

Results clearly demonstrate the high phenotypic diversity of the sessile oak: several measured leaf parameters differed significantly among populations, among trees within the same population and between branch positions. This coincides with the results of Kleinschmit et al. (1995) and Bruschi et al. (unpublished data) who examined morphological and molecular characters of *Q. petraea* populations.

However, if extensive variation has been reported for several studies of *Q. petraea* foliage, the magnitude and complexity of within- and among-tree variation has not been generally appreciated.

Two sources of within-tree variation were identified. First, a branch position effect was found. Branch position (innermost/outermost) accounted for more of the variance than trees within population. In anatomical and productivity traits, branch position accounted for four times as much variation as among trees. For 19 out of 32 variables, the within-tree variation explained by branch position was greater than the variation among trees (Table 3). The high intra-individual variation is a usual finding (Baranski 1975; Blue and Jensen 1988), although several species have been found to show little variation at this level and sometimes different populations of the same species have different patterns of varia-

Table 7 Cases correctly classified (P=0.091)

	% of correct cases	CR Light	CR Shade	MC Light	MC Shade	TT Light	TT Shade	MR Light	MR Shade	MAD Light	MAD Shade	*Q. pubescens* Light	*Q. pubescens* Shade
CR Light	45	9	2	2	0	6	1	0	0	0	0	0	0
CR Shade	55	1	11	1	2	0	5	0	0	0	0	0	0
MC Light	70	1	1	14	0	0	4	0	0	0	0	0	0
MC Shade	90	0	0	0	18	0	2	0	0	0	0	0	0
TT Light	50	7	0	1	0	10	0	2	0	0	0	0	0
TT Shade	25	2	4	5	3	0	5	0	1	0	0	0	0
MR Light	100	0	0	0	0	0	0	20	0	0	0	0	0
MR Shade	100	0	0	0	0	0	0	0	20	0	0	0	0
MAD Light	100	0	0	0	0	0	0	0	0	20	0	0	0
Mad Shade	100	0	0	0	0	0	0	0	0	0	20	0	0
Q. pubescens Light	100	0	0	0	0	0	0	0	0	0	0	10	0
Q. pubescens Shade	100	0	0	0	0	0	0	0	0	0	0	0	10
Total	75.90	20	18	23	23	16	17	22	21	20	20	10	10

tion. Second, variation among leaves within a branch and variation within leaves was found. Although the variation displayed by these factors was not explicitly addressed, it is part of the error term. Because random errors, due to imprecise measurements, were judged to be low, this source of variation also seems to be consistently high.

Traits that accounted for the highest percentage of the within-tree variation were related to sun-shade dichotomy. Environmental heterogeneity represents an extrinsic source of within-tree variation. Variability in the light or microclimate encountered by different plant structures can affect leaf characteristics. Presumably, the most significant differences between sun and shade leaves of *Q. petraea* are largely adaptive, enabling a more efficient use of resources. The outer crown is a more stressful environment for leaves than the inner crown. Leaves on the outside are subjected to greater cold, heat, sun radiation, water stress and herbivory load (Nielsen and Ejlersen 1977). Outer leaves radiate more heat than inner leaves and are more susceptible to radiational cooling (Treshow 1970), and outer leaves that experience water stress or cold temperatures may be more susceptible to photoinhibition (Demming-Adams and Adams 1992). The smallest but thickest leaves were at the top or outer part of the crown, and the largest were the shade leaves in the innermost part. In general, the development of the palisade layer of cells was markedly thicker in sun leaves. As reported by Gutschick (1999), the development of palisade layer(s) is most responsive to light level. The lower epidermal layer shows no difference among branch positions. According to Ashton et al. (1998) this could suggest that the abaxial surface is shielded by upper tissues from the extremes of radiation and humidity fluxes. The greater length of the petiole observed in sun leaves can have an important effect on the spatial distribution of leaves and therefore on light interception. Sun leaves are more deeply lobed than shade leaves. A highly dissected leaf margin decreases the effective size of the leaf and the control of assimilation rate and transpiration rate by stomata is kept higher than in whole leaves. Trends in stomatal density and dimension also reflected patterns in responsiveness to moisture within the crown.

Differences in plasticity were found among the populations. In particular, the Madonie population showed mean values lower than the others. This population is situated at the southernmost part of the sessile oak biogeographic range and isolation and environmental selection could explain the lower plasticity versus the accumulation of well adapted morphotypes. In effect, it is highly divergent from the other populations, both at a morphological and a molecular level and it shows also a lower average heterozygosity, that is the probable result of genetic drift forces (Bruschi et al., unpublished data). In controlled field provenance trial conditions it does not seem to show any plasticity for any character analysed in this study (unpublished observations). This finding suggests that most of the aspects of its phenotype could be canalized against environmental variation (at least for traits to which fitness is more sensitive), that is to say that this population remains phenotypically uniform even if it is exposed to wide fluctuations of the environment (Waddington 1941).

Large differences were observed between the neighbouring populations of TT and MR in plasticity values for macromorphology, pubescence and micromorphology. These two populations experience the same climate and they are genetically very similar (Bruschi et al., unpublished data), but they show great morphological differences probably due to edaphic factors. Monterufoli sessile oak grows on ophiolitic soils, where water stress, together with soil nutritional deficiencies, may have led to development of stable xeromorphic traits. We are planning further experiments to test the phenotypic plasticity of these two populations in controlled conditions.

Contrary to our expectations, among-tree variation has been found to be lower than within-tree variation. This finding could be dependent on the sampling modalities. Most natural populations of organisms represent a mixture of individuals of different genotypes or individuals from different growth environments. Statistical conclusions about properties of the entire heterogeneous population may differ from those relating to identifiable subsamples within the combined population.

In conclusion, our results may have general implications for the design of experiments involving trees. Although the optimal sampling design will depend upon the research objectives, it will always be influenced by the patterns of variance within and among trees. This is particularly true in the case of the genus *Quercus*, where the high intraspecific variation does not often allow a correct attribution of an individual tree or a population to one or other species with certainty (Bruschi et al. 2000). High intra- and inter-tree variation and erroneous sampling methods are the root of the great problems of classification in the genus *Quercus*. Many systematic studies estimate tree properties by sampling leaves haphazardly from throughout each tree. We suggest that leaves be selected either (1) at random with respect to position (inside/outside) of the branches within the crown or (2) consistently from the same position. A better strategy is to sample leaves from different regions of the crown, including information in the analysis about how many branches and shoots were sampled, how the samples were selected (randomly, haphazardly from those branches that could reached, etc.) and about the positional status of the leaves (light or shade).

We are conducting further studies to determine how these factors, especially position and compass direction, influence among-species comparisons in the subgenus *Quercus*.

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