

Geometric morphometric analysis as a tool to explore covariation between shape and other quantitative leaf traits in European white oaks

Vincenzo Viscosi, Anna Loy, Paola Fortini

Abstract — In this study, 2-Block Partial Least-Squares analysis was used to explore the pattern of covariation between two sets of leaf morphological data (landmarks and traditional measures), sampled on 273 trees in a mixed forest of *Quercus frainetto*, *Q. petraea* and *Q. pubescens*, in southern-central Italy. Two groups of related variables were detected and the three oak species were highly significant discriminated by CVA computed on dimensions extracted by 2B-PLS analysis. *Q. frainetto* was characterized by obovate leaf blade with a short petiole and leaves were greater than in the other species. *Q. petraea* was differentiated by acute basal and apical regions, while *Q. pubescens* has higher values of leaf compactness, pubescence and length of trichomes. The high degree of classification accuracy of this combined approach advocates its extension to other problematic species and highlights its importance as an exploratory tool in plant ecology, physiology and taxonomy.

Index Terms — geometric morphometrics, leaf shape, *Quercus*, Two-Block Partial Least-Squares.



1 INTRODUCTION

The morphological variability in subgenus *Quercus* has been studied for a long time. Several analyses, usually based on leaf morphological data, supported the existence of high variability within and among these oak species ([1], [2], [3], [4], [5]). Here a landmark based geometric morphometric approach is used to analyse the leaf morphological pattern of covariation

V. Viscosi is with the Museo Erbario del Molise, Department STAT, University of Molise, Pesche (IS) IT-86090. E-mail: vincenzo.viscosi@unimol.it.

P. Fortini is with the Museo Erbario del Molise, Department STAT, University of Molise, Pesche (IS) IT-86090. E-mail: fortini@unimol.it.

A. Loy is with Department STAT, University of Molise, Pesche (IS) IT-86090. E-mail: vincenzo.viscosi@unimol.it. E-mail: loy@unimol.it.

between shape and other traditional traits in three white oak species. We used Two-Block Partial Least-Squares analysis (2B-PLS) [6] (i) to summarize the greater part of leaf phenotypic variability in few linear dimensions, (ii) to detect the degree of differentiation among species, (iii) to obtain a useful classification method for unidentified specimens.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL AND DATA SAMPLING

A total 273 oaks were sampled in a woody community (Italy, Molise) hosting three hybridizing white oak species. Species assignment followed Schwarz (1996): *Q. petraea* (n=122), *Q. pubescens* (n=57), *Q. frainetto* (n=56), hybrids (38). Ten leaves for each tree were photographed for morphological analyses. Eleven landmarks and 25 traditional morphological characters were collected on each leaf [5]. Eight morphological characters (area, perimeter, roundness, elongation, feret diameter, compactness, major axis length, minor axis length) were automatically recorded on digital images by means of ImageTool ver. 5.1 [7]; five variables were recorded manually by a digital calliper: lamina length (LL), petiole length (PL), lobe width (LW), sinus width (SW), length of lamina at largest width (WP), number of lobes (NL) and number of intercalary veins (NV). Five variables were obtained by ratios between previously measured variables [1]: lamina shape (OB), petiole ratio (PR), lobe depth ratio (LDR), percentage venation (PV), lobe width ratio (LWR); four variables were observed and recorded as ordinal variables ([1], [8],): basal shape (BS), pubescence of abaxial surface, adaxial surface and petiole. Finally, the length of hairs was measured [9].

2.2 Statistical analysis

For each tree, the mean of 25 leaf variables and the landmark consensus configuration was computed. 2B-PLS analysis was used to investigate the covariation pattern among the 11 shape descriptors and the 25 traditional variables. Linear combinations of partial warps and other quantitative traits were visualized as deformation grids through tpsPLS 1.18 [10].

Finally, the linear dimensions, derived from 2B-PLS analysis, were analysed by Canonical Variate Analysis (CVA) to detect the degree of species differentiation among species and to obtain a classification rule for unidentified specimens.

3 RESULTS

The first two significant dimensions (2B-PLS) accounted for 95.34% and 2.96% of total covariance, respectively. The correlations between shape descriptors and morphological variables for the two first dimensions were 0.884 ($p < 0.001$) and 0.780 ($p < 0.001$), respectively.

The ordination plot showed a clear separation of *Q. frainetto*, *Q. pubescens*

and *Q. petraea* (Fig. 1): *Q. frainetto* is clearly distinguished from both *Q. petraea* and *Q. pubescens*, while the two latter partially overlapped. Moreover, two main groups of variables that covaried in relation to leaf shape were detected (Fig. 2). Weight along these dimensions in relation to each deformation grid are shown in Fig. 3.

Linear dimensions derived from 2B-PLS analysis were subjected to CVA (Fig. 4): the two CVs explained 76.8% (Wilks' $\lambda = 0,006$; $df = 22$; $p < 0.0001$) and 23,2% (Wilks' $\lambda = 0,136$; $df = 10$; $p < 0.0001$) of total variability, respectively. All three species were highly significant discriminated. The cross validation confirmed the result and 100% of specimens were correctly classified.

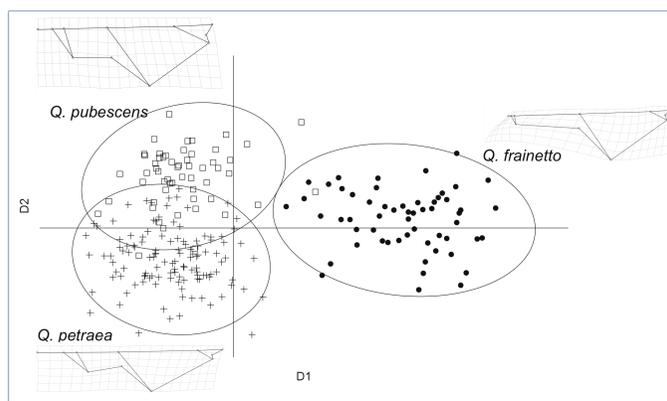


Fig. 1 – Ordination plot for the projections of the pure species onto first two significant dimensions. Filled circles = *Q. frainetto*; crosses = *Q. petraea*; empty squares = *Q. pubescens*. Hybrids are not shown.

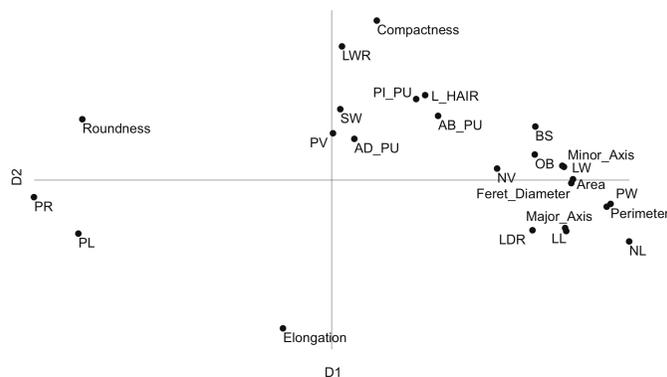


Fig. 2 – Loadings plot of 25 morphological variables onto the first two significant dimensions.

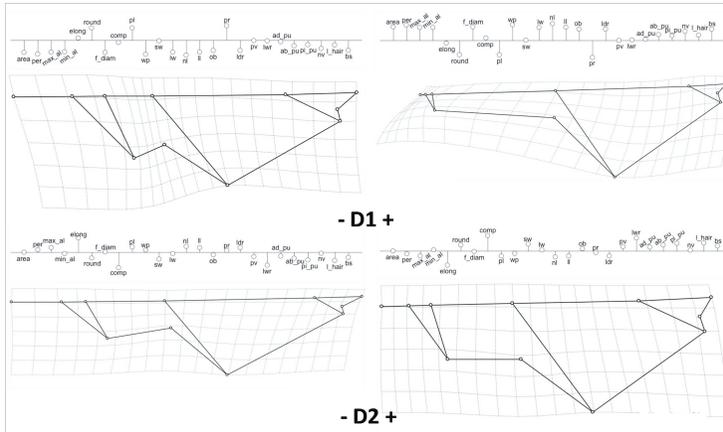


Fig. 3 – Shape variation of leaves along the first two significant dimensions of 2B-PLS. Deformation grids are shown for the negative (left) and positive (right) extremes of D1 (top) and D2 (bottom). The bar-plot show the degree of covariation between shape and the 25 traditional characters (see text for codes).

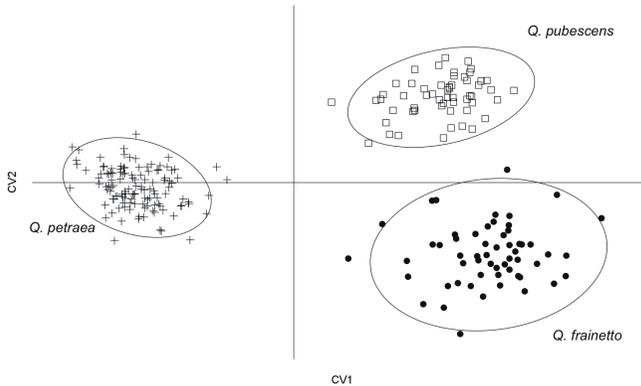


Fig. 4 – Ordination plot of the pure species onto the first two canonical variates. Filled circles = *Q. frainetto*; crosses = *Q. petraea*; empty squares = *Q. pubescens*. Hybrids are not shown.

4 CONCLUSION

This approach results as a useful tool to analyze traditional morphological variables in relation to leaf shape, one of the most important visual attributes to characterize white oak species, playing an important role in pattern of recognition, and in the systematics of this plant subgenus.

2B-PLS results allowed to define a typical leaf shape for each oak species, and to associate this shape to other morphological traditional features.

Results confirmed the high diagnostic power of the geometric morphometric approach. Moreover, the visualization of the leaf shape differences, associated

to other groups of correlated morphological traits, allowed to obtain a clear diagnosis of leaf morphology for each species.

Q. frainetto was characterized by grater leaves and short petiole, obovate leaf blade and high deepness of lobes. *Q. petraea* was characterized by a longer petiole, more acute basal and apical regions, and a more deeply lobed lamina than *Q. pubescens*, which has higher values of leaf compactness, pubescence and length of trichomes.

The high degree of classification accuracy of this combined approach advocates its extension to other problematic species and highlights its importance as an exploratory tool in plant ecology, physiology and taxonomy.

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