

Morphological variability of oaks (*Quercus robur* L, *Quercus petraea* (Matt) Liebl, *Quercus pubescens* Willd) in northeastern France: preliminary results

JL Dupouey, V Badeau

Laboratoire de phytoécologie forestière, Centre de recherches forestières,
INRA Nancy, 54280 Champenoux, France

Summary — Morphological variability of oaks in Lorraine (northeastern France), was studied. Eight hundred oaks were sampled in 80 stands covering a broad range of ecological variability; 10 leaves, fruits and current-year shoots were collected per tree. Thirty-four morphological variables were measured and analyzed by factorial correspondence analysis. It is concluded that *Q robur* and *Q petraea* are clearly separated with a few morphologically intermediate individuals (3.5%). *Q petraea* is more variable than *Q robur*. *Q pubescens* and *Q robur* are totally isolated from each other, while *Q petraea* and *Q pubescens* form a continuum. Many variables discriminate between these 3 species; some of them have been little known prior to now (pilosity, presence of intercalary ribs). These results are compared with those from other parts of Europe.

taxonomy / morphometrics / hybridization / introgression / *Quercus robur* / *Quercus petraea* / *Quercus pubescens*

Résumé — Variabilité morphologique des chênes dans le Nord-Est de la France; résultats préliminaires. Nous avons étudié la différenciation morphologique des chênes pédonculé, sessile et pubescent dans le Nord-Est de la France. L'échantillonnage a porté sur 80 populations provenant de stations représentant toute la gamme de variation des milieux de chênaies en Lorraine. Sur 10 arbres par population, 10 feuilles, infrutescences et rameaux de l'année ont été prélevés. Trente-quatre variables morphologiques ont été mesurées et analysées par analyse factorielle des correspondances. On observe une très nette séparation des chênes sessile et pédonculé, avec seulement 3,5% d'individus morphologiquement intermédiaires, ainsi qu'un isolement total du chêne pubescent et du chêne pédonculé. Par contre, les chênes sessile et pubescent forment un continuum. Le chêne pédonculé est moins variable que le chêne sessile. De nombreuses variables discriminent ces 3 espèces, dont certaines peu connues jusqu'alors (pilosité, présence de nervures intercalaires). Ces résultats sont comparés à ceux obtenus par ailleurs en Europe.

taxonomie / morphométrie / hybridation / introgression / *Quercus robur* / *Quercus petraea* / *Quercus pubescens*

INTRODUCTION

The distinction of species in the *Quercus* complex is still a matter of debate. In western Europe, several species have been reported as potentially interbreeding, the most widespread being *Quercus robur* and *Quercus petraea*. Until now, the prevalent opinion was in favor of the common occurrence of hybrids between the different species, producing many morphologically intermediate forms between pure parental species due to hybridization and introgression. A huge body of literature has been published with this thinking in mind, (see eg, Kissling, 1983; Minihan and Rushton, 1984, for the most recent papers). Only a few recent studies (Dupouey, 1983; Dupouey et Le Bouler, 1989; Dupouey *et al*, 1990; Grandjean and Sigaud, 1987; Aas, 1990) have come to different conclusions.

The aim of this study was to describe the actual morphological status of *Q robur* and *Q petraea* at a regional scale (Lorraine Plain), including *Q pubescens*. We studied inter- and intraspecific variations, and their link with ecological constraints. In this preliminary paper, only results on morphological differentiation at the interspecific level are presented. We have tried to answer the following questions: what is the organization of morphological variability among the 3 species? What is the degree of isolation of each species? What are the best discriminant morphological characters?

MATERIALS AND METHODS

Stands were selected from 8 forests in the Lorraine Plain, and a total of 80 populations were sampled in order to cover the whole ecological variability of oakwoods in this area. Coppices with standards were discarded, as were supposed plantations. In each of these populations, 10 dominant trees were marked at random. A

total of 761 trees were sampled during summer 1989, 655 of them had produced fruit during this year. Twenty leaves, fruits (including peduncles, cupules and acorns) and twigs of the current growth year were collected. To minimize positional variability within the tree (Blue and Jensen, 1988), leaves were collected from the external part of the canopy usually on the aspect facing south, and always in the middle part of the first flush shoot. Ten of these 20 samples were chosen at random for measurements, after the elimination of broken, incomplete or damaged units. Eighty variables were measured or calculated. These variables concern many aspects of foliar and fruit morphology: size, overall shape, color, pilosity on various parts of leaves or fruits (measured as in Grandjean and Sigaud, 1987), shape of some details (lobes, auricles at the lamina base). These data were acquired with a digitizing tablet hooked up to a microcomputer. Several features were obtained from distances and angles between different landmarks along the outline of the leaf.

Data were analyzed mainly by factorial correspondence analysis using the SPAD.N statistical package (Lebart *et al*, 1988). In the first stage of the analysis, the study of correlation coefficients between all pairs of the 80 initial variables allowed the elimination of 46 redundant variables. The comparison between results with or without fruit morphological characters showed no significant differences so only a subset of 29 parameters describing leaves and shoots was used for subsequent analysis. This allowed the use of the whole set of trees instead of only those which had fruited during the sampling year. Fruit variables were used as supplementary characters.

Variables were ranked by decreasing power of discrimination according to their *F* value in an unbalanced analysis of variance between the 3 species. Bonferroni *t*-tests of difference between means for each species were performed. Discriminant analysis was used to calculate a function for species recognition.

RESULTS

Figure 1 shows the projection of trees into the space of axes, 1, 2 and 3 of the factorial analysis. One can observe 3 poles of

distribution for these individuals. Table I gives the mean values of the most discriminant variables for each pole.

At the right-hand side of the first axis, the leaves are shortly petiolated, with well-developed auricles at the base of the lamina. The maximum width of leaves is located in the upper part of the lamina. Lobe sinuses are irrigated by numerous intercalary veins. Fruits have a long and thin peduncle. Pilosity is absent, very short on all parts. This group of individuals represents the typical *Q robur*.

At the left end of the first axis, and at the upper part of axis 2, leaves have a long petiole. The maximum width is at the middle of the lamina. Fruits are shortly pedunculate and pilosity is medium to dense. this pole is composed of *Q petraea* trees.

The last pole is also located on the left-hand side of the first axis, in the lower part of axis 2. It is composed of trees with leaves rather similar to those of the previous one. Lobes are sharper, often accompanied by lobules (lobes irrigated by nerves of the third order). The pilosity is

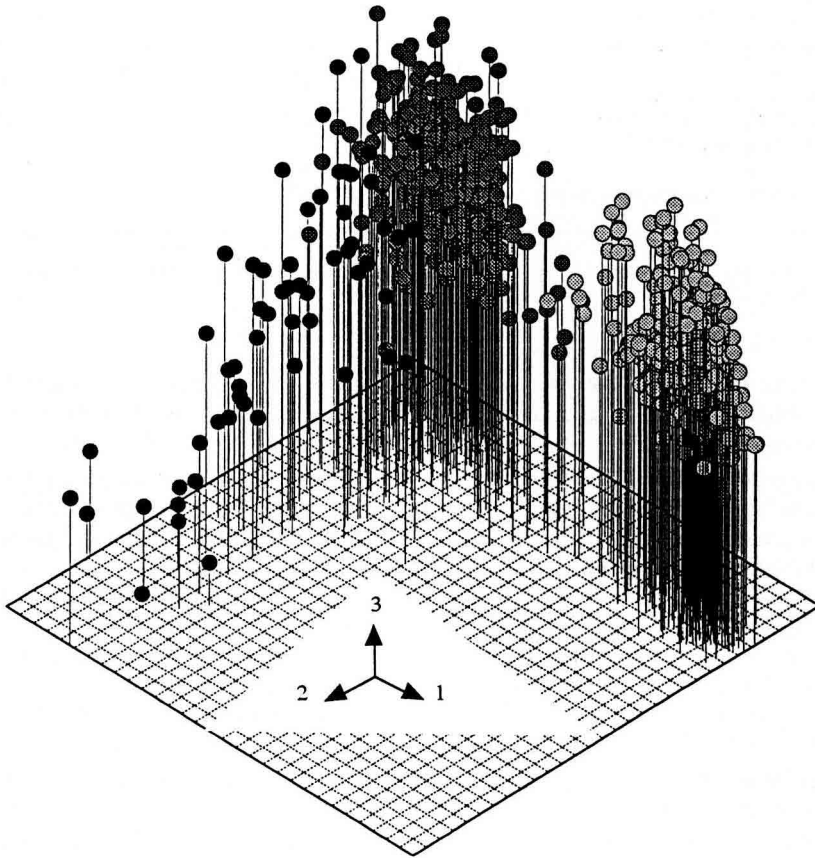


Fig 1. Projection of trees into the space of axes 1, 2 and 3 of the factorial correspondence analysis (black: *Q pubescens*, dark grey: *Q petraea*, light grey: *Q robur*).

Table I. Mean values for the most discriminant variables between species which are classified by decreasing order of *F* value of an analysis of variance between the 3 species.

<i>Parameter</i>	<i>Q robur</i>	<i>Q petraea</i>	<i>Q pubescens</i>	<i>F value</i>
Density of pilosity under lamina (%)	4 ^a	47 ^b	46 ^b	1591
Relative length of intercalary veins (%)	49 ^a	4 ^b	9 ^c	1522
Density of pilosity on midrib (%)	8 ^a	45 ^b	65 ^c	1484
Length of pilosity on petiole	0.1 ^a	1.9 ^b	2.4 ^c	1465
Density of pilosity on petiole (%)	1 ^a	24 ^b	59 ^c	1456
Number of intercalary veins	4.9 ^a	0.5 ^b	1.1 ^c	1429
Length of petiole (mm)	7 ^a	16 ^b	13 ^c	974
Length of peduncule (mm)	61 ^a	15 ^b	14 ^b	836
Density of pilosity on shoot (%)	0 ^a	9 ^b	53 ^c	818
Diameter of peduncle (mm)	1.2 ^a	2.5 ^b	1.9 ^c	776
Length of pilosity under lamina	0.3 ^a	1.7 ^b	2.1 ^c	712
Angle of auricle at lamina base (°)	21 ^a	74 ^b	77 ^b	581
Density of pilosity on peduncle (%)	4 ^a	36 ^b	60 ^c	450
Number of lobes	10.2 ^a	13.2 ^b	12.6 ^c	380
Length of pilosity on shoot	0.0 ^a	0.6 ^b	2.0 ^c	309
Length of pilosity on peduncule	0.4 ^a	1.7 ^b	1.8 ^b	268
Length of pilosity on midrib	0.9 ^a	2.1 ^b	2.7 ^c	245
Number of lobules	0.5 ^a	0.7 ^a	2.1 ^b	49
Relative height of maximum width (%)	57 ^a	54 ^b	55 ^b	38

Means with the same letter are not significantly different at the 5% level. Lengths of pilosity are measured on an arbitrary scale from 0 to 3.

more highly developed, both in terms of density and length. This group can be identified as *Q pubescens*.

Thus we find, with this analysis, that the 3 species have different morphological poles, and also that these 3 species differ widely in their degree of separation from one another. The *Q robur* cluster is completely separated from that of *Q pubescens*, and only a few morphologically intermediate individuals are found between *Q robur* and *Q petraea* (3.5% of the total number of trees).

On the other hand, *Q petraea* and *Q pubescens* form a continuum without any clear distinction between the 2 species. A number of morphologically intermediate individuals occurs. Consequently, *Q petraea* and *Q pubescens* exhibit much more intra-

specific morphological variability than *Q robur*, which appears to be more homogeneous.

Table I gives the variables by decreasing power of discrimination between the species. The best discriminant parameters are pilosity (density and length of pilosity on nerves, petiole and lamina), number and length of intercalary veins, length of the petiole and of the peduncle. Some classical features only appear after these variables, such as the development of an auricle at the lamina base.

Length of the petiole and number of intercalary veins are sufficient to separate the 2 species *Q robur* and *Q petraea* with 99% success rate. The discriminant function for tree recognition is:

$$I = (407 \times \text{nint}) - (130 \times \text{lpet}) + 357$$

where, *nint*: number of intercalary veins (mean of 10 leaves per tree); *lpet*: length of petiole in mm (mean of 10 leaves per tree); *I* species index; positive for *Q robur* and negative for *Q petraea*. Values between -1000 and +1000 indicate an intermediate tree.

DISCUSSION AND CONCLUSION

These results are different from those reported in a number of previous publications: Carlisle and Brown (1965), Wigston (1974), Olsson in Sweden (1975 a,b) Rushton (1978, 1979, 1983), Minihan and Rushton (1984) in the United Kingdom and Kissling (1980a,b, 1983) in Switzerland, all concluded that there was extensive hybridization between *Q robur* and *Q petraea*. Conversely, the authors of some more recent studies reached the same conclusions we did: Grandjean and Sigaud (1987) in France (including *Q pubescens*), Ietswaart and Feij (1989) in The Netherlands; Aas (1990) in Germany.

It is difficult to compare these results because they are based on different sample sizes, sampling regimes, characters and with different companion species. But several hypotheses could be advanced to explain the discrepancies. One possible explanation is that the extent of hybridization differs in the different parts of the distribution range of the species. Peripheral situations would be more favorable to hybridization than in the inner part. This could account for results from Sweden or the United Kingdom, but not those from Switzerland. More probably, one must look to the differences in the statistical approaches used for data analysis. All previous studies concluding that numerous hybrids which were present were based on statistical methods according to which the limits of each species are defined before the

analysis. The main methods used in these cases were hybridity indices for which you had to choose subjectively the range of each species for all the variables before calculation, principal components analysis (PCA) using 'pure' stands as references and discriminant function analysis. On the other hand, purely descriptive methods, such as factorial correspondence analysis (or principal components without reference populations) do not require the definition of the species before the analysis. They can be regarded just as a means of looking at the raw data from a particular point of view (the one with maximum variance explained).

Factorial correspondence analysis is preferable to PCA (even when a 'reference population' is not used) because it is able to deal with non-linear relationships between characters, whereas PCA only measures linear correlation coefficients.

The frequency of hybrids between *Q pubescens* and *Q petraea* has been underlined by other authors. Semerikov *et al*, (1988), studying populations from Dagestan (Russia), even conclude that such hybrids represent a unique species complex. This lack of isolation could explain the greater variability observed in *Q petraea* versus *Q robur*.

Variables used in previous taxonomic studies were not always the most highly discriminating ones, and sometimes not discriminant at all. The best features, in our sample, are intercalary veins and pilosity development.

Further studies in progress are exploring the persistence of these discriminant characters under homogeneous growing conditions (nursery) for the populations under consideration. Also, the structure of the intraspecific variability along ecological clines is of major importance. Finally, there is a need for standardization of the statistical methods used for the analysis of mor-

phological variability in the different parts of the range of distribution of these species.

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of Gilles Doucet during the collection of leaves. We also thank Patrick Behr for measurements of fruits and Hervé Cochard for 3D graphics. Support for this project was provided by the EEC (Research action Programme MA1B: Genetics and Breeding of Oaks).

REFERENCES

- Aas G (1990) Kreuzbarkeit und Unterscheidung von Stiel- und Traubeneiche. *Allg Forstztg* 9-10, 219-221
- Blue MP, Jensen RJ (1988) Positional and seasonal variation in oak (*Quercus*, Fagaceae) leaf morphology. *Am J Bot* 75, 939-947
- Carlisle A, Brown HF (1965) The assessment of the taxonomic status of mixed oak populations (*Quercus* sp). *Watsonia* 6, 120-127
- Dupouey JL (1983) Analyse multivariable de quelques caractères morphologiques de populations de chênes (*Quercus petraea* (Matt) Liebl et *Quercus robur* L) du Hurepoix. *Ann Sci For* 40, 265-282
- Dupouey JL, Le Boulter H (1989) Discrimination morphologique des glands de chênes sessile (*Quercus petraea* (Matt) Liebl) et pédonculé (*Quercus robur* L). *Ann Sci For* 46, 187-194
- Dupouey JL, Fougère V, Kremer A (1990) Variabilité génétique des chênes sessile et pédonculé. *Rev For Fr* 42, 198-203
- Grandjean G, Sigaud P (1987) Contribution à la taxonomie et à l'écologie des chênes du Berry. *Ann Sci For* 44, 35-66
- Ietswaart JH, Feij AE (1989) A multivariate analysis of introgression between *Quercus robur* and *Quercus petraea* in The Netherlands. *Acta Bot Neerl* 38, 313-325
- Kissling P (1980a) Un réseau de corrélations entre les chênes (*Quercus*) du Jura. *Ber Schweiz Bot Ges* 90, 1-28
- Kissling P (1980b) Clef de détermination des chênes médioeuropéens (*Quercus* L). *Ber Schweiz Bot Ges* 90, 29-44
- Kissling P (1983) Les chênaies du Jura central suisse. Thesis, Université de Lausanne, Switzerland, 400 p
- Lebart L, Morineau A, Lambert T (1988) SPAD.N: *Système Portable Pour l'Analyse des Données. Version 1.2: Manuel de Référence*. CISIA, Paris, 306 pp
- Minihan VB, Rushton BS (1984) The taxonomic status of oaks (*Quercus* ssp) in Breen Wood, Co Antrim, Northern Ireland. *Watsonia* 15, 27-32
- Olsson U (1975a) A morphological analysis of phenotypes in populations of *Quercus* (Fagaceae) in Sweden. *Bot Not* 128, 55-68
- Olsson U (1975b) On the size and microstructure of pollen grains of *Quercus robur* and *Quercus petraea*. *Bot Not* 128, 256-264
- Rushton BS (1978) *Quercus robur* L and *Quercus petraea* (Matt) Liebl: a multivariate approach to the hybrid problem. 1. Data acquisition, analysis and interpretation. *Watsonia* 12, 81-101
- Rushton BS (1979) *Quercus robur* L and *Quercus petraea* (Matt) Liebl: a multivariate approach to the hybrid problem. 2. The geographical distribution of population types. *Watsonia* 12, 209-224
- Rushton BS (1983) An analysis of variation of leaf characters in *Quercus robur* L and *Quercus petraea* (Matt) Liebl population samples from Northern Ireland. *Ir For* 40, 52-77
- Semerikov LF, Glotov NV, Zhivotovskii LA (1988) Example of effectiveness of analysis of the generalized variance of traits in trees. *Sov J Ecol* 18, 140-143
- Wigston DL (1974) Cytology and genetics of oaks. In: *The British Oak* (Morris MG, Perring FH, eds). Bot Soc Br Isles, London, 27-50