Genetic, Morphological, Ecological and Phenological Differentiation between *Quercus petraea* (Matt.) Liebl. and *Quercus robur* L. in a Mixed Stand of Northwest of France

By R. Bacilieri, A. Ducousso[1]) and A. Kremer

I.N.R.A., Laboratoire de génétique et d'amélioration des arbres forestiers,
B.P. 45, 33610 Gazinet, Cestas, France

Abstract

The sessile and pedunculate oaks are the most common tree species in Europe. The 2 taxons are closely related, and, in spite of their ecological differences, they form frequently mixed forests, where hybridisation is suspected to occur. In this paper, we describe a mixed forest stand of sessile and pedunculate oaks, in which a regular slope establishes an ecological gradient. Genetic, morphological, phenological and ecological differentiation between the 2 species and the existence of parallel variations among these different types of characters are analysed. The sessile and the pedunculate oaks studied here showed a weak differentiation for the genetic, morphological and ecological characters. The flowering of the 2 species was synchronous over 3 years. Morphological and ecological traits showed an overlapping distribution; with allozymes any specific marker was not identified. Within each species the diversity is not structured along the ecological gradient, we do not find associations among ecological, morphological, phenological traits and allozyme markers. Inferences about the taxonomic relationships of these species are discussed.

Key words: Quercus morphology, flowering period, ecological gradient, allozymes, differentiation.

FDC: 165.5; 176.1 *Quercus robur;* 176.1 *Quercus petraea;* (44).

Résumé

Les chênes sessile et pédonculé sont les 2 espèces les plus répandues à travers l'Europe. Les 2 taxons sont étroitement liés, et malgré leurs différences écologiques, ils forment souvent des forêts mixtes, où l'hybridation n'est pas exclue. Dans cet article, nous décriont un peuplement forestier mixte de chêne sessile et pédonculé, dans lequel une pente régulière crée un gradient écologique. La différentiation génétique, morphologique, phénologique et écologique entre les 2 espèces sera étudiée, ainsi que les associations entre ces différents types de caractères. Le chêne sessile et pédonculé que nous avons étudiés montrent une faible différenciation des caractères morphologiques, génétiques et écologiques; la floraison des 2 espèces est synchrone sur trois années. Les caractères écologiques et morphologiques montrent des distributions chevauchées; aucun marqueur spécifique n'a pu être identifié avec les allozymes. Au niveau intraspécifique, la diversité n'est pas structurée selon le gradient écologique; aucune association entre les caractères écologiques, morphologiques, phénologiques et les marqueurs enzymatiques a pu être mise en évidence. Les relations taxinomiques entre les deux espèces seront discutées.

Mots-clés: Quercus morphologie, phénologie, gradient écologique, allozymes, différentiation.

[1]) Correspondence: A. Ducousso, I.N.R.A., Laboratoire de génétique et d'amélioration des arbres forestiers, B.P. 45, 33610 Gazinet, Cestas, France: Tel.: 56680303; Fax: 56680223.

Introduction

Quercus petraea (Matt) Liebl. and *Quercus robur* L. are closely related species with a widely sympatric distribution in Europe. The boundary between the 2 species is not clearly defined, and a long debate on their taxonomic classification has maintained conflicts between botanists.

On one side, a series of morphological and physiological differences between the 2 species accounts for their different ecological preferences. *Q. petraea* has smaller vessels and is less prone to cavitation (Cochard et al., 1992), a smaller number of lenticels on the roots (Belgrand, 1983), a deeper and more developed root system than *Q. robur* (Bréda, 1993), hairy leaves (Dupouey, 1983), etc. As a result, *Q. petraea* is considered to be more drought resistant than *Q. robur*, as demonstrated by the decline that has preferentially affected pedunculate oaks in the past 10 years (Becker and Lévy, 1982 and 1983). On the contrary, *Q. robur* grows in soils with higher water tables, subjected to floods and on sites with higher pH (Grandjean and Sigaud, 1987). Differences are observed also in respect to light and social competitions (Fairbairn, 1954), *Q. petraea* being able to support denser and more shadowed conditions, partly because of its different morphology and architecture of the crown.

On the other side, all the morphological characters show an overlapping distribution between the 2 species (reviewed in: Gardiner, 1970), and in spite of their ecological differences, in many cases the 2 species coexist in the same mixed stands (Rushton, 1979; Dupouey, 1983; Grandjean and Sigaud, 1987). They have similar chromosomal structure and number (Ohri and Ahuja, 1990) and no specific molecular markers have been found until now neither in the nuclear (with allozymes: Kremer et al., 1991; Zanetto et al., 1993; Müller-Starck et al., 1993, Bacilieri et al., 1995, with RAPD-PCR technique: Moreau, 1993) nor in the cytoplasmic genome (Petit et al., 1993). The lack of diagnostic characters, in association with other observations such as: 1. the success of interspecific controlled crosses (Aas, 1990; Steinhoff, 1993), 2. the preferential finding of intermediate morphological forms in intermediate habitats (Rushton, 1979; Grandjean and Sigaud, 1987), 3. the diminished (but in very variable quantities among individuals) pollen viability of intermediary forms (Olsson, 1975; Rushton, 1978), and 4. the peculiar structure of chloroplast DNA polymorphism, that reflects a similar geographic differentiation for both species, but no specific differentiation (Petit et al., 1993), strongly support the hypothesis that sessile and pedunculate oaks can hybridize in nature.

Depending on the amplitude of interspecific gene flow, a series of evolutionary and ecological implications could concern this group of species. In particular, if the hypothesis of hybridization and introgression is true, the actual mixed populations could contain a number of introgressed individuals

distributed over a large ecological and morphological range. Moreover, if we admit that some morphological characters are in some way adaptative (as the hairiness of the leaves, that can be seen as an adaptation to drought, or the length of the petiole, that plays a role on the lymph conductivity and on the spatial distribution of leaves and therefore on the interception of light), we can expect an association between these traits and the ecological preferences of individuals. The correlation among characters could be reinforce by the slow decay of the linkage disequilibrium during the first generations of introgression (ANDERSON, 1953).

The success of the hypothetical hybridization between sessile and pedunculate oaks is largely dependent on the synchrony of flowering. Previous data addressing flowering phenology of these species are extremely scarce (BONNET-MASIMBERT, 1978), Moreover, if many aspects of the morphological, physiological and ecological differentiation between *Q. petraea* and *Q. robur* have been studied, only a limited number of studies were dedicated to the genetic variability of these species *(op. cit.)*, and only very rarely has it been attempted to relate genetic variability of natural populations with phenotypic characters.

A long term project focusing on *Q. petraea* and *Q. robur* was initiated in Bordeaux in 1988. The main theoretical interests were to describe the genetic diversity in these species, and to understand what are the mechanisms and the characters to be the most implicated in the organisation and in the maintain of this diversity. Practical issues concern the sylviculture and management of oak forests, that have a great economical and ecological interest across Europe. Here we will describe the differentiation between the two species, observed in morphological, phenological, ecological and biochemical traits in a mixed stand of Northwest France, and we will test if associations exist among these different types of characters.

Materials and Methods

The study area (250 m x 250 m) is located in Northwest France, in the Petite Charnie forest (Sarthe), 35 km west of the city of Le Mans. The climate is typically atlantic, temperate and wet. Mean rainfall is 800 mm per year, 50% of which is concentrated into 5 months, between November and March. Mean temperature is 11°C; temperature falls under 0°C for 60 days per year on average. The geological substratum is composed principally of sandstone, schist and lens of clay. The mean elevation of the stand is 140 m.

The parcel presents a regular slope which creates an ecological gradient, from a humid clay soil with a superficial water table (pseudogley) in the lower part (south-west) up to a relatively dry silt and sandy soil in the upper part (north-east) of the area. The stand consists of 426 oak trees (pedunculate and sessile), about 120 years old. The population comes from natural regeneration. A few ash trees *(Fraxinus excelsior)* are also present in the wet part of the stand. All the trees have been mapped and then sampled for morphological and genetic analyses. A series of pedological and phenological observations have also been conducted over the stand.

Morphology

During the summers of 1989, from 5 to 7 leaves were collected from the upper part of the crown of every tree, and then stored in a herbarium. Leaf characters were measured by means of a digitising tablet, with the help of a computer program developed by DUPOUEY and BADEAU (1993). Additional assessments of pubescence of leaves were made with a binocular microscope. These characters, described in *table 1,* concern measurements effectuated on the whole leaf, and meas-

urements of the three more developed lobes for each side of the leaf (in *table 1,* lobe 1 refers to the lobe, among the 3 measured, nearest to the base of the leaf, lobe 2 to the second, etc.).

For each character, repeatability coefficients were computed at the intraspecific level (FALCONER, 1981, chapter 8). The repeatability coefficient is the ratio of intra-individual variance (both genetic and environmental) to the total variance. The data from all the trees have been analysed by means of Factorial Discriminant Analysis, (FDA; LEGENDRE and LEGENDRE, 1984). FDA establishes a linear function of original data which maximises the repeatability.

Phenology

Male and female flowering phenology of trees was monitored every 3 days in 1989, every 14 days in 1991 and every 7 days in 1992. Floral development was observed in the upper part of the crown, using a telescope with 25x or 40x magnification. Male flowers were considered mature when the catkins began to release the pollen, and female flowers when they became receptive (the pistil exhibits a bright red colour). In each species, the trees were grouped in three precocity classes, with an approximately equal number of individuals, on the basis of the earliest and latest date of individual male flowering.

Allozymes

Electrophoretic analyses were carried out on winter buds of all trees, collected from the upper part of the crown of trees in 1990 and 1991. Primordial leaves and bracts were removed from 10 to 20 buds per tree. The remaining part of the buds was ground in a cold (2°C to 3°C) extraction buffer (MÜLLER-STARCK and ZIEHE, 1991). Extracts were stored in an ultra cold freezer (−90°C). Enzymes were separated from the crude homogenate by standard horizontal starch-gel electrophoresis (12%). The composition of the electrode and gel buffers and staining buffer formulations were adapted from ZANETTO *el al.* (1993) and from VALLEJOS (1983).

Twelve enzyme systems were routinely analysed: aminopeptidase (AAP, EC. Ref.: 3.4.11.1), acid phosphatase (ACP, EC. Ref.: 3.1.3.2), diaphorase (DIA, EC. Ref.: 1.6.4.3), glutamate oxaloacetate transaminase (GOT, EC. Ref.: 2.6.1.1), isocitrate dehydrogenase (IDH, EC. Ref.: 1.1.1.42), leucine aminopeptidase (LAP, EC. Ref.: 3.4.11.1), meniadione reducatse (MR, EC. Ref.: 1.6.99.2), phosphoglucoisomerase (PGI, EC. Ref. 5.3.1.9), phosphoglucomutase (PGM, EC. Ref. 2.7.5.1), shikimate dehydrogenase (SKDH, EC. Ref. 1.1.1.25), malate dehydrogenase (MDH, EC. Ref. 1.1.1.37), 6-phosphogluconate-dehydrogenase (6PGDH, EC. Ref. 1.1.1.44.). These have shown to be encoded by 15 loci (AAP, ACP-B, ACP-C, DIA, GOT, IDH, LAP, MR, PGI, PGM, SKDH, MDH-A, MDH-B, 6PGDH-A, 6PGDH-B), and to have mendelian inheritance in segregation analyses (ZANETTO, unpublished). Comparisons of allelic frequencies between groups have been carried out by means of a Chi-square test (SOKAL and ROHLF, 1981).

For both species, we calculated the genetic diversity, H_{es}, and the effective number of alleles, A_{es} (KIMURA and CROW, 1964). H_{es} corresponds to the probability that, in the total population, 2 randomly chosen individuals have, for the same locus, different alleles, and it is calculated as: $H_{es} = 1 - \Sigma\ p_i^2$, where p_i is the frequency of the allele p in the total population. A_{es} represent the actual number of alleles if they were present in equal frequenciesinthe total population, and is defined as:

$$A_{es} = 1 / (1 - H_{es}).$$

Genetic distance among species have been calculated with NEI's formulas (NEI, 1978). Multilocus analysis of gene diversity was done with the help of Multiple Factorial Correspondence Analysis (FCA; LEGENDRE and LEGENDRE, 1984).

Table 1. – List of morphological characters. The 16 characters indicated with a star were used to compare the phenological and the ecological groups (see text).

Abbreviation	Character
lpet*	= petiole length
llimb*	= lamina length
ltot	= lpet + llimb
largmax*	= maximal width of the leaf
hautmax*	= height of maximal width
peri	= perimeter
surf	= surface
tetmoy*	= mean of auricle angles
ll1, ll2	= mean length of lobes 1 and 2 (left and right)
llmoy*	= mean length of the 6 lobes
hl1, hl2	= mean height of lobes 1 and 2 (left and right)
hlmoy*	= mean height of the 6 lobes
el1, el2	= mean thickness of lobes 1 and 2 (left and right)
elmoy*	= mean thickness of the 6 lobes
an1, an 2	= mean angle of secondary veins 1 and 2 (left and right) with the primary vein
anmoy*	= mean angle of the 6 secondary veins with the primary vein
al1, al2	= mean angle of lobes 1 and 2(left and right)
almoy*	= mean angle of the 6 lobes
as1, as2	= mean angle of sinus 1 and 2(left and right)
asmoy*	= mean angle of the 6 sinus
apexmoy1, 2	= mean shape of apex of lobes 1 and 2 (left and right)
apexmoy123*	= mean shape of apex of the 6 lobes
nblob*	= number of lobes
nblub	= number of lobelets
nint*	= number of intercalary veins
pillim*	= density of leaf pilosity
defic	= $1-(4*\Pi*surface/perimeter^2)$

lmax/llimb, hlmax/llimb, lpet/llimb*, hlmoy/lmax, llimb/lmax, nblob/peri, nblob:llimb

Pedology and topography

Pedological observations have been made according to a systematic sampling *(Figure 1)* based on a 25 meters square mesh (points 11 to 55). We have intensified this sampling with observations (points 1 to 8) along a transept parallel to the main ecological gradient. In each sampling point, soil cores were extracted to determine texture at different depths, and the depth of the pseudogley horizon. Texture analyses have been made by the INRA laboratory of Arras (France). The discoloration of pseudogley horizon depends on the watertable level, which oscillates over the seasons (DUCHAFOUR, 1984). The upper part of the soil interested only by oscillation of water-

table, because of oxide-reductive processes, maintains saturated ochre colours but presents mottling bordered with rust, and iron and manganese concretions. In the part almost constantly engorged by water, iron is in the ferrous state, and grey or greenish colours are dominant. Observations of the colour and of the presence of concretions allowed us to identify the limits within which the water table oscillated. Moreover, for each pedological sampling point, elevation, relative to a reference point, was recorded. Pedological and topographic variables have been recorded in classes *(Table 2)* and then analysed with FCA.
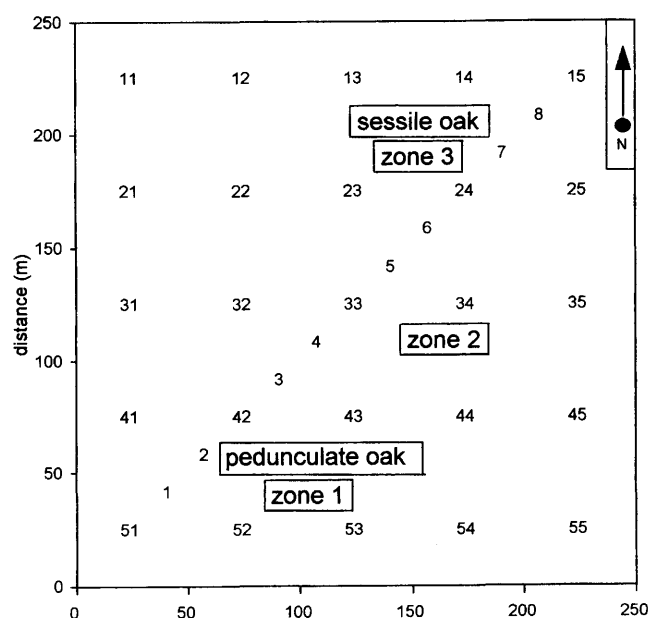

Figure 1. – Map of pedological sampling points. Subdivision of the stand in ecological zones.

Intraspecific relationships between genetic, morphological, phenological and ecological variables

We then tested the existence, at the intraspecific level, of parallel variations among the different types of characters presented above. First we subdivided the trees of each species into groups, according to the ecology, the morphology, and the phenology of the stand. Ecological subdivisions (based on *Figure 1*) were made up of 2 groups for each species: in sessile oaks 1 group was constituted with trees growing in zone 3, typical of this species, and the other with trees growing in zones 1 and 2 bulked together; in pedunculate oaks 1 group contained individuals growing in zone 1, typical of this species, and the other the individuals growing in zones 2 and 3 bulked together. For phenological traits, the subdivision of groups of precocity presented earlier was maintained. For morphological traits, the individuals of each species were subdivided into 2 groups on the basis of values on the first axis of FDA on morphology, 1 group composed of trees with more extreme characters and the other of trees with more intermediate characters.

Allelic frequencies of trees subdivided into ecological, morphological, and phenological groups were compared with a Chi-square test on single-locus 2-way contingency tables. Morphological data relative to the 16 most important characters (indicated with a star in *Table 1*) of phenological and ecological groups were compared with an one-way ANOVA; their variances were compared with BARTLETT's test (SOKAL and ROHLF, 1981). Differences in phenology between ecological zones were tested in contingency tables with Chi-square tests.

Results

Analysis of morphological characters

Normality of distribution of characters was verified for all variables except *nblub, nint* and *pillimb,* which have a strong left asymmetry. The distribution of these characters became more similar to the normal with a square root transformation.

Repeatability coefficients were particularly high for those characters normally used to discriminate between the 2 oak

Table 2. – Listing of ecological characters and their relative contributions to the determination of the FCA axis.

Characters	Definitions	axis 1	axis 2
Topo1	Elevation between 134 and 137.99 m	0.34	0.03
Topo2	Elevation between 138 and 140.99 m	0.08	0.07
Topo3	Elevation between 141 and 143.99 m	0.22	0.00
Topo4	Elevation between 144 and 147 m	0.32	0.02
H1-1	Horizon sometime saturated with water between 0 and 50 cm of depth	0.39	0.11
H1-2	Horizon sometime saturated with water between 50 and 100 cm of depth	0.00	0.24
H1-3	Horizon sometime saturated with water below 100 cm of depth	0.57	0.03
H2-1	Horizon permanently saturated with water between 0 and 50 cm of depth	0.26	0.36
H2-2	Horizon permanenlty saturated with water between 50 and 100 cm of depth	0.12	0.23
H2-3	Horizon permanenlty saturated with water below 100 cm of depth	0.64	0.01
t1-1	Texture between 0 and 50 cm: clay > silt > sands	0.42	0.14
t1-2	Texture between 0 and 50 cm: silt > clay > sands	0.06	0.47
t1-3	Texture between 0 and 50 cm: sand > silt > clay	0.26	0.16
t2-1	Texture between 50 and 100 cm: clay > 50%	0.68	0.00
t2-2	Texture between 50 and 100 cm: clay > silt > sand	0.02	0.46
t2-3	Texture between 50 and 100 cm: silt > clay > sand	0.36	0.39

species, such as: *lpet, tetmoy, pillimb, nblob, apexmoy123, nint.* An exception was hautmax, that was not informative because variability within the individual was greater than variability between individuals. The other variables with a repeatability coefficient smaller than 0.5 were: *hlmax:llimb, as2, as1, asmoy, an2, nblub, an1, al1, anmoy, lmax:llimb, hautmax, llmoy:lmax, apexmoy123, al2, hl1, el1.* The *F*-test (ANOVA, with tree as main effect) was significant for all the variables, meaning that in every case differences among trees could be observed.

The Factorial Discriminant Analysis separated the trees into 2 groups corresponding to the 2 species *(Q. robur* and *Q. petraea)* on the first axis, that accounts for the 48% of the total variance; all the other axis explained less than 8% of the total variance. A limited number of variables are strongly and highly significantly correlated with the first axis: *lpet:llimb, lpet, pillimb, nint, tetmoy, hlmoy:lmax, nblob, defic* (for all these variables $|r| > 0.5$; $P_{t-test} < 0.001$); these variables are those currently used to discriminate between the species. The distribution of individuals on the first axis of FDA is shown in *figure 2;* with this method, the level of separation between groups is improved if compared to that obtained with single characters. With FDA the classification of 12 individuals was uncertain; if a single character chosen among the most discriminant, such as *lpet,* was used to separate the species, the number of trees classified as uncertain would have risen to 39.
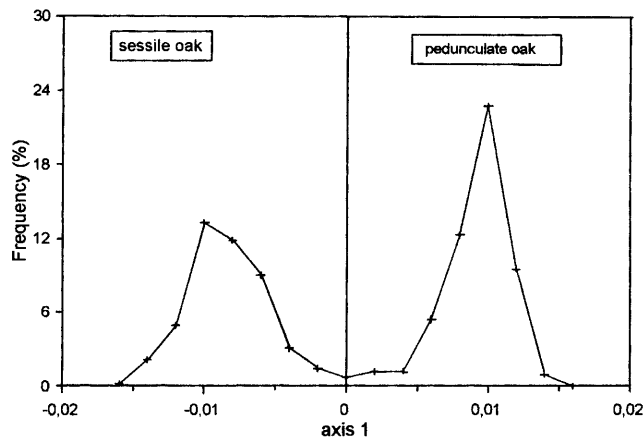

Figure 2. – Distribution of 426 oak trees on axis 1 of FDA on morphological characters. Individuals have been classified into species on the basis of this distribution. The 12 trees with values between –0.002 and 0.002 were considered intermediate and not included in subsequent analysis.

Analysis of flowering phenology

During 3 years (1989, 1991 and 1992), male and female flowering of sessile and pedunculate oaks were almost perfectly synchronous in the stand *(Figure 3).* In each tree, there was always a delay between male and female flowering: in sessile oak, female flowering on average occurred 4.5 days (± 5.21) after male flowering, and in pedunculate 7.5 days (± 6.01). Data relative to the mean length of flowering period reported in *table 3* refer to 1989 and 1992; in 1991, the flowering period was very short, and data are lacking for the final period of flowering on 128 trees. In 1989 and in 1992, there was a great variability among individuals for the length of flowering period that varied between 3 and 30 days. The proportion of trees that did not flower was 2% to 8%, according to year and to species.

Comparisons between 1989 and 1992 revealed that, in *Q. petraea* 40% of the trees could be classified in the same precocity class in the 2 years, 46% of trees changed from one

Table 3. – Mean length and standard deviation for male and female flowering time, in sessile and pedunculate oaks, in 1989 and in 1992.

YEAR		1989	1992
		Male flowering	
Sessile	mean	12,03	8,54
oak	st. dev.	6,75	4,25
Pedunculate	mean	10,60	8,08
oak	st. dev.	6,25	3,33
		Female flowering	
Sessile	mean	11,69	7,64
oak	st. dev.	6,03	3,04
Pedunculate	mean	10,10	9,23
oak	st. dev.	4,90	4,07

class to the adjacent one, and 15% changed from early to late flowering or vice versa. In *Q. robur* the 3 percentages were respectively 49%, 45% and 6%. Among the individuals that did not change phenological class, 22% were composed of early flowering trees and 48% of late flowering in sessile oak, and 26% early flowering and 26% late flowering in pedunculate oak.

Allozymic analysis

A total of 190 sessile oaks and 217 were included in this analysis. 12 trees were excluded because they were morphologically intermediate between the 2 species and 7 due to the bad quality of the extractions. The effective number of alleles (A_{es}) and the gene diversity (H_{es}) in the populations are respectively 1.45 and 0.249 in sessile oak and 1.541 and 0.256 in pedunculate oak. The 2 loci coding for 6PGDH and the 2 coding for MDH were monomorphic in our sample.

Significant differences in allelic frequencies between sessile and pedunculate oak were found for 6 out of 11 polymorphic loci; in this case, because we made simultaneously a number of comparisons, the probability level to reject the null hypothesis of no differences between species was fixed at 0.01 *(Table 4).* There were a few alleles present in *Q. robur* at extremely low frequencies that were absent in *Q. petraea.*

The FCA on 15-allelic data of the 2 species shows differentiation between the two species. The separation obtained is much weaker that the one obtained with morphological markers. For the isozyme data the first axis of FCA accounted only for 6% of the total variance *(Figure 4).* NEI's genetic distance between sessile and pedunculate oaks is 0.034 (± 0.016) over the 15 loci.

Ecological analysis

FCA on ecological variables resulted in the identification of a south-west to north-east gradient, parallel to the slope, within the experimental stand *(Figure 5).* The highest contributions to the first axis of FCA, which accounts for 26% of the total variance, were due to the presence of clay between 50 cm and 100 cm of depth and to the presence of the watertable below 100 cm of depth *(Table 2).* In the study area there was a strong and significant correlation between the frequency of sessile oaks within an area of 1250 m² around the sampling points and

the depth of the horizon permanently saturated with water ($r = 0.75$; $p < 0.01$).

On the basis of the distribution of sampling points in FCA and of the spatial distribution of trees of the 2 species, the stand can be subdivided into 3 ecological zones *(Figures 1 and 5)*: in zone 1, where the pseudogley horizon is superficial (~ 30 cm) and the soil contains a high quantity of clay, *Q. robur* is dominant; in zone 3 the pseudogley horizon is deeper (>100 cm), the soil is composed principally of sand and *Q. petraea* is dominant; in zone 2 the ecological variables were at intermediate values and the 2 species were mixed tree by tree.
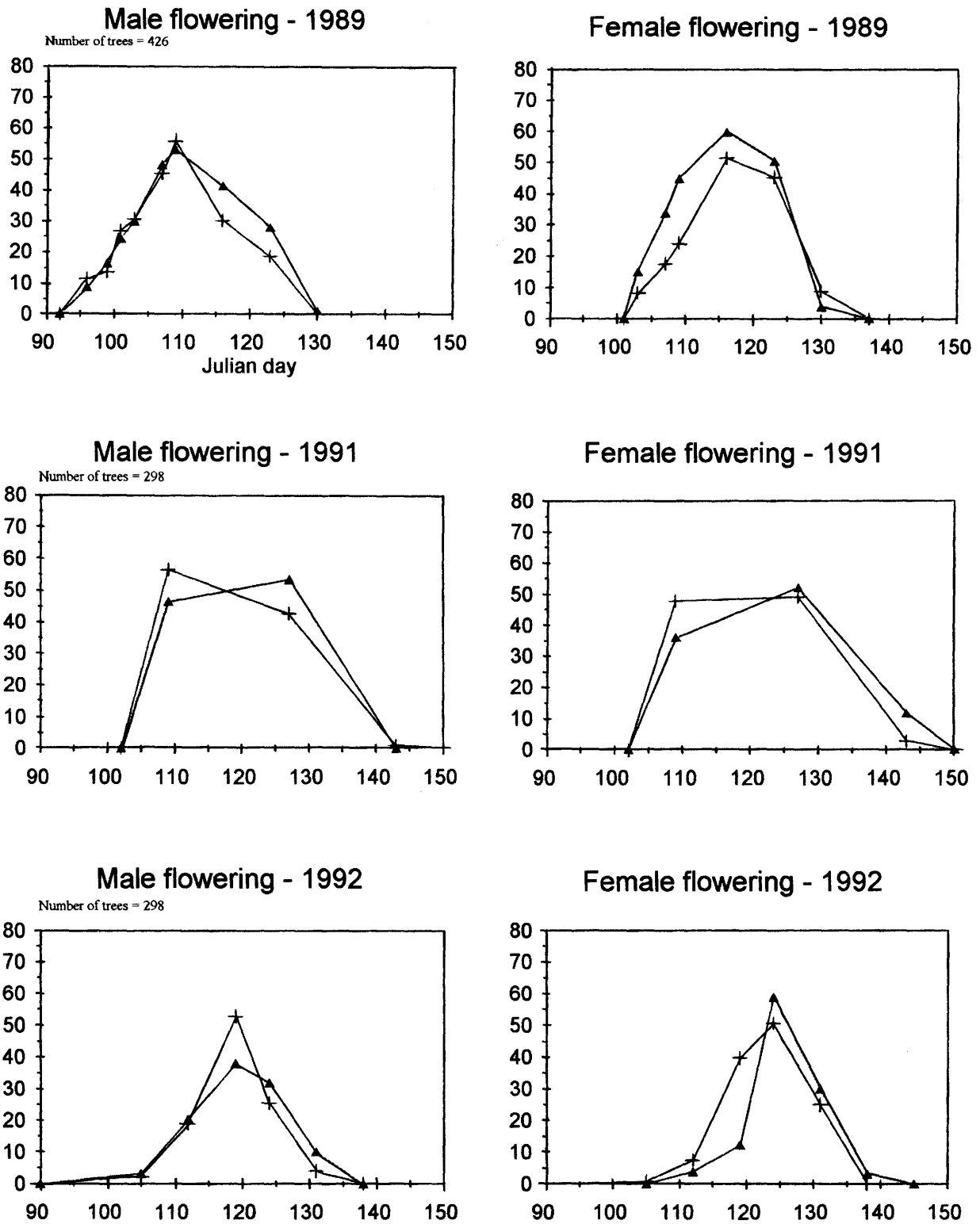

Figure 3. – Percent distribution of male and female flowering individuals, as a function of the Julian day, for 3 years and for the 2 species (sessile oak = (– ▲ –); pedunculate oak = (– + –); on abscissa: Julian Day; on ordinate: frequency (%)).

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Table 4. – Allelic frequencies in sessile and pedunculate oaks. N = number of individuals. X2 = Chi-square test of goodness of fit. d.f. = degrees of freedom. P levels: ns = non significant; (**) = 0.001.

|	LOCI	ALLELES							TESTS OF HOMOGENEITY			
		1	2	3	4	5	6	7	N	X2	d.f.	P level
Sessile oak	PGI	0,055	0,037	0,816	0,092	---	---	---	190	3,59	3	ns
Pedunculate oak		0,065	0,032	0,857	0,046	---	---	---	217			
Sessile oak	PGM	0,113	---	0,837	0,050	---	---	---	190	61,90	2	(**)
Pedunculate oak		0,468	---	0,482	0,038	0,012	---	---	217			
Sessile oak	SKDH	---	0,130	0,862	0,008	---	---	---	189	19,85	1	(**)
Pedunculate oak		---	0,007	0,979	0,014	---	---	---	217			
Sessile oak	IDH	0,074	0,048	0,841	0,021	0,016	---	---	189	14,18	2	(**)
Pedunculate oak		0,007	0,165	0,740	0,014	0,074	---	---	215			
Sessile oak	DIA	---	0,385	0,487	0,128	---	---	---	117	2,02	2	ns
Pedunculate oak		---	0,477	0,422	0,101	---	---	---	109			
Sessile oak	ACP-B	0,057	0,024	0,911	0,008	---	---	---	186	7,84	2	ns
Pedunculate oak		0,042	0,094	0,855	0,009	---	---	---	214			
Sessile oak	ACP-C	0,575	0,409	---	0,005	0,011	---	---	185	22,77	1	(**)
Pedunculate oak		0,795	0,200	---	0,002	0,002	---	---	217			
Sessile oak	GOT	0,034	0,961	0,005	---	---	---	---	190	0,19	1	ns
Pedunculate oak		0,018	0,952	0,028	0,002	---	---	---	217			
Sessile oak	AAP	---	---	---	0,597	0,090	0,261	0,053	190	24,24	3	(**)
Pedunculate oak		---	---	---	0,359	0,120	0,461	0,060	217			
Sessile oak	LAP	---	0,303	0,003	0,695	---	---	---	190	42,03	1	(**)
Pedunculate oak		---	0,623	0,005	0,373	---	---	---	216			
Sessile oak	MR	0,005	0,850	0,061	0,076	0,008	---	---	190	2,22	2	ns
Pedunculate oak		0,005	0,899	0,039	0,028	0,030	---	---	217			
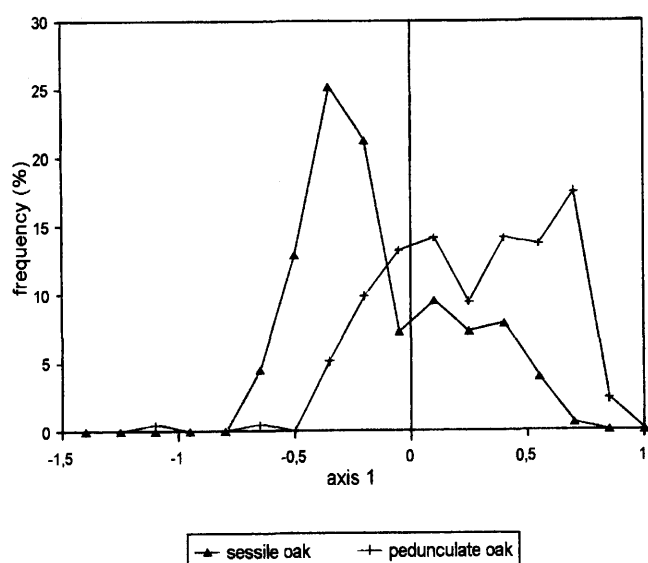
Figure 4. – Distribution of individuals of the 2 species on first axis of FCA on allozyme data. (407 individuals, 11 loci).

Figure 5. – Distribution of pedological sampling points on FCA axis.

Intraspecific relationships between genetic, morphological, phenological and ecological variables

All the results of such comparisons are summarised in *table 5*. In general, we can state that in both species there are no associations 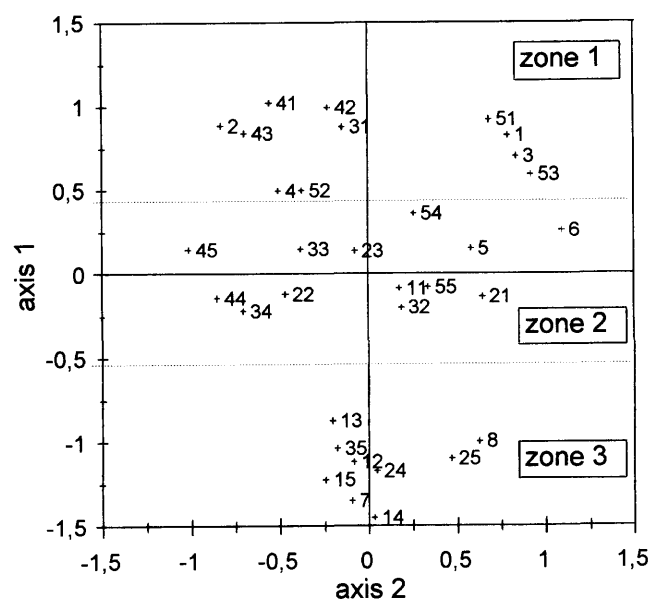among different types of characters. The few differences observed are apparently linked to the ecological conditions under which the trees grow. In pedunculate oak only 1 morphological characters *(anmoy)* out of 17 shows a highly significant (p < 0.001) difference between ecological zones. However, this character is not among those that discriminate the

species. Otherwise, in both species the variance of morphological characters was homogeneous over groups (BARTLETT's test). In both species weak differences (not significant) between ecological zones for the characters *lpet, llimb, largmax* indicate that in the upper and drier side of the stand the leaves were smaller.

Table 5. – Associations among ecological, morphological, phenological, and allozymic characters in sessile (190 trees) and pedunculate oaks (217 trees). Comparisons for sessile oaks are in the upper part of the table and for pedunculate oak in the lower part (further explanations see text).

	Ecology	Morphology	Phenology	Allozymes
Ecology	--	no	no	IDH, ACP-C
Morphology	anmoy	--	no	no
Phenology	no	no	--	no
Allozymes	MR	no	no	--

Comparisons of allelic frequencies between ecological zones showed statistical differences in 2 loci in sessile oak (IDH and ACP-C) and 1 (MR) in pedunculate oak. IDH and ACP-C are among the loci which presented differences in allelic frequencies between species *(Table 4);* this was not the case for MR. In sessile oak one of the loci (IDH) shows allelic frequencies more similar to those of pedunculate oak in the intermediate ecological zone as compared to typical sessile zone, but the other one (ACP-C) behaves in the opposite way.

Discussion and Conclusions

Morphological differentiation

Distributions of morphological characters of leaves of both species are approximately normal (in some a case square root transformation was necessary) allowing us to use the ANOVA and FDA methods. The sampling of leaves was optimised by testing the within tree repeatability. Mean number of leaves measured for each trees was 3.64. FALCONER (1981) showed that for a character with a repeatability coefficient $r = 0.5$, the maximal gain in precision is obtained with 3 to 4 measurements for each individual. When r is greater than 0.5, the number of observations per individual could be even smaller; as this was the case for most characters used, we can consider that our sampling is sufficiently accurate. The F-test showed that for all the characters a difference among trees was observable.

The two species are efficiently discriminated by the first axis of FDA with respect to morphological characters. Such discrimination is determined by a limited number of variables, which are those currently used in the botanical literature to distinguish the species; nevertheless, the FDA allows a much better separation than the one obtained using single characters. We confirmed that diagnostic characters do not exists, and that some individuals (about 3%) can not unequivocally be classified into one or another species, because of their intermediate features. This percentage is slightly inferior to the one obtained by other authors (WIGSTON, 1974; OLSSON, 1975; RUSHTON, 1978, 1979; KISSLING, 1983), but it is possible that this difference is caused by the use of different methods of analysis.

Lack of phenological differentiation between species

The phenological analysis showed that, in this stand and over 3 years, the flowering time did not constitute a barrier to hybridization. However, we observed a great variability among individuals in precocity and in mean length of flowering time; as a result, in a given year not all the crosses among individuals are realized. From the observations made on 2 non consecutive years, it is possible to state that about 50% of individuals change precocity class from one year to the other, probably under the influence of environmental factors. This variability over years inhibits the formation of mating groups that could lower the gene flow and the effective population size.

Genetic differentiation

Mean values for effective number of alleles and genetic diversity are very high in both species, agreeing with other studies on natural populations of oaks (KREMER *et al.,* 1991; MÜLLER-STARCK *et al.,* 1993; KREMER and PETIT, 1993). Such high values could be related to strategies of adaptation and survival of species which have a long life and that are exposed to an extraordinarily variable environment.

The differences in allelic frequencies between sessile and pedunculate oaks were of the same amplitude than what had been reported previously (KREMER *et al.,* 1991; ZANETTO, unpublished); these differences were found to be quite constant over France. In this case too we can not identify any species specific marker. With FCA, which uses the information given by all the alleles and by their associations due linkage disequilibrium, we can also observe a difference between species; but discrimination of the species is weaker than in case of the morphological markers.

It is generally admitted that differentiation for allozymes is much lower than differentiation for adaptative and morphological characters, because the allozymes are less subject to environmental selection (WHEELER and GURIES, 1982; CRAWFORD, 1989); nevertheless, LEWONTIN (1984) points out that the statistical power of comparison of allelic frequencies is much lower that for the comparisons of means of quantitative characters.

NEI's genetic distance between sessile and pedunculate oaks is of the same order as that obtained by ZANETTO (unpublished) for a large number of monospecific populations of the 2 species. It does not appear that the coexistence of the 2 species in the previous generations and the possible gene flow due to hybridisation has determined a diminution in the genetic differentiation between the species.

Ecological distribution of sessile and pedunculate oaks

Pedological analysis revealed the existence of an ecological gradient, determined mainly by the water conditions of the soil, within the experimental stand. The spatial distribution of sessile and pedunculate oaks followed this ecological gradient. These patterns, previously identified in other geographic locations (GRANDJEAN and SIGAUD, 1987), are probably due to ecophysiological differences between the 2 oak species, as was shown in comparative tests where juvenile plants were raised in controlled conditions (BELGRAND, 1983). A typical sessile oak zone, a typical pedunculate oak zone and an intermediate zone in which the 2 species are mixed tree by tree are evident.

Lack of intraspecific associations between morphology, phenology, genetic markers and ecology

In our experimental stand, at the intraspecific level, we did not observe associations among the different types of characters which could be attributed to the hybridization.

The morphological characters were homogeneous, in both species, over ecological zones, excepted anmoy in pedunculate oak. Because this character does not shown differences between species and because of the relatively large number of comparisons of means, this difference is probably not very significant. GRANDJEAN and SIGAUD (1987) found, on a regional scale, a correlation between ecology and those morphological characters that distinguish the species; however in our case such a correlation is not evident. Probably, the ecological gradient present in the stand is not sufficiently pronounced, at the spatial level, to establish a genetic differentiation among zones.

On the other hand, for both species, we can observe a trend in the dimensions of the leaves, which are slightly larger in the lower and more humid part of the stand. The observed difference is probably due to environmental factors and not to genetic factors.

Also the flowering phenology of trees was homogeneous through the stand, and among trees of different morphology. In addition, in both years, we did not observe genetic differences between phenological groups. These 2 kinds of observations, in association with the great variability observed in precocity over years, suggest that there were not groups of preferential mating based on precocity differences.

Within species, some differences in allelic frequencies were observed among ecological zones. If the 2 species hybridize and introgress, we could expect that hybrids and backcrosses have intermediate ecological preferences, and that they have also intermediate allelic frequencies between the 2 species. This does not seem to be the case, because in pedunculate oak the only locus that showed different allelic frequencies in the 2 ecological zones is not among those loci that showed specific differences, and in sessile oak the 2 loci that display differences behave in the opposite way. These differences may be due to chance, or to an ecological selection pressure, but apparently not in relation to hybridisation. Allelic frequencies are, on the contrary, clearly homogeneous within each species among the different morphological groups.

In conclusion, in spite of the lack of phenological differences between species that clearly offers them the possibility of hybridization, we did not find any evidence of interspecific gene flow. In other forest tree species in which hybridisation was detected, a number of diagnostic or quasi-diagnostic markers were usually found, as in *Aesculus* (DE PAMPHILIS and WYATT, 1989) and in *Populus* (KEIM et al., 1989). In 18 populations of 5 species of american white oaks, WHITTEMORE and SCHAAL (1991) did not found morphologically recognisable hybrids, in spite of the large interspecific gene flow they observed with chloroplast DNA markers. The overlapping distributions observed in all the characters examined here make the analysis of hybridization difficult; on the other hand, it is an argument that supports the hybridization hypothesis.

The lack of evidence of interspecific gene flow may be partially due to the fact that hybrids and backcrosses were progressively eliminated during the life history of this stand by natural selection. It must be noted that the individuals studied are survivors of a natural generation that was probably at least 100 times denser, and selection had material to act upon. Nevertheless, the observed pattern could also be due to other causes: 1) if hybrids and backcrosses were in small numbers, as was suggested in other sympatric *Quercus* species (NASON et al., 1992), they could have remained undetectable with the statistical tests used here; 2) the studied area was relatively small and the ecological gradient could be too weak in relation to the ecological sensibility of these species, or spatially too

restricted in relation to their dispersal ability; 3) hybrids and backcrosses could be morphologically or ecologically similar to one of the 2 parental species if long-lasting maternal effects were important; (4) recent studies here shown that correlation among characters in hybrid zones is not common, probably because there is no reason for species-specific genes to be strongly linked (RIESBURG and ELLSTRAND, 1993). The high levels of outcrossing that characterise the oak species (DUCOUSSO et al., 1993; BACILIERI et al., 1995) could accelerate the decay of linkage disequilibrium.

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Observed Selection Effects on Height Growth, Diameter and Stem Form in Maritime Pine

By F. DANJON

INRA, Laboratoire Croissance et Production, Pierroton
33610 Cestas, France

Summary

Effects of plus tree selection in local mature stands, and of early combined index selection performed in progeny trials, were assessed in a maritime pine (*Pinus pinaster* AIT.) test established in 1965 in 2 sites. Nonlinear regression was used for the characterization of selection effects on height growth curves. Diameter growth, stem straightness and the proportion of polycyclic annual shoots were also examined.

The plus tree selection was shown to be effective on the asymptote, i. e. on mature growth, whereas the early combined index selection was effective mainly on the maximal growth rate, i.e., on juvenile growth; both selections improved the stem form. No consistent genotype x environment interaction was observed. For height growth, provenance effects were found in the area studied.

It was hypothesized that, due to a low juvenile-mature correlation for vigour, selection at a given time altered the shape of the growth curves. Thus, several cycles of early selection could improve the stem volume at rotation age, though they could have a much greater effect on early height. Undesirable effects on wood structure and architecture were then expected.

Key words: height growth curves, nonlinear regression, polycyclism, genetic gain, plus tree selection, combined selection, *Pinus pinaster.*

FDC: 165.4; 165.62, 174.7 *Pinus pinaster.*

Introduction

The main objectives of forest tree breeding programs are usually the increase in wood production and in wood quality yielded at clearcut. Because of the rotation length, selection is made at young age and few genetic studies have been published for ages greater than 20 years (REHFELDT et al., 1991). In the same way, very little long-term data is available for improved stands (BIROT, 1986; TALBERT and HYINK, 1987). Genetic gains estimated at a young age are often assumed to be highly correlated with gains at rotation age (KNOVE and FOSTER, 1989).

Early maritime pine (*Pinus pinaster* AIT.) progeny tests are older now than half the rotation age. Taking advantage of the original structure of a progeny test established in 1965 (ILLY, 1966), effects of "plus tree" selection (*in-situ* phenotypic selection) and of early combined index selection in progeny test were assessed, and genetic gains on height at clearcut (age 40 to 60 years) were predicted, using a modelling technique. This work provides information on the performance of improved material, now widely used in the "Landes de Gascogne" forest, and some keys for the management of improved stands were inferred. As a result, genetic effects may then be incorporated into stand simulation models.

The selection program for maritime pine in Southwest-France (the "Landes de Gascogne" area) started in 1960 with selection of plus trees in the local provenance, in stands older than 30 years (ILLY, 1966). Then, a recurrent selection program was developed with height and stem straightness at approximately age 10 years as main selection criteria (BARADAT and PASTUSZKA, 1992). No extended evaluation of genetic gains at later ages have been made so far.

In some stands, seeds from trees of average performance (control trees) were collected in addition to plus tree seeds. One hundred plus tree and control tree progenies were established on 2 sites (trial B and trial C).