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Original article

Hybridization and mating system in a mixed stand of sessile and pedunculate oak

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Summary — Patterns of hybridization and of the mating system of *Quercus petraea* and *Quercus robur* have been inferred from examination of allozyme variation in 2 cohorts (adults and progeny) of a stand comprised of both species. Differences in allelic frequencies were found in each species between the pollen pool and the adult trees, but the pattern of hybridization was apparently asymmetrical. *Q petraea* and *Q robur* are almost exclusively allogamic, the multilocus outcrossing rate being 0.96 for both species.

allozymes / hybridization / mating system / pollen pool / *Quercus robur* / *Quercus petraea*

Résumé — Hybridation et système de reproduction dans une forêt mixte de chêne sessile et chêne pédonculé. *Les modalités d'hybridation et du système de reproduction de* Quercus petraea *et* Quercus robur *ont été étudiées à partir des variations allozymiques dans 2 cohortes (les adultes et leurs descendants) d'une forêt mixte composée des 2 espèces. Pour chaque espèce, des différences dans les fréquences alléliques entre le pool pollinique et les arbres adultes ont été trouvées, mais le sens de l'hybridation semble asymétrique.* Q petraea *et* Q robur *sont presque exclusivement allogames, le taux d'allofécondation multiloci étant de 0,96 pour chacune des 2 espèces.*

***allozymes / hybridation / système de reproduction / pool pollinique* / Quercus robur / Quercus petraea**

INTRODUCTION

Quercus petraea (Matt) Liebl and *Quercus robur* L have a largely sympatric distribution in Europe and it is suspected that they hybridize in nature. The species are anemophilous; a survey of phenology in the same mixed stand, described below, did not show any differences in flowering time between the 2 species (Expert, 1990). Differences in habitat preference can form a barrier to gene flow, but in the intermediate habitats the species are in contact and it is there that one can find the greatest number of intermediate forms (Grandjean and Sigaud, 1987). Nevertheless, in natural populations, adult trees with intermediate features seem to be quite rare, less than 5% of the total population (Dupouey, 1983; Dupouet and Badeau, 1993).

The possibility of hybridization between sessile and pedunculate oaks was proven by interspecific controlled crosses (Rushton, 1977). The success rate of artificial hybridization is higher when *Q robur* is fertilized with the pollen of *Q petreae* than *vice versa* (Aas, 1991; Steinhoff, 1993).

A few authors (Kremer *et al*, 1991; Müller-Starck *et al*, 1993) have investigated interspecific differentiation on a genetic basis using biochemical markers, but so far no conclusions have been drawn as to hybridization in nature. At present, the strongest evidence concerning active exchange of genes between pedunculate and sessile oaks can be deduced from the pattern of chloroplast gene diversity (Kremer and Petit, 1993).

The major questions are: 1) what is the real extent of hybridization? 2) how can the 2 species be mantained? In this paper patterns of hybridization and of the mating system of *Q petraea* and *Q robur* have been inferred from examination of allozyme variation in 2 cohorts of a stand comprised of both species.

MATERIALS AND METHODS

The population studied is a mixed adult stand of *Q petraea* and *Q robur* located in the Petite Charnie forest, in north-western France (Le Mans). The trees are about 120 years old. The study area was square (220 X 220 m), with a uniform slope. In this area, a good correlation was observed between hydromorphic layer depth and frequency of the 2 species. *Q robur* prefers more humid sites than *Q petraea.*

For genetic analysis, all plants of both species form the adult cohort. The young cohort was made up of the progenies of these adults (fig 1), taking a maximum of 6 open-pollinated seeds per family for sessile oak (160 individuals, 28 families) and 10 open-pollinated seeds per family for pedunculate oak (133 individuals, 16 families). This protocol was used to avoid bias due to local heterogeneity of the pollen pool.

The taxonomic status of the adults was determined using factorial correspondence analysis (FCA). The morphological characters used were: pubescence, number of intercalary and lobe veins, auricle form and embossing of the lobe.

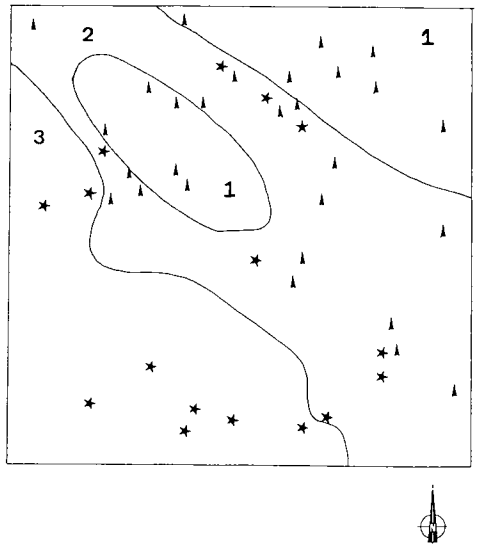

Fig 1. Position of mother trees in the stand. 1: pure sessile oak stand; 2: mixed stand; 3: pure pedunculate oak stand; ▲ : *Q petraea*; ⋆ : *Q robur.*

Allozymes extracted from buds of the adults and roots of the seedlings were electrophoresed. Seeds were collected directly from adult trees during the autumn of 1989, and germinated in an incubator. Technical procedures and genetic interpretations are described in detail in Kremer *et al* (1991) and Zanetto *et al* (1993). We stained and then scored 8 enzyme systems encoded by 8 putative loci: acid phosphatase (ACP), glutamate-oxalacetate transaminase (GOT), isocitrate dehydrogenase (IDH), menadione reductase (MR), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), leucine aminopeptidase (LAP) and alanine-aminopeptidase (AAP).

Allelic frequencies in the pollen pool and multilocus outcrossing rates *(t)* were estimated with Ritland's computer program (1990), based on the mixed-mating model. To obtain the best estimate of *t,* we used only the largest families, from 12 sessile oaks (332 individuals) and 10 pedunculate oaks (236 individuals).

Differences in allelic frequencies at each locus between adults and pollen pool were assessed by a *G*-test. The differences between adults and pollen pools over all loci were evaluated by a sign test (Sokal and Rohlf, 1981) that enables detection of directionality in changes of allele frequencies. For each of the 2 most frequent alleles at each locus, we assigned a positive sign if its frequency in the pollen pool was similar to that of the adults of the other species, and a negative sign if the opposite was the case. We then tested the hypothesis that the 2 signs were present in equal proportions; such sampling should exhibit a binomial distribution. The sign test is an exact test and does not require calculation of degrees of freedom.

RESULTS

Morphological analysis (performed by FCA, not shown here), failed to identify the taxonomic status of 2% of the trees. Trees that did not produce seeds in 1989 were excluded from subsequent analysis. The adult cohorts were then made up of 186 sessile oaks and 212 pedunculate oaks.

In adult trees, significant differences in allelic frequencies were found between sessile and pedunculate oaks in 7 out of 8 loci (table I). As in other studies (Kremer *et al,* 1991; Müller-Starck *et al*, this volume), we did not find any species-specific alleles.

There were significant differences in gene frequencies between the pollen pool and the adult trees (table I). In spite of the pollen environment, which is composed of similar proportions of conspecific *versus* foreign plants of the 2 species (mother trees are encircled by 32 and 37% of trees of the other species, for *Q robur* and *Q petraea*, respectively), the gene frequencies in the seeds of both species showed an asymmetrical shift towards more pronounced *Q petraea* genetic characters. For *Q robur*, this shift was significant for 4 loci (ACP, PGM, LAP and MR) out of the 7 with interspecific differences; AAP showed the same pattern, but the difference was significant only at the 0.10 level. For *Q petraea*, gene frequencies in the pollen pool were significantly different from those of the adults for 2 loci (MR and PGI).

The sign test for all the loci showed that the directionality of changes was significant for both species, at the 0.011 propability level for *Q petraea* and 0.038 for *Q robur.* Progenies of *Q robur* are therefore genetically closer to the genetic pool of *Q petraea.*

Since incorrect taxonomic determination can be a source of error in allele frequency estimates, we repeatedly calculated gene frenquencies in adult groups by restricting the sample size of the parent trees. Those with intermediate morphological characters were progressively excluded from the estimation of allele frequencies. However, no significant changes in gene frequencies were found in these new groups.

Estimates of multilocus outcrossing rates were 0.96 (± 0.08) and 0.96 (± 0.05) for *Q petraea* and *Q robur* respectively. Neither of these estimations is significantly different from one.

Table I. Gene frequency differences between species and between adults and pollen pools. The significance level of the differences between young and adult samples and between species were tested by a *G*-test. The total shifts in pollen-pool gene frequencies were tested with a sign test. A (+) is marked if frequency in the pollen pool of 1 species is closer to that of adults of the other species, and a (–) if the reverse is the case.

Loci	*Allele*	Q petraea			Q robur			
		Adult 1	*Pollen pool 2*	*2–1 (sign)*	*Pollen pool 3*	*Adult 4*	*4–3 (sign)*	*4–1*
ACP	1	0.575 (0.026)	0.547 (0.044)	NS (–)	0.643 (0.054)	0.790 (0.020)	0.01 (+)	0.01
	2	0.409 (0.025)	0.441 (0.045)	(–)	0.357 (0.054)	0.206 (0.019)	(+)	
	4,5	0.016 (0.006)	0.012 (0.009)		0.000 (0.0000)	0.004 (0.003)		
AAP	4	0.599 (0.025)	0.635 (0.055)	NS (–)	0.443 (0.051)	0.353 (0.023)	NS (+)	0.01
	6	0.262 (0.022)	0.215 (0.045)	(–)	0.413 (0.061)	0.470 (0.024)	(+)	
	5,7	0.139 (0.018)	0.150 (0.029)		0.143 (0.042)	0.177 (0.018)		
PGM	3	0.832 (0.019)	0.836 (0.033)	NS (–)	0.619 (0.054)	0.486 (0.024)	0.01 (+)	0.01
	1	0.115 (0.016)	0.112 (0.027)	(–)	0.313 (0.057)	0.465 (0.024)	(+)	
	2,4,5	0.053 (0.011)	0.052 (0.020)		0.068 (0.031)	0.049 (0.010)		
LAP	4	0.696 (0.023)	0.764 (0.043)	NS (–)	0.473 (0.073)	0.362 (0.023)	0.05 (+)	0.01
	2	0.301 (0.023)	0.229 (0.044)	(–)	0.504 (0.069)	0.633 (0.023)	(+)	
	3	0.003 (0.003)	0.008 (0.008)		0.023 (0.014)	0.005 (0.003)		
MR	2	0.856 (0.018)	0.723 (0.037)	0.01 (–)	0.847 (0.053)	0.898 (0.015)	0.05 (+)	0.01
	4	0.071 (0.013)	0.180 (0.033)	(–)	0.081 (0.039)	0.028 (0.008)	(+)	
	1,3,5	0.073 (0.013)	0.097 (0.030)		0.072 (0.025)	0.074 (0.013)		
IDH	3	0.842 (0.019)	0.803 (0.038)	NS (+)	0.681 (0.061)	0.744 (0.021)	NS (–)	0.01
	1	0.074 (0.013)	0.111 (0.024)	(+)	0.000 (0.000)	0.007 (0.0004)	(–)	
	2,4,5	0.084 (0.014)	0.086 (0.029)		0.319 (0.061)	0.249 (0.021)		
PGI	3	0.812 (0.020)	0.658 (0.039)	0.01 (–)	0.874 (0.053)	0.860 (0.017)	NS (–)	0.05
	4	0.094 (0.015)	0.188 (0.034)	(–)	0.071 (0.033)	0.044 (0.010)	(+)	
	1,2	0.094 (0.015)	0.154 (0.036)		0.055 (0.021)	0.094 (0.14)		
GOT	2	0.958 (0.010)	0.957 (0.022)	NS (+)	0.929 (0.033)	0.947 (0.011)	NS (–)	NS
	1	0.037 (0.010)	0.030 (0.019)	(+)	0.053 (0.028)	0.021 (0.007)	(+)	
	3,4	0.005 (0.004)	0.013 (0.010)		0.018 (0.014)	0.032 (0.008)		

DISCUSSION

In the Petite Charnie forest, the frequency of intermediate individuals at the adult stage, as deduced from FCA on morphological characters, was low, in spite of the apparent lack of spatial or phenological barriers to hybridization.

Differences in allele frequencies between adult populations of the 2 species were large and, within each species, were stable over morphological classes. These results are in agreement with the findings of other authors (Dupouey, 1983; Grandjean and Sigaud, 1987; Dupouey and Badeau, 1993).

The observed shift in gene frequencies of *Q robur* progeny could be explained by the fertilization of a portion of female flowers with pollen of *Q petraea*; on the contrary, the causes of the shift in frequencies of *Q petraea* progeny are more difficult to understand.

Different pre- and postzygotic mechanisms may explain this asymmetry. For the moment, we can only exclude the effects of differential proportion of selfing. On the contrary, we cannot exclude that, in 1989, male flowering of *Q petraea* was heavier or more effective than that of *Q robur*, contributing in that way to the largest part of the fertilization of both species. Indeed, strong temporal and spatial differences in the genetic composition of the pollen pool have been found in other species, such as *Fagus sylvatica* (Merzeau *et al*, 1989) and *Picea mariana* (O'Reilly *et al*, 1982).

Moreover, large differences can be observed between loci. This shift may then result not only from asymmetric hydridization but also from various differentiating forces.

Nevertheless, the hypothesis of asymmetric gene flow is confirmed by the results of interspecific controlled crosses (Aas, 1991; Steinhoff, 1993) showing a preferential pollen gene flow from *Q petraea* to *Q robur*, while the success in reciprocal crosses is close to zero. A similar unidirectional introgression has been described in *Populus* (Keim *et al*, 1989) and in *Eucalyptus* (Potts and Reid, 1988).

If the pattern of unidirectional hybridization occurs in the future, which needs to be confirmed, the gene pool of the next generation of the Petite Charnie oak stand would comprise a greater number of *Q petraea* genes.

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