

## MATING SYSTEM AND ASYMMETRIC HYBRIDIZATION IN A MIXED STAND OF EUROPEAN OAKS

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**Abstract.**—The sessile (*Quercus petraea* [Matt.] Liebl.) and pedunculate (*Quercus robur* L.) oaks are two closely related species having a wide sympatric distribution over Europe. Under natural conditions, they frequently form mixed forests, where hybridization is suspected to occur. In this paper, two different approaches have been applied to the study of the mating system and the interspecific gene flow in a mixed stand formed by the two species. The mating systems of both species have been studied separately by means of the mixed-mating model. The relative contribution of the parental species to the progenies have been estimated with two different methods. The first uses the admixture model. The second is an extension of the mixed-mating model and subdivides the outcrossing rate into intra- and interspecific components. The two species were almost completely outcrossing. This high level of outcrossing and interspecific gene flow could play an important role in the maintenance of the genetic diversity in these long-lived forest tree species. The contribution of the sessile oak to the pedunculate oak progenies varied from 17% to 48%. In contrast, ovules of sessile oak trees appear to be preferentially fertilized by other extreme sessile genotypes. We suggest that interspecific and directional gene flow was responsible for such patterns. Pedunculate oak is considered as a pioneer species and is progressively replaced by sessile oak. Our present findings add a further genetic component to this succession scheme, suggesting that unidirectional gene flow reinforces succession between the two species.

**Key words.**—Gene flow, hybridization, mating system, mixed-mating model, *Quercus*.

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Hybridization and introgression are common phenomena in many plant and animal groups and have been proposed as important processes in evolution. Interspecific gene flow has received much attention not only as a potential source of genetic variation in plant populations, but also for its implication in the speciation process (Anderson 1949; Stebbins 1969; Grant 1981; Rieseberg and Brunsfeld 1992; Avise 1994). Since Darwin (1859), the genus *Quercus* has attracted the attention of evolutionists for its very poor development of reproductive barriers between species (Grant 1981; Rushton 1993). Many oak species grow in mixed stands over large zones of sympatry. In these mixed populations, hybridization seems common (Rushton 1993). However, with the exception of a small proportion of intermediate individuals, separate morphological entities remain discernible even in conditions of sympatry and hybridization.

Sessile oak (*Quercus petraea* [Matt.] Liebl.) and pedunculate oak (*Quercus robur* L.) are two closely related species that share a wide sympatric distribution over Europe. A number of morphological and physiological differences account for their different ecological preferences. *Quercus petraea* has smaller vessels (Cochard et al. 1992), a smaller number of lenticels on the roots, a deeper and more developed root system (Bréda et al. 1993), and is considered to be more drought resistant than is *Q. robur* (Becker and Lévy 1982). On the contrary, *Q. robur* grows in soils with high water tables and frequent flooding (Grandjean and Sigaud 1987). Differences are also observed with respect to light tolerance and competitive ability (Fairburn 1954). *Quercus petraea* tolerates denser and more shaded conditions. *Quercus robur* has greater light tolerance, a higher capacity for germination on disturbed soils, and wide seed dispersal due to preferential

transport by jays (Bossema 1979). These ecophysiological differences contribute to the succession between the two species, *Q. petraea* replacing progressively *Q. robur* in mesic conditions (Rameau 1990). In studying hybridization, we are addressing the genetic component of species succession, especially if unidirectional crossings occur in mixed stands.

The hypothesis of hybridization between sessile and pedunculate oak is supported by different kinds of observation: (1) the systematic finding, in mixed forests, of intermediate morphological forms (Gardiner 1970; Rushton 1978, 1979; Minihan and Rushton 1984; Semerikov et al. 1988; Ietswaart and Feij 1989; Dupouey and Badeau 1993), which are more abundant in intermediate habitats (Rushton 1979; Grandjean and Sigaud 1987); (2) the lack of diagnostic characters either in morphology or in molecular markers (Kremer et al. 1991; Zanetto et al. 1994; Müller-Starck et al. 1993; Moreau et al. 1994; Ferris et al. 1993; Petit et al. 1993a,b); (3) the success of interspecific controlled crosses (Aas 1990; Steinhoff 1993); (4) the diminished pollen viability of some of the intermediary forms (Olsson 1975; Rushton 1978); and (5) the peculiar structure of chloroplast DNA polymorphisms, which reflects a similar geographic differentiation for both species but no specific differentiation (Kremer et al. 1991; Petit et al. 1993a; Ferris et al. 1993). However, most of these studies focused on patterns of variation, and conspicuously lacking are direct observations on mating and admixture in natural oak populations.

It is the objective of this study to estimate the rate of hybridization between sessile and pedunculate oak in a mixed stand using two complementary approaches. The first approach uses the ML (maximum likelihood) procedure of Ritland and El-Kassaby (1985), based on the mixed mating model to estimate the allelic frequencies in the pollen pools of the two species. These allele frequencies are then used to estimate the genetic admixture proportions (Roberts and

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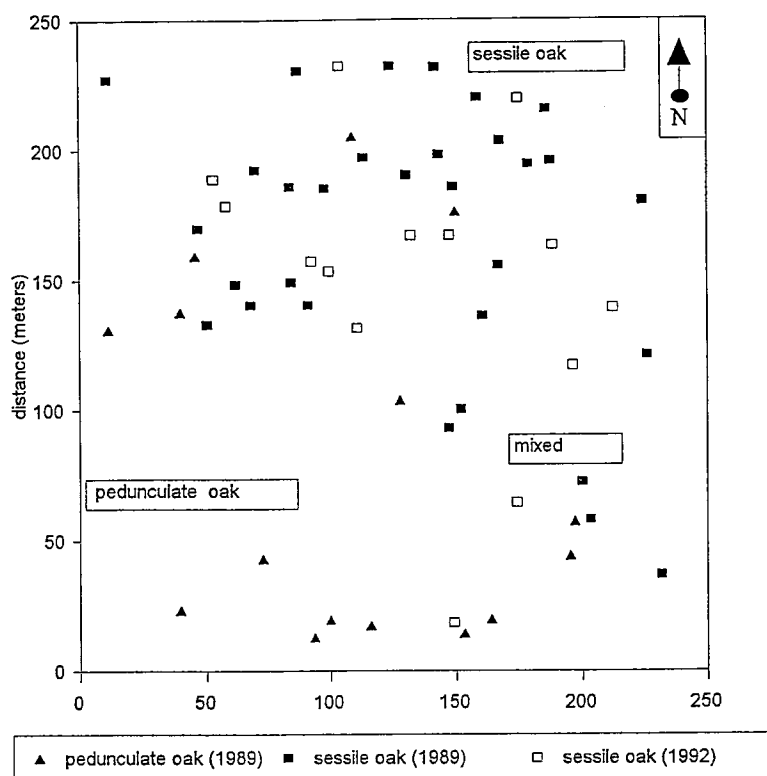


FIG. 1. Spatial distribution of the trees in the stand. The stand is composed of 426 trees of sessile and pedunculate oak. In the figure, only the mother trees from which seeds have been collected are represented.

Hiorns 1965; Elston 1971) by comparison with adult gene frequencies. The second approach presented here extends the mixed-mating model to the case of two interfertile species growing in a mixed population (two species mixed-mating model). On the basis of progeny arrays, our model estimates the selfing rate and subdivides the outcrossing rate into intra- and interspecific components. This new model, relaxing the assumption of homogeneity of gametic pools implicit in the original mixed-mating model (Fyfe and Bailey 1951), has a more general validity and could be used to study the admixture proportions at mating in hybrid zones or in zones of contact between cultivated and wild species.

#### MATERIALS AND METHODS

The experimental oak stand covers an area of 250 m  $\times$  250 m in the Petite Charnie Forest, near Le Mans (France). In the study area, 426 trees of the two species, about 120 yr old, grow along an ecological gradient generated by a gradual slope. The density of the stand is homogeneous, though the relative densities of the two species follow the ecological gradient, with sessile oak more frequent in the upper part and pedunculate oak more frequent in the lower. The ecology of the stand, the phenology of flowering, the taxonomic discrimination based on morphology, and the allele frequencies at 11 allozyme loci for 190 sessile and 217 pedunculate adult oak trees of the stand are described elsewhere (Bacilieri et al. 1994, 1995). Nineteen adult trees were excluded from the biochemical analyses either because they were morphologically intermediate between the two species or because the

quality of their tissue did not allow for extraction of proteins. The morphological intermediate forms did not bear fruits in the years of sampling (1989–1992). In 1990 and 1991, neither species set fruit. In 1989 and 1992, seeds were collected from the crown of a number of open-pollinated trees of the two species (Fig. 1). In addition, within a circle of 1 m in diameter around each of the adult trees, we randomly chose one seedling of the natural regeneration, from which leaves were collected for morphological and biochemical analyses. These seedlings were three to six yr old.

#### *Morphological Assignments of Seedlings to the Two Species*

Taxonomic classification of the seedlings was based on six morphological characters of leaves chosen from among those that showed the greatest discriminating power in adult trees, that is, density of leaf pilosity, number of lobes, petiole length, height of the maximal width of the leaf, mean of the auricle angles, and mean shape of the apex of the lobes. The morphological characters were measured on three leaves per seedling. The repeatability coefficient (Falconer 1981) was greater than 0.5 for all the morphological characters. The first axis of a Factorial Discriminant Analysis (FDA; Legendre and Legendre 1984) accounted for 73% of the total variance (in adult trees, the first axis of a FDA on the same six characters used here accounted for 69% of the total variance). On the basis of the FDA, it was possible to identify two groups, composed of 221 and 186 seedlings of sessile and pedunculate oak, respectively (Fig. 2).

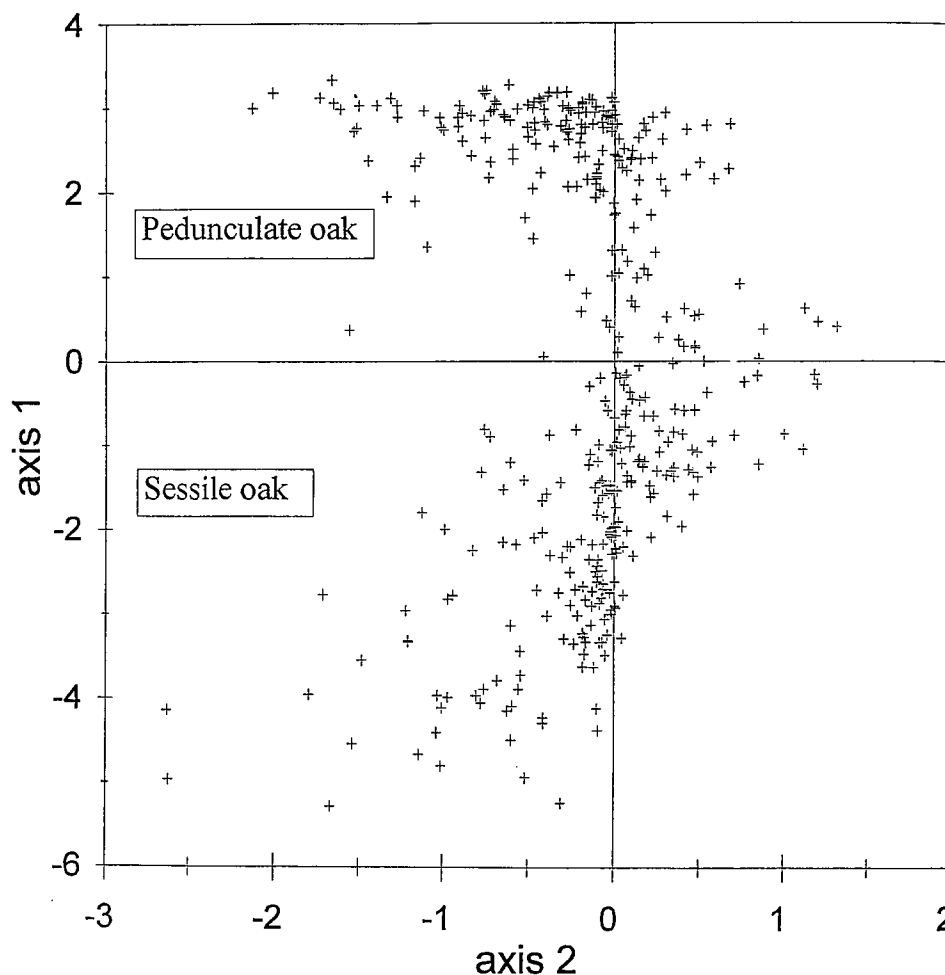


FIG. 2. Discriminant analysis of six morphological characters of seedlings originating from natural regeneration. Observations greater than zero on the first axis were classified as pedunculate oak, and those less than zero were classified as sessile oak.

#### Biochemical Analyses

Seeds collected from adult trees were germinated in an incubator; the enzymes were extracted from seedling roots with the procedure described by Kremer et al. (1991). To extract enzymes from leaves of the regeneration sample, we used the procedure described by Bacilieri et al. (1995). Seven enzymes were studied by starch-gel electrophoresis in adult trees, seeds, and seedlings. These enzymes were encoded by seven loci: *PGM-A* (phosphoglucumutase, EC. Ref: 3.4.11.1), *ACP-C* (acid phosphatase, EC. Ref: 3.1.3.2), *AAP-A* (alanine aminopeptidase, EC. Ref: 3.4.11.1), *IDH-A* (isocitrate dehydrogenase, EC. Ref: 1.1.1.42) *PGI-B* (phosphoglucoisomerase, EC. Ref: 5.3.1.9), *MR* (menadione reductase, EC. Ref: 1.6.99.2), and *GOT-B* (glutamate-oxaloacetate transaminase, EC. Ref: 2.6.1.1). The technical procedures and genetic interpretations have been described elsewhere by Kremer et al. (1991) and Zanetto et al. (1996), respectively. The homology of enzyme forms extracted from leaves and roots was tested by comparing, in a representative subsample, the band patterns obtained for different tissues of the same individual.

The multilocus genotype was obtained for a total of 407 adult trees, 407 seedlings, and 1479 seeds. Of these seeds, 702 were collected from sessile oak (31 families) and 307

on pedunculate oak (15 families) in 1989, and 470 were collected on sessile oak (15 families) in 1992. The pedunculate oak did not bear fruit in 1992. To simplify the computations, the two most frequent alleles were coded as allele 1 and 2, and the sum of the other alleles as allele 3.

#### Estimation of Mating-System Parameters

Population single- and multilocus outcrossing rates ( $t_i$  and  $t_m$ ) were estimated jointly with the pollen pool allelic frequencies ( $p$ ) using the maximum-likelihood procedure of Ritland and El-Kassaby (1985), for sessile and pedunculate oaks and for both years of sampling. Departures from the assumptions of the mixed-mating model were tested using Ritland's (1983)  $\chi^2$  procedure.

The allele frequencies in the pollen pools that contributed to the seed cohorts were estimated with a procedure reducing the bias due to (eventual) local heterogeneity of pollen clouds. Within the original data set, five to 10 seeds were randomly selected from each family, to minimize the family variance in the number of seeds. After this sampling, the three data sets were made up of 155 seeds and 124 seeds for sessile oak collections of 1989 and 1992, respectively, and of 127 seeds for pedunculate oak collection of 1989. This

TABLE 1. The probability of progeny genotypes for a given maternal genotype, under the "two-species" mixed-mating model. The model has been constructed for a diallelic locus.  $p_s$ , frequency of allele A in species  $s$ .  $p_p$ , frequency of allele A in species  $p$ .  $S_s$ , selfing rate of species  $s$ .  $Tm_s$ , intraspecific outcrossing rate of species  $s$ .  $(1 - Tm_s - S_s)$ , interspecific outcrossing rate of species  $s$ . A similar set of seven equations can be written for species  $p$ .

	Maternal genotype	Progeny genotype	Probability
Species $s$	AA	AA	$S_s + p_s Tm_s + p_p(1 - S_s - Tm_s)$
		Aa	$(1 - p_s)Tm_s + (1 - p_p)(1 - S_s - Tm_s)$
	aa	aa	$S_s + (1 - p_s)Tm_s + (1 - p_p)(1 - S_s - Tm_s)$
		Aa	$p_s Tm_s + p_p(1 - S_s - Tm_s)$
	Aa	AA	$S_s/4 + (p_s Tm_s + p_p(1 - S_s - Tm_s))/2$
		aa	$S_s/4 + (1 - p_s)Tm_s + (1 - p_p)(1 - S_s - Tm_s)/2$
		Aa	$1/2 S_s + p_s Tm_s + p_p(1 - S_s - Tm_s) + (1 - p_s)Tm_s + (1 - p_p)(1 - S_s - Tm_s)$

procedure of random sampling was repeated 100 times, and the averages of the allelic frequencies obtained for each sample with the ML procedure of Ritland and El-Kassaby (1985) were then considered as estimations of the allelic frequencies of the pollen cloud in the stand.

To assess the homogeneity of the pollen pool among maternal parent trees, the number of homozygous and heterozygous embryos borne by each homozygous female was entered into a  $2 \times f \chi^2$  contingency table, where  $f$  is the number of homozygous females (Brown et al. 1975). An estimate of effective selfing caused by consanguineous matings was obtained by means of a regression of individual tree outcrossing pollen allele frequencies ( $p_f$ ) on the additive value of the ovule genotype (Ritland and El-Kassaby 1985). For families with an effective number greater than 30, estimates of  $p_f$  and of individual outcrossing rate ( $t_f$ ) were obtained. Heterogeneity of  $t_i$  among loci and  $t_f$  among individuals were tested using the  $\chi^2$  test of Kahler et al. (1984).

#### Estimation of Admixture

Differences in allelic frequencies at each locus between the pollen pools, seedlings, and adults were assessed by a  $\chi^2$  test. Multilocus differences were evaluated by a sign test (Sokal and Rohlf 1981), which enabled detection of directionality in changes of allelic frequencies. For a given species at each of the two most frequent alleles at a locus (the frequency of the third allele being dependent on these), we assigned a positive sign if the allelic frequency in the progenies changed toward the frequency of the adults of the other species and a negative sign in the opposite case. We then tested the hypothesis that the two signs were present in equal proportion; such sampling should exhibit a binomial distribution.

The relative genetic contribution of each parental species to the progeny (seeds and seedlings) was estimated using the least-squares procedure developed to describe admixture in human populations (Roberts and Hiorns 1965; Elston 1971). The least-squares estimate of gene admixture (the proportion of genes derived from each parental population),  $\mathbf{m}$ , is a row vector defined as

$$\mathbf{m} = (\mathbf{X}'\mathbf{X})^{-1}\mathbf{X}'\mathbf{y},$$

where  $\mathbf{X}$  is the matrix of allele frequencies in the two populations and  $\mathbf{y}$  the vector of allele frequency differences between the progeny and one of the parental population, pro-

vided  $\mathbf{X}'\mathbf{X}$  is nonsingular. For each locus, the frequencies for all but the least common allele in the reference taxon were included in the calculations (Roberts and Hiorns 1965). We imposed that all elements of  $\mathbf{m}$  sum to 1.0.

We calculated  $\mathbf{m}$  on the basis of the allelic frequencies in adults and in progenies. The reference populations first included all the adult trees of each species. Then, to check whether a bias was generated by correlated matings, we used as female references the female parent trees only. For each species, the admixture proportions were computed for three data sets, that is, (1) the paternal gametic contribution in seeds, calculated with the model of Ritland and El-Kassaby (1985); (2) the seeds (in which paternal and maternal contributions were added); and (3) the seedlings. The admixture proportion were calculated also for families with an effective number greater than 30, in which it was possible to estimate the individual pollen pool allelic frequencies ( $p_f$ ) with the ML procedure of Ritland and El-Kassaby (1985).

#### Estimation of Inter- and Intraspecific Outcrossing Rates

To construct our two-species mixed mating model, we assumed, for each species, that the pollen pool involved in producing viable zygotes was composed of a proportion  $S$  derived from selfing, a proportion  $Tm$  derived from intraspecific outcrossing, and a proportion  $Th$  derived from interspecific outcrossing (hybridization). The allelic frequencies in the pollen pool were assumed to be known in both species (represented by the allele frequencies in adult trees). If, for a given locus,  $S_s$ ,  $Tm_s$ , and  $Th_s$  are the three mixed-mating parameters of species  $s$  then the probability of progeny genotypes for a given maternal genotype may be as shown in Table 1.  $S$  and  $Tm$  were calculated by solving simultaneously the equations with the maximum-likelihood method. For one locus, the likelihood function may be written as

$$L(S, Tm) \propto \prod_{i=1}^7 [P(g_i)]^{N_i},$$

where  $P(g_i)$  is a general form of the seven equations in Table 1, relative to one species, and  $N_i$  is the size of the progeny array for each of the seven classes. Minimum variance average estimates over all loci for the selfing and outcrossing rates can be obtained by calculating the maximum likelihood for all loci and both species simultaneously (Ritland 1986):

TABLE 2. Single locus ( $t_i$ ), multilocus ( $t_m$ ), minimum variance mean of single-locus ( $t_s$ ) estimates of outcrossing rate and their standard errors (SE) in sessile and pedunculate oaks. Heterogeneity of  $t_i$  among loci was verified with a  $\chi^2$  test (see text).  $N$  is the sample size of seed analyzed.

Locus	Sessile oak		Pedunculate oak		Sessile oak	
	Year	$N$	Year	$N$	Year	$N$
	1989	702	1989	307	1992	470
	$t_i$	SE	$t_i$	SE	$t_i$	SE
<i>PGI-B</i>	1.141	(0.046)	1.466	(0.483)	1.075	(0.057)
<i>PGM-A</i>	1.116	(0.032)	0.766	(0.087)	0.830	(0.140)
<i>IDH-A</i>	1.043	(0.056)	1.116	(0.065)	0.768	(0.056)
<i>ACP-C</i>	0.974	(0.055)	1.097	(0.118)	1.052	(0.078)
<i>GOT-B</i>	0.590	(0.037)	0.747	(0.185)	0.697	(0.225)
<i>AAP-A</i>	0.853	(0.045)	0.717	(0.083)	1.125	(0.041)
<i>MR</i>	1.113	(0.035)	0.625	(0.180)	0.881	(0.170)
$t_m$	0.995	(0.012)	0.976	(0.032)	1.009	(0.022)
$t_s$	0.976	(0.021)	0.933	(0.053)	0.918	(0.044)
$t_m - t_s$	0.019	(0.023)	0.043	(0.036)	0.091	(0.089)
Heterogeneity						
$\chi^2$ of $t_i$	$P < 0.001$		$P < 0.001$		$P < 0.001$	

$$L(S_s, Tm_s, S_p, Tm_p) \propto \prod_{k=1}^n \prod_{j=1}^2 \prod_{i=1}^7 [P_k(g_{i,j})]^{N_{k,i,j}},$$

where  $P_k(g_{i,j})$  is the general form of equation  $i$  for species  $j$  and locus  $k$ , and  $N_{k,i,j}$  is the size of the progeny array of species  $j$  and locus  $k$ .

To maximize this function, we choose a quasi-Newton numerical procedure in the NAG Fortran Library (1990).

## RESULTS

### Mating System

In progeny arrays,  $\chi^2$  tests of departure from the assumptions of the mixed-mating model (Ritland 1983) showed significant differences between observed and expected genotypic frequencies at *AAP-A* locus; the discrepancy was due to a lack of heterozygotes between alleles 1 and 2. Because

a significant departure was observed only in one data set (collection of sessile acorns in 1992) and this test has a tendency to be conservative, this locus was not excluded from further calculations. Single-locus outcrossing rates ( $t_i$ ) were heterogeneous among loci both in sessile and in pedunculate oaks (Table 2). Multilocus outcrossing rate ( $t_m$ ) was not significantly different from  $t_m = 1$  in all three data sets. Allelic frequencies of the pollen pools and the ovule pools did not differ significantly at the 95% level (results not shown). In both species, the differences between the multilocus outcrossing rate and the mean of the single-locus outcrossing rates were not significant (Table 2).

Departures from the assumptions of the mixed-mating model were observed for the sessile oak sample of 1989 with the contingency  $\chi^2$  test (Brown et al. 1975), which detected significant heterogeneity of pollen pool allele frequencies across female homozygous mother trees at *PGI-B* ( $P < 0.01$ ), *PGM-A* ( $P < 0.01$ ), and *IDH-A* ( $P < 0.001$ ). Individual outcrossing rates ( $t_i$ ) were also heterogeneous between trees ( $\chi^2$  test of Kahler et al. 1984) in both species, varying from 0.75 to 1.26 in sessile oak ( $P < 0.01$ ) and from 0.86 to 1.29 in pedunculate oak ( $P < 0.01$ ). However, with the regression method (Ritland and El-Kassaby 1985), we did not detect significant relationships between the allele frequencies in the pollen pool and the additive values of maternal genotypes, except for a negative association in pedunculate oak at the *AAP-A* locus ( $b = -0.03$ ;  $P < 0.05$ ).

### Genetic Admixture Proportions

The allelic frequencies of the pollen clouds, the natural regeneration, and the adult trees are shown in Table 3. In the adult population, significant differences between sessile and pedunculate oak were observed at *ACP-C*, *AAP-A*, *PGM-A*, and *IDH-A*. In sessile oak, no differences were observed between the allelic frequencies in the pollen cloud during the two years of observations (data of 1992 not shown). The gene frequencies in the pollen pool of both species showed

TABLE 3. Frequencies of the two most common alleles in pollen pools, in natural regeneration, and in adults, and test of differences between frequencies ( $\chi^2$ ). a, sessile oak pollen cloud (1989); b, pedunculate oak pollen cloud (1989); c, sessile oak seedlings; d, pedunculate oak seedlings; e, adult sessile oak trees; and f, adult pedunculate oak trees. The data of the sessile oak pollen cloud of 1992 were not represented here, as they did not show significant differences with those of the sessile oak pollen cloud of 1989.

Locus	Allele	Allelic frequencies						Significance level of the $\chi^2$ test				
		a	b	c	d	e	f	a-e	c-e	b-f	d-f	e-f
		$N = 155$	$N = 127$	$N = 221$	$N = 186$	$N = 190$	$N = 217$					
<i>ACP-C</i>	1	0.541	0.735	0.633	0.744	0.575	0.795	(NS)	(NS)	(NS)	(NS)	(***)
<i>ACP-C</i>	2	0.449	0.265	0.362	0.253	0.409	0.200					
<i>AAP-A</i>	1	0.732	0.490	0.630	0.452	0.597	0.359	(*)	(NS)	(*)	(NS)	(***)
<i>AAP-A</i>	2	0.163	0.345	0.251	0.436	0.261	0.461					
<i>PGM-A</i>	1	0.884	0.663	0.892	0.678	0.837	0.482	(NS)	(*)	(***)	(***)	(***)
<i>PGM-A</i>	2	0.067	0.266	0.042	0.216	0.113	0.468					
<i>MR</i>	1	0.771	0.858	0.875	0.923	0.850	0.899	(NS)	(NS)	(NS)	(NS)	(NS)
<i>MR</i>	2	0.161	0.057	0.095	0.032	0.076	0.028					
<i>IDH-A</i>	1	0.878	0.769	0.882	0.835	0.841	0.740	(NS)	(NS)	(NS)	(**)	(**)
<i>IDH-A</i>	2	0.052	0.228	0.049	0.123	0.064	0.240					
<i>PGI-B</i>	1	0.755	0.828	0.766	0.895	0.816	0.858	(NS)	(NS)	(*)	(NS)	(NS)
<i>PGI-B</i>	2	0.141	0.110	0.145	0.055	0.092	0.046					
<i>GOT-B</i>	1	0.959	0.946	0.895	0.955	0.961	0.952	(NS)	(*)	(NS)	(NS)	(NS)
<i>GOT-B</i>	2	0.033	0.036	0.091	0.037	0.034	0.021					

Significance levels of Chi-square tests: NS, nonsignificant; \*, 0.05; \*\*, 0.01; \*\*\*, 0.001.

TABLE 4. Admixture contribution of adult sessile and pedunculate oak trees to the progeny (seeds and seedlings) of the two species. The contribution ( $m$ ), the standard deviation (SD), and the probability of the  $t$ -test ( $P$ ) were calculated in relation to the allelic frequencies of all adult trees.

	Sessile oak contribution (Adult trees)			Pedunculate oak contribution (Adult trees)		
	$m$	SD	$P$ $ t $	$m$	SD	$P$ $ t $
Sessile oak						
Pollen pool 1989	1.24	0.067	0.000	-0.24	0.067	0.004
Pollen pool 1992	1.21	0.041	0.000	-0.21	0.041	0.000
Seeds 1989	1.23	0.054	0.000	-0.22	0.054	0.001
Seeds 1992	1.30	0.073	0.000	-0.30	0.073	0.000
Seedlings	1.09	0.067	0.000	-0.09	0.067	0.212
Pedunculate oak						
Pollen pool 1989	0.47	0.047	0.000	0.53	0.047	0.000
Seeds 1989	0.27	0.043	0.000	0.73	0.043	0.000
Seedlings	0.48	0.068	0.000	0.52	0.068	0.000

an asymmetrical shift toward sessile oak frequencies. In pedunculate oak, this shift was significant for three loci (*AAP-A*, *PGM-A*, and *PGI-B*). In sessile oak, the shift was observed for locus *AAP-A*. The sign test over all the loci showed that the directionality of changes was significant for both species, at the 0.001 probability level for pedunculate oak and at the 0.006 probability level in sessile oak (pollen clouds of 1989 and 1992).

In the regeneration sample, the probability of this directional change was 0.029 in pedunculate oak and 0.09 in sessile oak (Table 3). Allelic frequencies in progenies of pedunculate oak were therefore intermediate between frequencies of adult parental populations. Because incorrect taxonomic determination could be a source of error in allele frequency estimates, we repeatedly calculated gene frequencies 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. A similar procedure of exclusion of the most intermediary morphological forms was applied for seedlings. However, no significant changes in gene frequencies were observed in these new groups (results not shown).

The genetic contribution of the sessile oak to pedunculate

oak progenies, calculated with the method of Elston (1971), was estimated to vary from 27% to 48%, depending on the population used as a reference (Table 4). Similar proportions of admixture were obtained excluding the adult trees and the progenies collected at the border (50 m) of the plot (results not shown). A smaller estimation ( $m = 0.17$ ;  $SD = 0.044$ ,  $P|t| = 0.002$ ) of the sessile oak contribution to the pedunculate oak progenies was found using the maternal parent trees (pedunculate oak) as the female population, in the place of the whole pedunculate oak population. All of these estimations differed significantly from zero, as indicated by the  $t$ -test (Table 4). The genetic contributions of pedunculate oak to sessile oak progenies were estimated to be negative; some of these estimations were significant (Table 4). In *Q. petraea* the admixture proportion for single progenies varied between -0.42 and 0.22 (15 families). The range of variation in *Q. robur* was 0.38 to 0.73 (four families).

#### Estimation of Intra- and Interspecific Outcrossing Rates by Means of the Two-Species Mixed Mating Model

Maximum-likelihood estimations of the selfing and the intraspecific outcrossing rates were obtained, for the two species, for each locus and for all loci simultaneously (Table 5). Single-locus estimates exceeding unity were probably generated by sampling error. Nevertheless, these values were necessary to obtain unbiased average estimates (Brown et al. 1985). Single-locus estimates were affected by large standard deviations; in one case (*GOT-B*), the ML procedure did not converge. When all the loci were considered simultaneously, the gain in information was large, as indicated by the small standard deviations of the minimum variance average estimates over loci. The average estimates of selfing rate did not differ substantially from the estimations obtained with the ML method of Ritland and El-Kassaby (1985); the estimations of the interspecific outcrossing rates gave results similar to those obtained with the admixture method. The genetic contribution of sessile oak to pedunculate oak progenies was estimated to be 31.7%. The contribution of pedunculate oak to sessile oak progenies was negative (-20%).

TABLE 5. Estimations of selfing and intraspecific outcrossing rate in sessile and pedunculate oak.  $S_s$  and  $S_p$ , selfing rates in sessile and pedunculate oak respectively;  $Tm_s$  and  $Tm_p$ , intraspecific outcrossing rates in sessile and pedunculate oak, respectively. In parentheses, standard errors of estimates. Interspecific outcrossing rates for sessile oak were calculated as  $Th_s = (1 - Tm_s - S_s)$  and similarly for pedunculate oak.

	$S_s$	$Tm_s$	$Th_s$	$S_p$	$Tm_p$	$Th_p$
<i>PGI-B</i>	0.000 (0.126)	2.000 (0.523)	-1.000	0.000 (0.634)	1.180 (0.866)	-0.180
<i>PGM-A</i>	0.000 (0.072)	1.153 (0.098)	-0.153	0.200 (0.086)	0.450 (0.090)	0.350
<i>IDH-A</i>	0.000 (0.185)	1.439 (0.457)	-0.439	0.000 (0.086)	1.150 (0.263)	-0.150
<i>ACP-C</i>	0.021 (0.053)	1.117 (0.103)	-0.138	0.000 (0.135)	0.746 (0.192)	0.254
<i>GOT-B</i>	this estimate did not converge					
<i>AAP-A</i>	0.050 (0.052)	1.390 (0.098)	-0.440	0.050 (0.121)	0.288 (0.142)	0.662
<i>MR</i>	0.000 (0.099)	1.346 (0.346)	-0.346	0.000 (0.632)	1.194 (0.608)	-0.194
Single-locus mean	0.012 (0.100)	1.408 (0.300)	-0.419	0.042 (0.348)	0.835 (0.423)	0.124
Minimum variance average estimate over loci	0.000 (0.026)	1.203 (0.041)	-0.203	0.050 (0.041)	0.633 (0.063)	0.317

## DISCUSSION

*Mating System and Maintenance of Diversity*

The analysis of the mating system of the sessile and pedunculate oak conducted either with the ML method of Ritland and El-Kassaby (1985) or with our two-species mixed-mating model demonstrated that both species are completely outcrossing, although variations of outcrossing rate were observed between trees. The heterogeneity of outcrossing rates among loci and among trees, and of individual pollen allelic frequencies, may be generated by (1) different spatial or temporal distributions of adult tree genotypes and, as a consequence, of pollen pool genotypes; (2) variation in mating behavior among individuals; and (3) statistical inefficiency of the method when only one locus or one family is considered (Brown et al. 1985). Spatial heterogeneity in the allele frequencies of pollen clouds tends to cause a downward bias in single-locus and in individual outcrossing rates (Ennos and Clegg 1982; Shaw and Allard 1982). Here, those loci in which some heterogeneity of pollen pool among trees were detected, also show a very high single-locus outcrossing rate. The first hypothesis could then be excluded. On the contrary, we could not separate the effects due to the inefficiency of the ML method when based on single-family arrays and those due to variation in mating behavior among individuals.

In a number of studies, the mating system emerges as the key indicator of the level of genetic diversity in plants (Hamrick and Godt 1989). Oak species exhibit a level of diversity that is among the highest of all woody species (Kremer and Petit 1993). Our study shows that the maintenance of genetic diversity in these species is associated with a complete outcrossing. The absence of selfing may be due to a self-incompatibility system (Wright 1964). Steinhoff (1993) has found in controlled crosses that autocompatibility for both species is very rare and that some intra- and interspecific crosses are nearly incompatible. A self-incompatibility system is also suspected in *Quercus ilex* L. (Lumaret et al. 1989); its presence has been shown in other deciduous forest tree species such as *Castanea mollissima* (McKay 1942), *Betula* ssp (Hagman 1975), and *Corylus avellana* (Thompson 1979). The same autoincompatibility system could also determine the asymmetric interspecific incompatibility, as suggested by Hiscock and Dickinson in *Brassica* (1993).

*Directional Gene Flow and Succession*

In pedunculate oak, significant differences in allelic frequencies are observed among pollen clouds and adult trees. A significant directionality is present in these changes, 93% of alleles showing a shift toward sessile oak gene frequencies. Similar directional changes in gene frequencies were observed also in pedunculate oak seedlings of the regeneration. Directionality was also detected for allelic frequencies in sessile oak progenies, though to a lesser extent. It is unlikely that these results can be explained by random effects alone. We suggest that the directional changes in gene frequencies are due to directional intra- and interspecific gene flow. An alternative hypothesis is that these changes were generated by differential flowering of genotypes at the intraspecific level. We could not quantify the pollen production of the trees.

However, during three years of observation, the proportion of trees that did not flower was small, varying from 2% and 8% according to year and species; the allelic frequencies of trees that flowered were not different from those of the whole stand. Moreover, no correlations were found between phenology of flowering (precocity and duration) and taxonomic or genotypic status of individuals (Bacilieri et al. 1995). These considerations contradict the hypothesis that differential flowering of genotypes accounts for biased allele frequencies of pollen and seedlings.

The contribution of the sessile oak to the pedunculate oak progenies has been estimated to vary between 17% and 47%, according to the year and to the population used as a reference. The smaller value of admixture was found using the maternal parent trees as the female reference population. The maternal contributions were not considered in the estimations of pollen cloud allele frequencies, as in these species no selfing was observed. Elevated admixture estimates could be generated by correlated matings if the pedunculate oak maternal plants (15 trees) had, by chance, allelic frequencies more similar to those of sessile oak than the rest of the pedunculate population. However, we have not found evidence for correlated matings in either of the two species, as discussed above. More probably, the small estimate obtained using the maternal parent trees as the female reference population was caused by chance alone. The admixture contribution of pedunculate oak to sessile oak progenies is estimated to be negative. This apparently incongruous result is due to a shift of gene frequencies in sessile oak toward still more pronounced "sessile oak frequencies." We did not follow the suggestion of Elston (1971) and dePamphilis and Wyatt (1990) to impose the restriction that all  $m > 0$ . Doing so would have given an estimate of the contribution of sessile oak equal to 1 and of pedunculate oak equal to 0. We found it more interesting to consider the possibility that directional mating events also occur in sessile oak. Sessile oak ovules are apparently preferentially fertilized by pollen of other extreme sessile genotypes. There is no experimental evidence for such assortative matings; however, controlled crosses indicate that the success of matings varies dramatically between trees (Steinhoff 1993).

Overall, a trend to become more sessile is present. The similar shift observed in the seedling cohorts (3–6 yr old) parallels patterns seen in the seed cohorts and indicates that the hybrids have not been eliminated by selection in establishment. Asymmetrical hybridization has been documented previously in other forest tree species, that is, *Aesculus* (dePamphilis and Wyatt 1990), *Eucalyptus* (Potts and Reids 1988), *Populus* (Keim et al. 1989), and *Pinus* (Wheeler and Guries 1987). In *Zea mays*, the asymmetric incompatibility of crossing between lines is controlled by three recessive alleles (Rashid and Peterson 1992). Controlled crosses made in Germany on local oaks (Aas 1991; Steinhoff 1993) have indicated that compatible crosses are more frequent in the direction (*Q. robur* [female]  $\times$  *Q. petraea* [male]) than in the reciprocal. The concordance of these results with ours, obtained in a natural population situated more than 1000 km apart, suggests that asymmetry of gene flow in oaks is also under genetic control.

In *Aesculus*, dePamphilis and Wyatt (1990) proposed that

colonization of new habitats could take place by means of pollen dispersion, hybridization with a related species, and successive repeated backcrossing. This hypothesis could also apply to sessile and pedunculate oak. Furthermore, in these oak species, the directional inter- and intraspecific gene flow may reinforce the dynamic of species succession. Pedunculate oak, a pioneer species, is progressively replaced by sessile oak in mesic habitats, because of the ability of the latter to grow in denser and more shaded conditions (Rameau 1990). Our finding adds a further genetic component to the succession scheme. Replacement of *Q. robur* by *Q. petraea* may not only be the consequence of their differential response to site conditions (Rameau 1990) but also the result of asymmetric hybridization followed by repeated unidirectional backcrosses. This intriguing hypothesis needs to be confirmed by studying the mating system of hybrids in mixed populations, although the persistence of directional matings within *Q. petraea* is already indicative of asymmetrical backcrosses.

#### *Interspecific Gene Flow and Coexistence of the Two Species*

Hybridization between oak species of the same section is not rare (see references in Rushton 1993). The proportion of interspecific gene flow between sessile and pedunculate oak seems rather large, though similar values have been found in *Pinus* (Wheeler and Guries 1987), *Aesculus* (dePamphilis and Wyatt 1990), and *Alnus* (Bousquet et al. 1990). Because we have studied only a fraction of the life cycle of the stand, gene flow observed at this early stage does not really equal introgression. Hybrid progenies may be subsequently eliminated. However, the similar regional patterns of differentiation observed for both species in chloroplast DNA polymorphisms, which otherwise show a very weak interspecific differentiation, strongly supports the hypothesis of an extensive introgression between the two species (Petit et al. 1993a; Ferris et al. 1993). Analogous results obtained by Whittemore and Schaal (1991) for American white oaks suggest that extensive introgression is common within the genus *Quercus*.

For most plants, introgression is confined to limited geographic zones, among the pure species habitats (Barton and Hewitt 1989). By contrast, sessile and pedunculate oaks are nearly completely sympatric over most of their geographic range. Reliable quantification of hybridization between these species is not available for other regions. However, putative hybrid individuals or populations have been described on the basis of morphological characters from all over Europe, that is, Netherlands (Ietswaart and Feij 1989), United Kingdom (Wigston 1975; Rushton 1978, 1979), France (Grandjean and Sigaud 1987), Russia (Semerikov et al. 1988), Sweden (Olsson 1975), and Germany (Elsner 1993). Despite the high level of interspecific gene flow shown by our study, *Q. petraea* and *Q. robur* are still differentiated throughout their natural distribution (Zanetto et al. 1994). How can separate species be maintained in the context of such extensive gene flow? Further studies are required to test whether selection against hybrids and introgressed forms can account for maintenance of species integrity in these oak species

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