emasculated with no pollination bag treatment. If, for example, contaminants were only one-half what we observed based on germination, *i.e.*, approximately 0.3%, the maximum number of unwanted seeds produced would be about 3 per 1,000, or one seed for every 13 controlled pollinations, assuming an average yield of 25 filled seeds per pollination as found in this study.

In a *large-scale*, applied breeding program designed to produce large quantities of hybrid seed, this level of contamination would be acceptable, and generally no greater than would be expected by accident. It is certainly less than the level of contamination found in wind-pollinated seed orchards of other species. TAFT (1962) estimated that a worker can perform 15 to 20 controlled pollinations per hour using this technique. An average of 1,000 pollinations per day could thus result in the production of 35,000 to 75,000 control-pollinated seeds per man-day, of which 100 to 200 might be contaminants.

In our study the effects of environmental events such as rain following pollination were not evaluated. It is possible that such events would interfere with normal pollen germination and pollen tube growth into stigmas of gynoecia not protected by pollination bags, and may have been responsible for the significant difference between bagging treatments on trees #7 and #8, located 80 km north of the other study trees.

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Genetic Diversity in Holm-Oak (Quercus ilex L.): Insight from Several Enzyme Markers

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(Received 20th June 1988)

Summary

Genetic variation using three alloenzyme markers (respectively loci PGI1, ADH1 and IDH1) was studied in the holm-oak (*Quercus ilex* L.), an anemophilous tree which is characterized by high phenological variability. At a regional scale, the results from genetic distance analyses showed that the closer the population sites, the higher the gene flow between the populations. The determination of pollen flow seems to depend essentially on inter-site phenological divergence.

At a larger scale (which corresponds to a substantial part of the whole distribution of this species), analysis of the genetic differentiation using F-statistics revealed, on one hand, that one or very few genenations would have passed since the time of the original founding of the populations and, on the other hand, that these populations were probably differentiated from one another a long time ago.

Key words: *Quercus ilex* L., genetic differentiation, phenology, F-statistics, allozymes.

Zusammenfassung

Anhand dreier alloenzymischer Merkmale (Enzymloci PGI1, ADH1 und IDH1) wurde die genetische Diversität der Stech-Eiche (*Quercus ilex* L.) untersucht, einem anemophilen Baum mit großer phänologischer Variabilität. In einem regionalen Rahmen erwiesen genetische Distanzanalysen, daß der Genfluß zwischen den Populationen umso bedeutender ist, je dichter deren Standorte beieinander liegen. Der Pollen-Fluß scheint wesentlich durch die phänologische Divergenz zwischen den Standorten bestimmt.

In einem weiteren Rahmen (der einen guten Teil der gesamten Verbreitung dieser Art erfaßt) erwies die Analyse der genetischen Differenzierung mittels F-Statistik zum einen, daß erst eine oder nur sehr wenige Generationen seit der Gründung der Populationen vergangen sind, zum anderen, daß diese Populationen bereits seit langer Zeit voneinander differenziert waren.

Introduction

Theoretical studies on population genetic structure predict generally the occurrence of higher intra-population variability in species that are distributed continuously than in those which are made up of small isolated populations; these, on the other hand, would possess a higher inter-population variability (WRIGHT, 1931; KIMURA and CROW, 1964; NEI *et al.*, 1975). WRIGHT (1946, 1965) underlines the importance of the reproductive system on the genetic structure of populations and shows that limited gene-flow can generate consanguinity and thereby genetic structuring within those populations. These theoretical concepts were also supported by experimental studies. Thus, from the survey of 110 plant species, HAMRICK *et al.* (1979) showed that those species which have a wide distribution, long life-span and are allogamous as well as wind-pollinated, possess the greatest intra-population genetic variability.

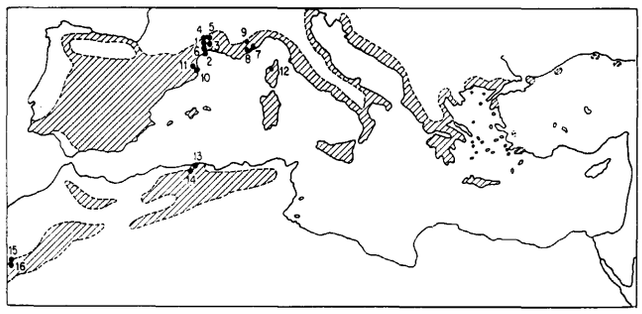

Figure 1. — Distribution area of the holm-oak and mapping of the studied sites (for coordinates and identification of the sites, see *Table 1*).

The genetic structure of only a limited number of forest-tree species have been studied up to now. Most of these species belong to the Gymnospermae. Results from anemophilous tree populations showed that these could no longer be considered as being panmictic and genetically homogeneous. However, in Fagus sylvatica which has a high rate of allogamy and substantial gene flow, the genetic variation was found to be more pronounced within populations than between them (Cuguen, 1986).

The present paper examines the case of the holm-oak (*Quercus ilex* L.). It is a diploid (2n = 24), long-lived, monoecious, anemophilous Angiosperm which is distributed primarily in the Mediterranean area. It often shows a dense and continuous distribution with low seed regeneration and often resprouts from the stump. In addition, the holm-oak is characterized by a great phenological variability (Du Merle, 1983).

The aim of the present study is to analyse the intra- and inter-population variation in *Quercus ilex* using enzyme markers. In forest-tree populations, such markers have been used to study genetic diversity which was found generally to be globally high and often more important within than between the populations (Tigerstedt, 1973; Hamrick *et al.*, 1979). Allele and genotype frequencies obtained from those workers were also used to analyze the population structure by means of Wright's (1951, 1965, 1969) F-statistics. Their results often showed that genetic variation is sometimes higher within forest-tree populations than between them (Mitton *et al.*, 1977; Knowles and Grant,

Table 1. — Location and principal abiotic characteristics of the sixteen studied sites. (Data from Code, J. (1977); Commission de Météorologie de l'Hérault (1950 à 1980); Enregistrements du Laboratoire Arago, Banyuls-sur-Mer; Garnier, M. (1967); Ionesco, T. (1965)).

n° Site	Locality	Coordinates	Altitude (m)	P (mm)	m (°C)	M (°C)	Parent-rock
1	Seranne (F)	43°49'N 03°36'E	260	1274	+0.5	+31.5	limestone
2	Lagardiole (F)	43°28'N 01°60'E	30	628	+4.2	+27.0	colluvia
3	La Cardonille (F)	43°52'N 03°44'E	340	1152	-0.2	+28.3	limestone
4	Pont d'Hérault (F)	44°89'N 03°41'E	270	1782	+0.4	+27.2	schist
5	Estrechure (F)	44°05'N 03°46'E	830	1518	+1.3	+28.5	crystalline
6	Puéchabon (F)	43°44'N 03°35'E	300	1075	+0.8	+30.3	limestone
7	Menton (F)	43°47'N 07°30'E	200	819	+4.4	+27.2	sandstone
8	Pas de la Faye (Grasse) (F)	43°45'N 06°50'E	860	1078	+0.4	+23.2	limestone
9	Thoard (F)	44°06'N 06°06'E	700	756	-0.3	+28.8	crystalline
10	Vallée Baillaurie (F)	42°27'N 03°06'E	160	976	+5.3	+28.3	schist
11	Gorges de la Fou(F)	42°27'N 02°35'E	350	860	+2.1	+28.9	schist
12	Vallée du Fango (Corse) (F)	42°22'N 08°45'E	370	940	+3.9	+25.3	crystalline
13	Tigzirt (DZ)	36°52'N 04°07'E	180	936	+5.9	+32.5	schist
14	Meurdja (DZ)	36°30'N 03°09'E	900	1129	-	-	limestone
15	Amizmiz (MA)	31°13'N 08°14'W	1650	481	+2.4	+31.1	schist
16	Ounein (MA)	31°02'N 08°01'W	2100	544	+1.8	+29.8	limestone

P: average annual rainfall; m: average daily minimum temperature for the coldest month; M: average daily maximum temperature for the hottest month.

1985; SHEA; CUGUEN *et al.*, 1985). The intensity of gene flow and the age of the populations, in terms of the number of generations, are frequently cited as factors which may explain such results. Both of these parameters are taken into account in the present study.

Materials and Methods

1. Plant material

The study has been carried out using naturally regenerated populations. Locations of the sixteen sampled populations are shown in *Figure 1*. Six are located in the region of Montpellier (from n^0 1 to n^0 6), the distances between them ranging from 6 km to 34 km. Two other populations are located in the Oriental Pyrenees, one more in the "Alpes de Haute Provence" region, two along the french Riviera and one in the Fango valley, in Corsica. Four other studied sites are located in North Africa: two of them in the Moroccan High Atlas and the two others in the Algerian Tellian Atlas. Geographical coordinates, altitudes and several climatic characteristics of the sixteen sites are given in *Table 1*.

Leaves from small branches collected on each tree were used for the enzyme analyses. The number of trees studied per population ranged from 27 to 158, the average being 51 individuals.

Seeds from controlled crosses or from deliberate self fertilization were not available for assay. A total of 490 offsprings from open-pollinated progenies were therefore used to study the genetic control of the isozymes. These individuals originated from acorns collected on 9 mother-trees located in site n^0 6 (Puechabon), 40 km from Montpellier. The method of AISSA (1981) was used for the acorn germination. Quick and simultaneous germinations were obtained by destroying the pericarp and by positioning the acorns, under one centimetre of compost, in boxes located in a green house.

2. Analysis of enzyme polymorphism

Of the 8 enzyme systems investigated and analysed using starch gel electrophoresis, only those which showed no change in relation to age of leaves and the position of leaves on branches, variability in environmental conditions or collecting season, and which possessed a very simple genetic control, were selected as markers. These systems are: phosphoglucose isomerase (PGI), isocitrate dehydrogenase (IDH) and alcohol dehydrogenase (ADH). The protein extraction was carried out using 350 to 700 mg leaves crushed in 1 ml Tris-HCl buffer (0.1 M, pH 7.6) (LUMARET, 1982), 100 mg of sterile sand and 30 mg of insoluble polyvinylpyrolidone (PVP). The homogenate was centrifuged for 15 min. at 40,000 x g and the supernatant was stored at -80^0 C before analysis. Enzyme separation (using electrophoresis) and staining were carried out using SECOND's methods (1982), with the difference that the starch concentration in gels was changed to 12.5%.

3. Genetic parameters

3.1. Genetic distance

Genetic distances between populations were estimated from their allelic distributions using three different indices: (1) NEI's distances (1978) derived from a "mutation drift" model; (2) distance of REYNOLDS *et al.* (1983) based on the coancestry coefficient, which is generally used for short-term differentiation; (3) The "X^2 distance" which is the square root of a weighted X^2. This has thereby the property of stabalizing variances (DE VIENNE and DAMERVAL, 1985) and of maximizing the effect of rare alleles. It was proposed by BALAKRISHNAN and SANGHVI (1968) and can be written as follows:

$$(1) \qquad d = \sqrt{\sum_{i=n}^{i=1} (1/pi)\,(xi - yi)^2}$$

where xi and yi are the respective frequencies of the ith allele in the two compared populations, and pi the average frequency of the ith allele in all the populations.

The respective positions of the populations estimated by the distances between them were plotted in multidimensional space and then projected upon a plane by non-metric multidimensional scaling (or proximity analysis) (ESCOUFIER, 1975). The same distances were also used as the basis for a cluster analysis (UPGMA) with the "average distance" as the clustering criteria, which preserves the initial space (LEGENDRE and LEGENDRE, 1979).

3.2. Genetic structure

Allelic diversity was estimated using the entropy measure of SHANNON and WEAVER, in LEWONTIN (1972):

$$(2) \qquad H = \sum_{i=1}^{i=n} - pi \log_2 pi,$$

where pi is the frequency of the ith allele and n the total number of alleles. This index combines diversity from both the numbers and frequencies of the alleles.

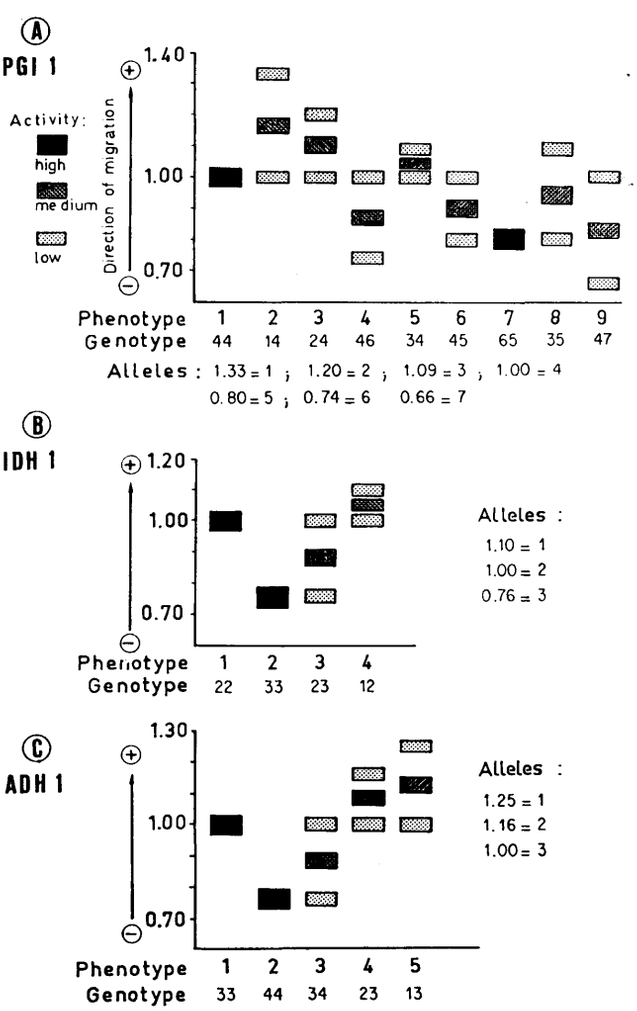

Figure 2. — Schematic pattern of PGI (A), IDH (B) and ADH (C) zymograms, and genetic interpretation of the phenotypes.

Analysis of genotypic structure was carried out using WRIGHT's F-statistics (1951, 1965, 1969) modified by KIRBY (1975) and NEI (1977). In each population, the fixation index was first estimated for each allele using the KIRBY's formula (1975) and then for each locus using an average measure weighted by the allele frequencies. Average F-statistics were estimated by the method of WEIR and COCKERHAM (1984) which minimizes the effects due to population size variation.

Results and Discussion

1. Genetic interpretation of zymograms

Phenotypes obtained in zymograms from individuals belonging to the same family were compared among themselves and to the maternal parent phenotype. Several maternal parents suspected of being either heterozygotes or homozygotes a loci coding for PGI or IDH enzymes were used. For the ADH system, only putative homozygote maternal parents were available.

The individuals that constitute the progeny of a given homozygote mother are expected to possess in common the allele present in the maternal genotype. Descendants from heterozygote mother possess at least one of the two alleles which are present in the maternal genotype. Both these postulates were taken into account when examining the phenotype distributions in the progenies. Moreover, the patterns of variation of the systems in other plant species and the occurrence of hybrid bands (heteromultimers) in putative heterozygous phenotypes also supported our interpretation.

Great consistency was obtained from the examination of the total results. That is PGIs from the holm-oak were observed to be dimers (either single or three banded phenotypes) and to be specified by one locus (PGI1) showing seven codominant alleles (*fig. 2a*). The index value 1.00 was given to the most frequent allele; other indexes were calculated as relative migration distances. IDH enzymes were found to be specified by one locus with three codominant alleles and to be of dimeric structure (*fig. 2b*). The ADH enzymes were also dimers specified by a locus showing four codominant distinct alleles (*fig. 2c*). No gene duplication was indicated in our experimental conditions.

2. Allele frequencies

Allele frequencies at each of the three studied loci in each population are given in *Table 2*. Polymorphism at locus PGI1 was found to be high (4.69 alleles per population in average). Alleles 1.00, 1.09 and 0.80 are present in all the studied populations. These populations could be distinguished from one another by the occurrence of one or several of the other alleles which have low frequencies (from 1% to 7%). Thus the allele 1.33 is only present in North-African populations, whereas 0.66 (which never exceeds 2%) was only found in the populations from Oriental Pyrenees and in populations n° 2 and 5 from the region of Montpellier. The French Riviera populations showed the lowest polymorphism at this locus with the occurrence of only the ubiquitous alleles (1.00, 1.09 and 0.80). Among the 16 studied populations, 4 (n° 2, 5, 10 and 11) possess 6 out of the 7 known alleles.

The average number of alleles per population at locus IDH1 is 2.19 and the allelic frequencies show variation between the populations. Those from North Africa and from the region of Montpellier are characterized by low frequencies (from 0 to 11%) for $IDH1^{0.76}$, whereas this allele ranges from 18% to 36% in the other populations. The low frequency allele 1.10 (from 0.3% to 2%) is only present in populations n° 4, 5, 6 and 15.

The lowest degree of polymorphism is found at locus ADH1 with an average number of alleles per population of 2.06. Five populations are monomorphic for the allele $ADH1^{1.00}$. Populations from Morocco are characterized by high frequencies (21% and 40% respectively) of allele 0.76; whereas this never exceeds 8 elsewhere. $ADH1^{1.16}$ is present in 7 populations. Six of them have frequencies that range from 1% to 4%. The $ADH1^{1.16}$ frequency is 9% in population n° 9 which also possesses a peculiar and rare allele ($ADH1^{1.25}$).

Table 2. — Allele frequencies (%) of holm-oak at loci PGII, IDHl in the sixteen studied sites. N1, N2, N3 are the numbers of individuals analysed respectively at the PGII, IDHl and ADHl loci.

	LOCUS															
Population N°	PGI-1								IDH-1				ADH-1			
	Alleles															
	N1	1.00	0.66	1.33	0.74	1.09	0.80	1.20	N2	1.00	0.76	1.10	N3	1.00	0.76	1.16	1.25
1	30	72	-	-	3	22	3	-	30	82	18	-	30	96	2	2	-
2	50	71	1	-	5	13	5	5	50	94	6	-	49	94	5	1	-
3	50	77	-	-	1	13	9	-	50	80	20	-	50	99	-	1	-
4	52	80	-	-	2	15	3	-	46	92	6	2	50	95	5	-	-
5	50	82	1	-	1	5	10	1	48	94	5	1	46	100	-	-	-
6	153	74	-	-	5	8	12	1	158	87	12	1	158	94	5	1	-
7	45	62	-	-	-	35	3	-	48	81	19	-	45	100	-	-	-
8	50	70	-	-	-	21	9	-	47	80	20	-	50	100	-	-	-
9	49	78	-	-	7	4	11	-	48	93	7	-	49	90	-	9	1
10	50	79	1	-	5	6	7	2	44	78	22	-	50	100	-	-	-
11	50	69	2	-	5	8	12	4	47	78	22	-	50	99	1	-	-
12	49	85	-	-	1	9	5	-	49	64	36	-	52	100	-	-	-
13	38	83	-	3	5	3	5	1	31	97	3	-	38	93	3	4	-
14	40	71	-	1	5	15	8	-	40	99	1	-	40	90	9	1	-
15	34	76	-	3	6	15	-	-	32	94	5	1	33	62	38	-	-
16	27	68	-	6	7	15	-	4	23	100	-	-	26	79	21	-	-

Table 3. — Descriptive statistics over the three distance matrices.

Genetic distance	Average	Variance	Standard-deviation	min.	max.	Amplitude
Nei (1978)	0.023	0.000	0.023	0.000	0.119	0.012
Reynolds et al.(1983)	0.056	0.002	0.054	-0.003	0.240	0.024
X^2	0.496	0.053	0.231	0.015	1.100	0.109

3. Genetic distances

3.1. At the large scale

Descriptive analyses were carried out from the three distance matrices (NEI, 1978; REYNOLDS *et al.*, 1983; and "X^2"). The results are plotted in *table 3* and in *figure 3*. The diagrams constructed from both multidimentional scaling and cluster analysis treatments established from these three matrices are shown in *figs. 4a* and *4b*. The estimates of genetic distance using NEI's and REYNOLD's methods give very similar results. They can be distinguished only by their absolute values as well as by their range of variation between the populations (*table 3*). As the synthetic diagrams from these two methods were also very similar, only that obtained from REYNOLDS *et al.* distances is presented (*fig. 4a*).

Histograms that divided the whole range of distance values into 10 equal classes were also constructed from the three distances matrices. The highest values are on the right of the histograms (*fig. 3*). It can be noticed that the class representing the shortest distances is very important (87%) for the NEI's and REYNOLD's distances whereas it represents only 3% of all distances for the "X^2" distances. This has obviously consequences for the cluster analyses where the clustering levels are very different depending upon the kind of distance used. However, the population composition within the groups were observed to be similar when obtained from the different treatments. This is not very surprising as significant positive correlations ($p < 0.05$) were found between the three matrices.

It is obvious from the whole results that the greatest genetic distances as well as the greatest geographical distances are found between the populations of the Fango valley in Corsica (n^0 12) and those from Morocco (n^0 15 and 16).

Populations from the Oriental Pyrenees (n^0 10 and 11), the french Riviera (n^0 7 and 8) and populations n^0 1 and 3 from the region of Montpellier are genetically the closest to the Corsican populations. Populations n^0 13 and 14 from Algeria, n^0 9 from the "Alpes de Haute Provence" region and populations n^0 2, 4, 5, 6 near Montpellier are intermediate. The extent and the organization of gene flow among local populations determine their potential for genetic differentiation. Thus, genetic distance analyses at a regional scale were estimated to be more appropriate than those at a very large scale to examine such effects.

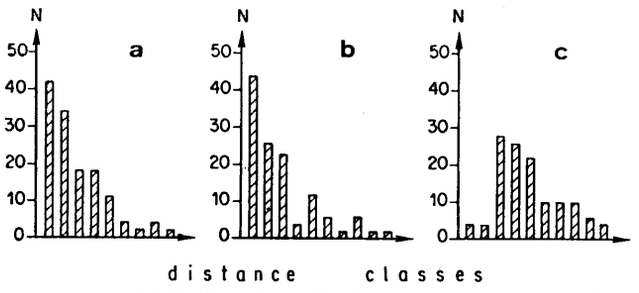

Figure 3. — Distribution of genetic distance values obtained from NEI, 1978 (a), REYNOLDS *et al.*, 1983 (b) and X (c) estimations.

3.2. Genetic distance at a regional scale

Populations from n^0 1 to n^0 6 were only considered at this scale. Distances (as the crow flies) between two suc-

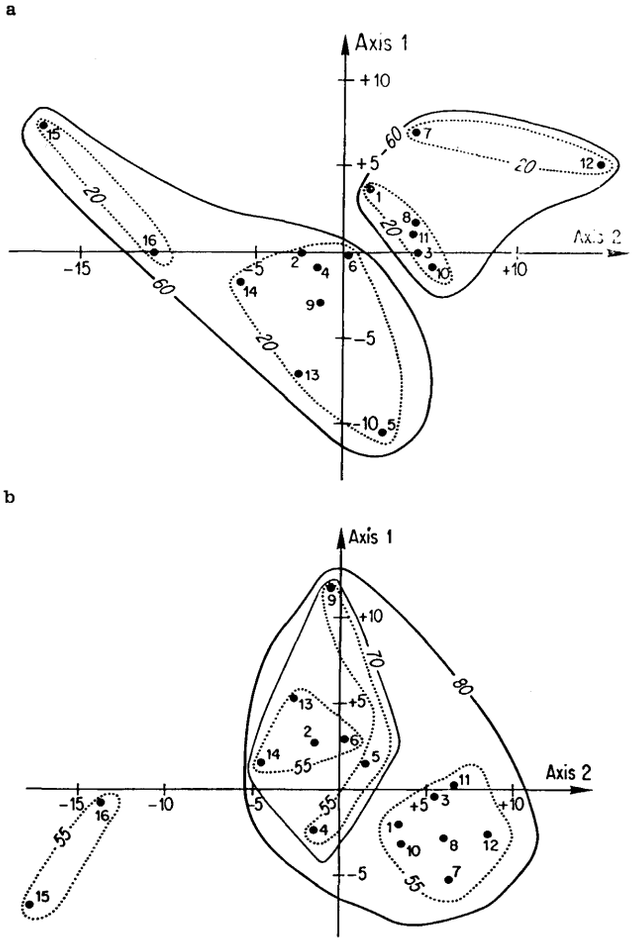

Figure 4. — Position of holm-oak populations according to polymorphism at loci PGI1, IDH1 and ADH1. Multidimensional scaling from NEI's (1978) distance (4a) (populations are clustered at levels 20 and 60 of the hierarchical clustering) and from X^2 genetic distances (4b) (populations are clustered at levels 55 and 80 of the hierarchical clustering).

cessive populations ranged between 6 km and 34 km. Results from the correlation tests between genetic and geographic distances showed that no significant correlations ($p < 0.05$) could be found between the NEI's or REYNOLDS's genetic distances and the geographic distance. On the contrary, a positive correlation ($p < 0.05$) was observed between geographic distance and genetic distances estimated from the "X^2" method. From a selection-mutation-drift model, SLATKIN (1981, 1985) demonstrates that the average frequency of rare and more especially "private" alleles (those which are present in particular demes) is approximately independant of either selection intensity or mutation rate but depends essentially on the global gene flow. Thus, according to this model, the positive correlation between "X^2" and geographic distances could be interpreted as evidence that gene flow increases, the closer the populations are to one another. It should be noticed, however, that the geographical transect established for this regional study also corresponds to a climatic gradient (*table 1*) which may well have some effect on the phenology of the studied populations.

4. Genetic structure

4.1. Allelic diversity

For each locus the index of allelic diversity in each population (Hpop), in the species considered as a whole (Hsp), and its average value per population and per locus ($\bar{H}pop$) were calculated and are given in *Table 4.*

PGI1 has both the highest allele number and the greatest diversity but also the lowest ability to discriminate populations. The diversity index at loci IDH1 and ADH1 show a wide range of variation, the highest values being obtained in populations 10, 11 and 12 for IDH and in the Moroccan populations for ADH.

The total diversity was then apportioned to within populations ($\bar{H}pop/Hsp$) and between populations ($Hsp - \bar{H}pop/Hsp$) and the results are plotted in *Table 5.* They show clearly that most of the total diversity at the three loci is present within populations. However, the part of the diversity which is attributable to the interpopulation level varies substantially between loci. The highest values were obtained for IDH1 and more especially ADH1 which, as stated previously, possesses the greatest differentiation ability. Similar results were obtained using the same method in several forest-tree species such as *Pinus ponderosa* (LINHART *et al.*, 1981), as well as in allogamous grass species such as *Phlox drumondii* (LEVIN, 1977), *Desmodium nudiflorum* (SHAAL *et al.*, 1980) and *Dactylis glomerata* (LUMARET, 1985).

Table 4. — Genetic diversity in holm-oak from the SHANNON-WEAVER index in each population (Hpop) averaged over the 16 populations ($\bar{H}pop$) and in the species taken as a whole (Hsp).

Locus	PGI-1	IDH-1	ADH-1
Population N°			
1	1.15	0.69	0.24
2	1.45	0.33	0.37
3	1.05	0.72	0.08
4	0.93	0.45	0.29
5	0.98	0.38	0.00
6	1.27	0.54	0.37
7	1.12	0.70	0.00
8	1.15	0.73	0.00
9	1.10	0.38	0.52
10	1.18	0.75	0.00
11	1.54	0.77	0.08
12	0.81	0.94	0.00
13	1.03	0.21	0.41
14	1.33	0.10	0.52
15	1.09	0.39	0.95
16	1.47	0.00	0.74
Hpop	1.16	0.50	0.29
Hsp	1.28	0.58	0.41

Table 5. — Apportionment of the total allelic diversity within (1) and between (2) the populations at the three studied loci.

Locus	(1) $\bar{H}pop/H\ sp$	(2) $(H\ sp - \bar{H}pop)/H\ sp$
PGI-1	0.912	0.088
IDH-1	0.873	0.127
ADH-1	0.703	0.297
Average	0.829	0.171

Table 6. — Fixation index (Fis) per population and per locus as well as for the loci taken as a whole (T) in the sixteen studied populations.

Site	PGI-1	IDH-1	ADH-1	T
1	0,027	-0.214	0.380	0,064
2	0,150	-0.053	0.130	0,043
3	0,221	-0.113	0.000	0.036
4	0,213	0.560	-0.031	0.154
5	0.321	0.095	0.000	0.139
6	0.068	0.091	-0.190	-0.010
7	0.237	-0.230	0.000	0.002
8	0.133	-0.227	0.000	-0.031
9	-0.006	-0.095	0.120	0.006
10	-0.025	-0.257	0.000	-0.094
11	0.047	-0.286	-0.005	-0.081
12	0.272	-0.008	0.000	0.088
13	-0.020	-0.090	-0.015	-0.042
14	0.212	0.240	-0.069	0.128
15	0.092	0.021	0.050	0.054
16	0.056	0.000	-0.250	-0.065

4.2. Genotype structure

The fixation index per population (Fis) calculated for each locus are indicated in *table 6.* The values of this index (quantity and sign) are directly dependent on the degree of polymorphism. At each locus, this was defined as the expected heterozygote frequency (He) assuming panmixia. For each locus, a sign-test was carried out. Thus, at locus PGI1 which is highly polymorphic (He values range between 0.25 and 0.50), more populations show an excess (Fis < 0) than deficiency (Fis > 0) of heterozygotes. This deviation is significant ($X^2_{(1)} = 6.25$, $p < 0.05$). At both loci ADH1 and IDH1, no significant deviation between heterozygote excess or deficency was observed.

Average values of F-statistics obtained from the method of WEIR and COCKERHAM (1984) over the whole populations are indicated in *table 7.* The Fis fixation index calculated over all the populations shows variation between loci. Thus a 12% heterozygote deficiency is found for PGI1 whereas a slight heterozygote excess is observed at loci IDH1 and

Table 7. — Average F-statistics at each the three loci (genotype data from the sixteen studied populations).

Locus	Fis	Fst	Fit
PGI-1	0.120	0.018	0.136
IDH-1	-0.092	0.065	-0.021
ADH-1	-0.050	0.135	0.091
Average	-0.008	0.073	0.069

ADH1. These non-significant differences are likely to be due to variation in allele frequency and number in the polymorphism between the studied loci. Similar effects were noticed previously by CUGUEN (1986) in his study of numerous European populations of beech where he stated: "the mode of Fis variation differs according to the level of polymorphism of the studied samples. When this is low, with or without selfing, the negative values of Fis are more numerous than the positive ones. In case of high polymorphism, in a panmictic situation, the positive and negative values are equifrequent". These observations constitute only a partial explanation of the results obtained in our study. At the highly polymorphic locus PGI1, indeed, the estimated Fis do not show equifrequency in heterozygote excess and deficiency.

This inter-locus Fis variation could result also from selection acting differently on the loci. According to WRIGHT (1965, 1978), neutral loci under the action of the same reproductive process are expected to show the same response and, consequently, to have similar (Fi) indices. This would not be the case with selected alleles. Such a situation cannot be completely ruled out at least for PGI1 because, at the regional scale, this locus shows a decrease of the allelic diversity and an increase of homozygotes for the predominant allele ($PGI1^{1.00}$) as the degree of continentality increases. Selection might be acting on this locus or on a tightly linked locus.

The average Fis value calculated over all the loci which was found to be very close to zero (−0.008) shows that no differentiation is occurring within the populations. Several hypotheses can be suggested as explanation:

(1) The species possesses a high rate of allogamy. This is very likely the case as no offspring was obtained from 37 controlled-self pollinated trees (unpublished data);

(2) The neighbourhood size is at least equal or even exceeds the size of the studied samples and/or very few successive generations (overlapping or not) have occurred since foundation the population. In that case, even with limited gene flow, a long time would be needed before some differentiation would be noticeable. In a plant species such as *Quercus ilex* that possesses a long life-span and that is able to produce suckers from the stump quite continuously (this characteristics increases even more the plant's longevity), the number of successive generations since the foundation of the population is likely to be very low. The extensive vegetation clearing that occured in the region of Montpellier at the end of the eighteenth century (DUGRAND, 1964) could indicate a possible time of foundation for the populations observed nowadays in this region.

The Fst value calculated over all the loci is 0.073. This may indicate an heterozygote deficiency which could be attributable to inter-population differentiation. This result supports the hypothesis that only limited gene flow occurs between the populations. Several observations about phenological variability in this species provide further information to explain the differentiation between the populations by utilizing the idea of efficient pollen-flow. From numerous observations made during three consecutive years, DU MERLE (1983) showed that the length of the period of flowering maturity varied, depending upon the population location. He also demonstrated that the greater the environmental differences between the populations, the higher the flowering period divergence between them. As a consequence, even if the putative pollen flow is substantial, the efficient part of this flow will decrease depending upon the phenological divergence between the populations, which itself is related to differences in characteristics of the environment.

STAM (1983) proposed a model of the evolution of reproductive isolation in adjacent plant populations resulting from flowering period divergence. The results can be summarized as follows: genetic divergence for the reproductive period which is derived from environmental differences between adjacent populations is likely to occur in particular circumstances. Conditions for a non selective divergence are (1) initial occurrence of substantial genetic variability in flowering time; (2) short (or no) overlap of the flowering period between early and late genotypes; (3) restricted seed flow between the populations. These mechanisms lead automatically to a substantial degree of reproductive isolation the effects of which will be increased by microgeographical differentiation.

When our results are compared to those obtained from other grass or anemophilous-tree species, holm-oak seems to be one of the very few species not to show any intrapopulation differentiation. The reproductive system could not explain completely this situation. In a completely allogamous species such as *Liatris cylindracea* (SCHAAL, 1975), the Fis value was equal to 0.41. such genetic structuring was thought to be attributable to restricted seed dissemination, reduced pollen flow which generates crosses between relatives and a large number of generations since the original foundation of the population. So, the eventuality that, in holm-oak populations, internal structuring could be established after numerous generations, despite complete allogamy, is not completely ruled out for the two following reasons. (1) In this species, from the few studies on acorn dissemination and predation, it could be concluded from the occurrence of consequent acorn predation (according to VINCENT (1977) from 65% to 78% of the acorns would be collected by several micromammals) and their short dissemination as, from SORK's observations (1984) about 99% of the acorns carried more than 20 metres apart from the producting-source would be consumed. (2) According to the model of STAM (1983), phenological variability between individuals from a same site associated with short dissemination of the acorns escaped from predation would lead to produce some intrapopulation differentiation. This may well be the case in three of the studied populations (n^0 4, 5 and 14) for which the Fis values are respectively 0.15, 0.14 and 0.13.

The genetic differentiation between the holm-oak populations is high when compared with that of other anemophilous-tree species such as *Pinus rigida* (GURIES and LEDIG, 1982), *Pinus banksiana* (DANIK and YEH, 1983) or *Fagus sylvatica* (CUGUEN, 1986). This particular situation in holm-oak seems to be attributable to phenological cariability which

produces temporal isolation between the populations, adding to the spatial isolation and thus increasing inter-population differentiation.

Conclusion

In models devoted to describing plant population structure, the following parameters are generally taken into account: (1) characteristics of the reproductive system (selfing and outcrossing rate essentially); (2) intensity of gene flow which includes both seed and pollen flow (the latter being related to entomophilous or anemophilous characteristics); (3) number of generations since the founding of the population; (4) selective forces.

The results obtained from this study suggest that an extra parameter can be decisive in terms of the way the populations become structured. When the phenological variability on population level is indeed very substantial, the opportunities for inter-population crosses are restricted so that only a small part of the pollen flow is efficient, whatever its dispersal ability. Similar observations were found in the study of metal-tolerant grass species such as *Agrostis tenuis* and *Anthoxanthum odoratum* (McNeilly and Antonovics, 1968). These authors showed that variation in the flowering period can lead effectively to some degree of isolation which reduces the homogenizing effect of the observed high genic flow, without completely eliminating the occurrence of gene exchange between the populations.

In the particular case of the holm-oak, it has been observed that the divergence for the flowering period increases with the differences of environmental conditions between the sites (Lumaret et al., unpublished). The inter-annual climate variation reduces or increases the duration of the flowering-time but the respective divergence between the populations is globally maintained. Thus, results from the regional scale study showed that the closer the sites (and therefore the more simiilar their environmental conditions), the higher the gene flow.

On the contrary of what would be expected from an allogamous species with a long life-span and generally a continuous distribution, the holm-oak is characterized by absence of intra-population genetic differentiation, whereas the populations are highly differentiated with respect to one another. These results can only be attributed to both the low number of successive generations from the original foundation and the occurrence of some ancient differentiation between the populations. The historical events which depend essentially on man's activity (vegetation clearing and forestry management) may have contributed significantly to fashion the present structure of the holm oak populations.

Acknowledgements

We are grateful to Dr. C. Gliddon (School of Plant Biology, Bangor, U. K.) for providing critical discussions and comments on the manuscript, and to Dr. J. Cuguen for providing several computer-programs. We thanks also Dr.F. Romane(Centre Emberger, CNRS, Montpellier) for taking part actively in field-survey and the plant material collection. J. Panis (CNRS, Montpellier) provided extensive technical assistance.

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Breeding Strategies for Coppice Production in a Eucalyptus grandis Base Population with Four Generations of Selection

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(Received 25th July 1988)

Summary

A genetic base population (GPOP77) of *Eucalyptus grandis* (HILL) *ex.* MAIDEN planted in July 1977 with 529 families representing four generations of selection was partially harvested in August 1978. Regrowth through December 1983 was evaluated to assess genetic improvement potential for coppice productivity.

Four generations of selection have produced impressive genetic gains. At 64 months after harvest, first-, second-, third- and fourth generation families averaged 7.04 dm^3, 21.54 dm^3, 25.91 dm^3 and 40.16 dm^3 per tree, respectively. Fourth-generation families also had the best frost resilience and coppice quality. In individual tree volume, the best trees were more than three times larger than the fourth generation trees at 64 months after harvest. Provenances from Queensland, Australia, grew better than other sources studied. High individual and family tree heritabilities were observed for all growth traits at different ages.

The potential inbreeding depression resulting through mating of related families was examined. The mean inbreeding coefficients in the offspring of all possible matings of selected individuals for six different selection strategies ranged from 0 to 1%. The predicted genetic gains, adjusted for any inbreeding through relatedness and selfing, were high. The highest gain of 90% was predicted by the selection of thre trees from the top 100 families.

Key words: *Eucalyptus grandis* (HILL) ex. MAIDEN, genetic variation, frost resilience, heritabilities, inbreeding, genetic gains, provenance.

Introduction

Although eucalypts occupy perhaps one-fifth of the world's plantations (LOGAN, 1967), only a small part of the world's tree breeding effort has been devoted to this genus. In Florida, eucalypts were first planted in 1878, and industrial plantations were first established in 1972 (GEARY *et al.*, 1983). The ability of *E. grandis* to coppice, combined with its exceptional growth rate in low quality soils, makes it an attractive species for short rotation biomass production.

Eucalyptus grandis has revealed significant provenance variation around the world (ASSIS and BRUNE, 1983; ADES and BURGESS, 1983; DARROW and ROEDER, 1983; KING, 1983; BORGES and BRUNE, 1983). In Florida, appreciable genetic variation has been observed in *E. grandis*, and successful selection programs have been reported (ROCKWOOD and MESKIMEN, 1981; MESKIMEN, 1983). Limited information is available on the genetic variation for coppicing ability in *E. grandis*. Significant genetic variation in this trait has been reported by GEARY *et al.* (1983). In southern Florida, four progenies at two different sites showed no significant differences in coppicing ability (ROCKWOOD and GEARY, 1982).

Due to the absence of annual resting buds in *Eucalyptus*, indeterminate shoots grow continuously year round (FAO, 1979). In the southern United States temperatures drop suddenly from well above freezing to well below (HUNT and ZOBEL, 1978). This sudden drop has a devastating impact on the survival of trees. Of all the *Eucalyptus* species examined in Florida, *E. grandis* was notably frost sensitive (HUNT and ZOBEL, 1978). MESKIMEN *et al.* (1987) found significant relationship ($r = 0.33$) between tree height and frost resilience among clones of *E. grandis*.

In recent years research has been done on the pollination biology of the eucalypts. The amount of natural selfing occurring in eucalypts is higher than the 7% that is reported for most pines (WRIGHT, 1976). Published estimates for the degree of natural selfing occurring in eucalypts vary somewhat among species: 24% in *E. obliqua* L'HERIT. (BROWN *et al.*, 1975), 37% in *E. pauciflora* SIEB. ex SPRENG (PHILLIPS and BROWN, 1977), 23% in *E. delegatensis* R. T. BAK. (MORAN and BROWN, 1980) and 18% in *E. stoatei* C. A. GARDN. (HOPPER and MORAN, 1981). In *E. grandis* ELDRIDGE (1978) reported 20% to 40% selfing, and VAN WYK (1981) estimated this to average about 30%. In *E. grandis* HODGSON (1974, 1976, 1977) studied the extent of inbreeding depression for inbred individuals where he observed height of selfed progenies to be 8% to 49% less than that of outcrossed progeny.

Materials and Methods

Eucalyptus grandis in Florida constitutes a landrace developed through four generations of selection and progeny testing in local environments (GEARY *et al.*, 1983) (*Figure 1*).GPOP77, the fourth-generation base population planted in July 1977, had a total of 529 (144 first-genera-