Therefore, the squared sum of orchard pollen father's contribution will be

$$\sum_{i=1}^{N_w} n_i^2 = N_w \left[\frac{(1-M-S)N}{N_w} \right]^2 (CV^2 + 1)$$

$$= \frac{\psi_w N^2 (1-M-S)^2}{N_w}$$

From the same way, the squared sum of alien pollen father's contribution becomes

$$\sum_{m=1}^{N_a} a_m^2 = \frac{\psi_a N^2 M^2}{N_a}$$

Comparison of Levels of Genetic Diversity Detected with AFLP and Microsatellite Markers within and among Mixed *Q. petraea* (MATT.) LIEBL. and *Q. robur* L. Stands

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Summary

The aim of this study was to compare genetic diversity within and among *Quercus* spp. populations based on two contrasting types of nuclear markers. Seven mixed stands of *Quercus petraea* and *Quercus robur* were analysed using six highly polymorphic and codominantly inherited microsatellite markers as well as 155 dominant AFLP markers. Genetic differentiation and genetic diversity within each population were assessed. The intra- and inter-locus variances were calculated, and the results were used to compare the genetic diversity between populations. Both classes of markers revealed similar results: the genetic diversity within population and the genetic differentiation among populations is greater in *Q. petraea* than in *Q. robur*. The genetic differentiation is generally higher when AFLP markers were used in comparison to microsatellites. For AFLPs, the inter-locus variance is always much higher than the intra-locus variance, and explains why it was not possible to distinguish populations using this marker system. Finally, no significant positive correlation was found between the level of within-population diversity assessed with the two markers.

Key words: microsatellite, AFLP, genetic diversity, genetic differentiation, *Quercus robur*, *Quercus petraea*.

Introduction

The assessment of genetic diversity using nuclear markers can follow two different strategies for the species tested: sampling different populations and individuals as well as analysing an expanding number of loci. The associated components of sampling variance have been termed "intra-locus variance" and "inter-locus variance", respectively, and, from the theoretical point of view, the inter-locus variance is considered to be much higher than the intra-locus component (NEI, 1987). Several PCR-based marker technologies are available now to characterise the genetic diversity of a species directly at the level of DNA (KARP et al., 1997). Microsatellite markers are provided by sequence-specific primer pairs and this makes their development time-consuming and expensive. However, once developed, they yield a maximum of information with refer to their codominant inheritance which enables to distinguish between both alleles of a single heterozygous locus. In contrast, markers such as Random Amplified Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP) are more efficient since no species-specific sequence information is required for the development of PCR primers. They provide information on many loci which are commonly randomly distributed throughout the genome; however, such markers are usually dominantly inherited (BREYNE et al., 1997). Consequently, for a given investment of time and money, multilocus profiling techniques provide information on an enlarged number of loci in contrast to one-locus specific microsatellites, but the genotypic information obtained from each locus is reduced due to the dominant inheritance. Two contrasting sampling strategies can

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be adopted to assess genetic diversity using molecular markers: (*i*) selection of highly informative markers at a few loci (e.g. microsatellites), and (*ii*) sampling of numerous less informative markers randomly distributed within the genome (RAPD or AFLP technology). It is not yet clear that these two strategies will produce similar results when used to measure within- and among-populations diversity. Most of studies that compare different types of markers have been carried out by allozymes and RAPD markers, and do not provide consistent results (Baruffi et al., 1995; Cagigas et al., 1999; Isabel et al., 1995; Lannér-Herrera et al., 1996; Le Corre et al., 1997).

Genetic variation within and among *Q. petraea* and *Q. robur* populations has previously been analysed in great detail by allozymes (Müller-Starck and Ziehe, 1991; Kremer et al., 1991; Müller-Starck et al., 1993; Kremer and Petit, 1993; Zanetto et al., 1994). These studies showed that *Q. petraea* is more variable than *Q. robur*, and that both species share the same marker alleles, exhibiting only small differences in their allele frequencies. Analysis of total protein patterns has provided results comparable with allozymes, indicating that the level of genetic differentiation between *Q. petraea* and *Q. robur* is very low (Barreneche et al., 1996). Bodenes et al. (1997b) investigated the geographic variation of the species differentiation between *Q. petraea* and *Q. robur* on the basis of RAPD markers. They found only two per cent of the amplified fragments, exhibiting significantly different frequency distribution between both species, and none of them was species specific.

We report here on the comparison of the levels of genetic diversity within and among populations of two closely related white oak species, *Q. petraea* (Matt.) Liebl. and *Q. robur* L., with respect to two contrasting types of nuclear DNA markers above-mentioned, namely microsatellites and AFLP markers. Within the framework of a research project, supported by the European Union, seven mixed *Q. petraea* and *Q. robur* stands were selected in six different countries. For each stand, every tree was analysed with respect to six microsatellite markers by each laboratory within the project. In addition, approximately 45 samples of each species were screened per stand, using 155 AFLP loci, which has been done by the INRA laboratory. These data sets were used to compare the levels of diversity within and between both oak species. The objectives of this study were twofold: (*i*) to compare the above-mentioned contrasting marker systems for the assessment of gene diversity in oaks. The main goal was to verify whether these markers would provide the same genetic relationships when populations within each species were ordered by their level of diversity and (*ii*) to compare the level of diversity among populations within each species by taking into account the whole genome. Furthermore, a new method was developed to assess both the inter-locus and the intra-locus sampling variance of gene diversity to compare the level of diversity between populations.

Material and Methods

Sampling of stands

Seven stands, representing six European countries, were selected under following conditions: (*i*) the stands are mixed, comprising *Q. petraea* and *Q. robur* in approximately equal proportions, as well as consisting of three zones, namely two monospecific zones and one zone where the two species are growing tree by tree; (*ii*) the stands are of natural origin; (*iii*) the stands are characterised by adult trees (more than 120 years old); and (*iv*) the population size for each species should be close to 200. Although these are the features of an ideal site, it was not possible that each population meets all the criteria mentioned. A standard protocol (Dupouey and Badeau, 1993; Kremer et al., 2002) was used to distinguish *Q. petraea* from *Q. robur* by morphological characters. A principal component analysis (PCA) with refer to 14 leaf characters enabled the species classification for each oak tree tested. Trees exhibiting intermediate morphology were excluded from further analysis.

The location of stands is given on *Figure 1*. The list of stands and their composition are given in *Table 1*.

Table 1. – List of the stands and their composition.

Country	Location	Number of trees (*Q. petraea*)	Number of trees (*Q. robur*)	Number of trees revealing intermediate morphology
France	Petite Charnie	199	215	8
Germany	Escherode	110	206	5
United Kingdom	Dalkeith Old Wood	21	351	27
The Netherlands	Meinweg	181	184	15
Austria	Sigmundsherberg	228	159	8
United Kingdom	Roudsea Wood	205	56	11
Spain	Salinasco Mendia	233	45	-

Fig. 1. – Location of stands.

Microsatellite markers

All trees were genotyped using six microsatellite loci: ssrQpZAG9, ssrQpZAG36, ssrQpZAG104 and ssrQpZAG1/5 (Steinkellner et al., 1997) as well as MSQ4 and MSQ13 (Dow et al., 1995). DNA extraction, PCR-based amplification and detection of microsatellites were performed according to Streiff et al. (1998). A test was carried out to validate the genotype identification across different laboratories. Each regional laboratory has sent 10 DNA extracts to the INRA reference laboratory who performed the comparison between own results and the data obtained from the other participants. Five scoring differences among the laboratories were found:

Error 1 reflects a systematic shift in the estimated allele sizes, constant across the range of allele sizes. For example, the allele of microsatellite ssrQpZAG9 that was scored 202 bp by

the INRA laboratory was scored 205 bp by the Austrian laboratory.

Error 2 reflects a systematic shift in allele size, but not constant across the range of allele sizes. The difference amounted to a certain value when the allele size was lower than a given threshold, and then changed above this threshold.

Error 3 reflects a random variation of allele sizes. There were occasionally discrepancies between allele sizes.

Error 4 reflects differences in genotype identification, especially inconsistencies in differentiating heterozygotes and homozygotes. This mainly occurred when the two alleles exhibited a small difference in size.

Error 5 reflects a miscoding of rare, high molecular weight alleles. For a few loci, there were alleles of unusually extreme size corresponding most likely to either a deletion or insertion in flanking regions. In general, there were small discrepancies in the assessment of the size of these alleles scored across the laboratories.

AFLP markers

For each stand, approximately 45 randomly chosen trees from both species were screened. Using four AFLP Primer-Enzyme Combinations [P*st*I+CAG / M*se*I+CAA, P*st*I+CAG / M*se*I+GCA, P*st*I+CAG / M*se*I+GGA and P*st*I+CCA / M*se*I+CAA], AFLP analysis was performed as has been described by GERBER et al. (2000). The RFLPscan version 3.0 (Scanalytics) software was used for scoring the AFLP fragments. The STR marker (purchased by LI-COR, Biotechnology Division) was employed to determine the accurate sizes of individual fragments.

Data analysis for microsatellite markers

The following standard parameters of population genetics were calculated for each species and each stand (BROWN and WEIR, 1983): allelic richness (A), effective number of alleles (A_E=1/[1-H_E]), observed heterozygosity (H_O), expected heterozygosity (H_E) from Hardy-Weinberg proportion and fixation index (F_{IS}=1-H_O/H_E). In addition, the within-population gene diversity (H_i), the mean within-population gene diversity (H_S), the total diversity (H_T) and the genetic differentiation (G_{ST}) were calculated according to NEI (1987). Parameters were indicated by mean values. The coefficient of gene differentiation among populations (G_{ST}) was calculated between both species in each stand and among populations of each species. Parameters were computed using the DIPLOIDE program (ANTOINE KREMER, Equipe de Génétique et Amélioration des Arbres Forestiers, Cestas, France).

Taking into account the discrepancies obtained for microsatellite data between different laboratories, the estimation of genetic diversity was performed in two different ways: the comparison of diversity between *Q. petraea* and *Q. robur* was done separately for each stand, using the scoring procedure which has been developed by the laboratory in charge of the given stand (1st analysis). On the other hand, the comparison of diversity of *Q. petraea*/*Q. robur* populations across sites was performed after transforming the original data by taking into account the above-mentioned discrepancies, which were found between the genotypes estimated in the reference laboratory and in the regional laboratory (2nd analysis). Basic corrections were introduced according to the results obtained from the master PCRs. Furthermore, alleles differing by one base pair and present in low frequencies were pooled. In this case, the comparison of diversity was restricted to the estimations of expected heterozygosity (H_E) and the within-population gene diversity (H_i). Both parameters are known to be less sensitive to small changes in allele frequencies than allelic richness A, simply because the square of the allelic frequencies is involved in the calculation of H.

Data analysis for AFLP markers

The analysis of the AFLP markers was done under the assumption that each amplified band, regardless of its relative intensity, corresponds to a dominantly inherited allele at a single locus. Polymorphic loci were scored as "1" for the presence and "0" for the absence of the marker band.

Both phenotypic and genotypic types of analysis were performed in case of the AFLP data. Phenotypic analysis considered two types of variants: the individuals exhibiting a band (frequency P) and those without the band (frequency Q). P and Q were obtained directly from the DNA profiles and used for calculating H_i, H_S, H_T, and G_{ST}.

The genotypic analysis depends on the frequencies p and q of allelic variants, which are responsible for the presence or the absence of bands, respectively. The following hypothesis of genetic structure allows p and q to be deduced from Q: if the deficiency of heterozygotes (indicated by F_{IS}) is known, then Q results from the formula $Q = q^2\ (1–F_{IS}) + qF_{IS}$. Following the calculation of F_{IS}, an asymptotically unbiased estimation of q is obtained by the use of a second order Taylor function (KENDALL and STUART, 1977):

$$\hat{q} = \frac{-F_{IS}}{2(1-F_{IS})} + \frac{\Delta^{\frac{1}{2}}}{2(1-F_{IS})} - 2(1-F_{IS})\Delta^{-\frac{3}{2}}\left[\frac{Q(1-Q)}{N}\right] \qquad (1)$$

with Δ resulting from the formula $\Delta = F_{IS}^2 + 4(1–F_{IS})\ Q$ and N indicating the number of trees sampled per population.

The genotypic analysis (G1) was performed using the F_{IS} value, which was estimated from the average data represented by the gene pool of six microsatellite loci. As recommended by LYNCH and MILLIGAN (1994), another genotypic analysis (G2) was performed, taking into account only such loci that showed an observed frequency smaller than (1-[3/N]), where N indicates the population sample size. Any fragment that exhibited a higher frequency than (1-[3/N]) in a single population was therefore removed from the data set. LYNCH and MILLIGAN (1994) demonstrated that the bias affecting the estimation of Q in case of a small sample size is substantial when the null allele reveals a low-frequency distribution. Gene diversity statistics were computed by using the allelic frequencies as estimated by formula (1).

The phenotypic and genotypic analyses were performed, using both the HAPLOID and the HAPDOM program (ANTOINE KREMER, Equipe de Génétique et Amélioration des Arbres Forestiers, Cestas, France), respectively.

Intra- and inter-locus sampling variances

The total variance of gene diversity statistics (A, A_E, H_O, H_E, H_i, H_S, H_T, F_{IS} and G_{ST}) depends from both sampling procedures: sampling of individuals within populations ($V_{intra\text{-}locus}$) and sampling of loci within the genome ($V_{inter\text{-}locus}$). The total sampling variance is $V_{total} = V_{intra\text{-}locus} + V_{inter\text{-}locus}$. Both components were estimated by using bootstrap methods. All resampling procedures were done with replacement. One thousand bootstrap samples were made each time for estimating the sampling variances.

$V_{intra\text{-}locus}$ was estimated by resampling individuals within populations.

For calculating G_{ST}, $V_{intra\text{-}locus}$ was estimated by resampling populations as has been suggested by PETIT and PONS (1998). $V_{inter\text{-}locus}$ was estimated by resampling loci across individuals.

Statistical test of differences between populations

The distributions of the diversity statistics, estimated by bootstrapping, were used to test the difference between two populations a and b (a and b being the two species populations from the same stand or being populations of the same species from two different stands). H_{ia} and H_{ib} were computed for each population by bootstrapping, and the difference, existing between two populations ($H_{ia} - H_{ib}$), was calculated. The distribution of ($H_{ia} - H_{ib}$) was then compared with the null hypothesis ($H_{ia} - H_{ib} = 0$) and the associated probability p was calculated.

Statistical test of AFLP markers allelic frequencies differences between species

For the species level and within each stand, the frequencies of AFLP markers were compared between the two species by performing FISHER's exact tests (SOKAL and ROHLF, 1995).

Comparison of diversity statistics between markers

The *Q. petraea* and *Q. robur* populations were compared according to the parameters A, A_E, H_O and H_i for microsatellites and according to H_i for AFLP markers. The value of these parameters were then compared by computing SPEARMAN's rank coefficient correlation: r_S (SOKAL and ROHLF, 1995).

Results

1-Microsatellite and AFLP markers both revealed a higher genetic diversity in Q. petraea than in Q. robur

1a-Analysis of *Q. petraea* and *Q. robur* populations with microsatellites

For almost all stands, the genetic diversity assessed by means of microsatellites was somewhat higher within the *Q. petraea* populations than within *Q. robur*. The analysis depicted in *Table 2* shows that at least one diversity parameter (among A, A_E, H_E, H_i and H_O) per stand revealed significantly higher values in *Q. petraea* than in *Q. robur*. The only site in which *Q. robur* indicated a higher value of genetic diversity was Dalkeith Old Wood, but this was probably due to the very low numbers of *Q. petraea* trees growing at this site (*Table 1*). Note that the differences in the levels of diversity were altogether small, but supported statistical relevance when the intra-locus standard deviation was used.

Similarly, differences in the diversity (H_E) for each species were statistically significant among stands and the rankings of stands were very similar for *Q. petraea* and *Q. robur* (r_S=0.769, p=0.043). Extreme values were the same for both species, Roudsea Wood exhibiting the highest value of diversity and Sigmundsherberg the lowest (*Table 2*).

For two stands (Sigmundsherberg and Meinweg), the fixation index (F_{IS}) was significantly higher in *Q. robur* than in *Q. petraea*, indicating an excess of homozygotes.

1b-Analysis of *Q. petraea* and *Q. robur* populations with AFLP markers

The four Primer-Enzyme Combinations tested were able to provide 155 scorable marker bands. Based on the phenotypic analysis and the all band-scoring G1 analysis of AFLP markers, the within-population gene diversity (H_i) indicated a somewhat higher value for five (Petite Charnie, Escherode, Dalkeith Old Wood, Meinweg and Salinasco Mendia) of the seven *Q. petraea* populations tested in comparison with *Q. robur* populations (*Table 3*). However, none of the results were significant when the total standard deviation was used to compare between both species of each stand. However, when the comparison was performed on the basis of the intra-locus standard deviation (sd 1), the phenotypic analysis indicated significantly higher genetic diversity for *Q. petraea* in populations Petite Charnie, Escherode and Meinweg (*Table 3*). No significant deviations were found between *Q. petraea* and *Q. robur* using the G2 analysis of AFLP marker bands. The standard deviation, derived from sampling different loci within the genome, was always higher than the standard deviation derived from sampling of individuals, independently from the type of analysis employed.

No significant difference was detected among stands when the total standard deviation of H_i was analysed. For each analysis, the correlation between the ranking of stands for *Q. petraea* and for *Q. robur* was always positive but never significant.

1c-Microsatellite and AFLP analyses at the species level

When the data were pooled from the seven populations on a species-specific basis, both microsatellite and AFLP markers indicated a significantly higher genetic diversity for *Q. petraea* on the basis of H_i, H_O, A and A_E values (*Table 4*). The G2 analysis of AFLP markers was the only exception, showing nearly identical H_i values for both oak species (0.252 vs 0.256 for *Q. petraea* and *Q. robur*, respectively). The genetic diversity in

Table 2. – Microsatellites diversity statistics in *Q. petraea* and *Q. robur* stands.

Petite Charnie	***Q. petraea***	**sd 1**	***Q. robur***	**sd1**	**p1**
H_i	0,877	0,004	0,866	0,004	**0,036**
H_O	0,819	0,014	0,804	0,013	0,209
A	18,67	0,39	18.00	0,44	0,077
A_E	8,14	0,26	7,46	0,22	**0,046**
F_{IS}	0,063	0,016	0,068	0,015	0,616
Escherode	***Q. petraea***	**sd 1**	***Q. robur***	**sd1**	**p1**
H_i	0,878	0,005	0,835	0,004	**0,001**
H_O	0,884	0,011	0,832	0,010	**0,001**
A	19,50	0,41	18.00	0,39	**0,024**
A_E	8,23	0,29	6,04	0,16	**0,001**
F_{IS}	-0,011	0,013	0.000	0,012	0,671
Dalkeith Old Wood	***Q. petraea***	**sd 1**	***Q. robur***	**sd1**	**p1**
H_i	0,869	NC	0,868	0,002	NC
H_O	0,881	NC	0,815	0,009	NC
A	12,67	NC	19,50	0,36	NC
A_E	7,63	NC	7,57	0,12	NC
F_{IS}	-0,038	NC	0,058	0,010	NC
Meinweg	***Q. petraea***	**sd 1**	***Q. robur***	**sd1**	**p1**
H_i	0,867	0,003	0,860	0,004	0,079
H_O	0,790	0,011	0,748	0,014	**0,007**
A	18,17	0,37	17,83	0,42	0,278
A_E	7,50	0,18	7,12	0,20	0,108
F_{IS}	0,086	0,012	0,128	0,015	**0,016**
Sigmundsherberg	***Q. petraea***	**sd 1**	***Q. robur***	**sd1**	**p1**
H_i	0,883	0,004	0,882	0,004	0,306
H_O	0,819	0,010	0,757	0,014	**0,001**
A	26,67	0,44	24,67	0,51	**0,005**
A_E	8,54	0,17	8,45	0,14	0,351
F_{IS}	0,071	0,012	0,142	0,017	**0,000**
Roudsea Wood	***Q. petraea***	**sd 1**	***Q. robur***	**sd1**	**p1**
H_i	0,908	0,002	0,899	0,005	**0,003**
H_O	0,781	0,013	0,775	0,022	0,434
A	26,50	0,45	19,50	0,56	**0,004**
A_E	10,85	0,24	9,90	0,43	**0,003**
F_{IS}	0,136	0,014	0,131	0,026	0,501
Salinasco Mendia	***Q. petraea***	**sd 1**	***Q. robur***	**sd1**	**p1**
H_i	0,862	0,004	0,866	0,007	0,354
H_O	0,840	0,009	0,812	0,020	0,107
A	19,33	0,34	14,50	0,55	**0,003**
A_E	7,22	0,20	7,44	0,33	0,349
F_{IS}	0,023	0,010	0,052	0,023	0,928

sd1 indicates the standard deviation associated to the intra-locus variance; **p1** values are the associated probabilities; significant values at 5% are presented in bold numbers; NC: not calculated, because bootstrap mean values differed markedly from the observed values, indicating that the bootstrap procedure is not adequate in case of low sample sizes.

Table 3. – AFLP markers diversity statistics in *Q. petraea* and *Q. robur* stands.

Petite Charnie	***Q. petraea***	**sd1**	**sd2**	**total sd**	***Q. robur***	**sd1**	**sd2**	**total sd**	**p1**	**p2**	**p**
$H_i^{(P)}$	0.194	0.006	0.018	0.019	0.179	0.005	0.018	0.019	**0.030**	0.319	0.575
$H_i^{(G1)}$	0.191	0.006	0.018	0.019	0.172	0.004	0.018	0.018	**0.006**	0.232	0.465
$H_i^{(G2)}$	0.234	0.012	0.022	0.025	0.252	0.010	0.022	0.024	0.123	0.277	0.610
Escherode	***Q. petraea***	**sd1**	**sd2**	**total sd**	***Q. robur***	**sd1**	**sd2**	**total sd**	**p1**	**p2**	**p**
$H_i^{(P)}$	0.207	0.006	0.016	0.017	0.192	0.005	0.015	0.016	**0.028**	0.230	0.522
$H_i^{(G1)}$	0.189	0.005	0.016	0.017	0.185	0.006	0.015	0.016	0.322	0.471	0.865
$H_i^{(G2)}$	0.211	0.007	0.017	0.018	0.233	0.008	0.020	0.022	**0.023**	0.205	0.441
Dalkeith Old Wood	***Q. petraea***	**sd1**	**sd2**	**total sd**	***Q. robur***	**sd1**	**sd2**	**total sd**	**p1**	**p2**	**p**
$H_i^{(P)}$	0.214	NC	NC	NC	0.189	0.004	0.015	0.016	0.098	0.168	NC
$H_i^{(G1)}$	0.204	NC	NC	NC	0.180	0.005	0.014	0.015	0.180	0.223	NC
$H_i^{(G2)}$	0.234	NC	NC	NC	0.236	0.006	0.017	0.018	0.384	0.490	NC
Meinweg	***Q. petraea***	**sd1**	**sd2**	**total sd**	***Q. robur***	**sd1**	**sd2**	**total sd**	**p1**	**p2**	**p**
$H_i^{(P)}$	0.200	0.006	0.016	0.017	0.186	0.005	0.015	0.016	**0.038**	0.259	0.549
$H_i^{(G1)}$	0.194	0.008	0.015	0.017	0.189	0.005	0.015	0.016	0.300	0.380	0.834
$H_i^{(G2)}$	0.247	0.008	0.019	0.021	0.261	0.008	0.020	0.022	0.127	0.257	0.646
Sigmundsherberg	***Q. petraea***	**sd1**	**sd2**	**total sd**	***Q. robur***	**sd1**	**sd2**	**total sd**	**p1**	**p2**	**p**
$H_i^{(P)}$	0.189	0.006	0.016	0.017	0.196	0.006	0.015	0.016	0.189	0.377	0.764
$H_i^{(G1)}$	0.189	0.006	0.016	0.017	0.199	0.008	0.015	0.017	0.180	0.300	0.682
$H_i^{(G2)}$	0.262	0.010	0.020	0.022	0.262	0.008	0.020	0.022	0.450	0.527	1.000
Roudsea Wood	***Q. petraea***	**sd1**	**sd2**	**total sd**	***Q. robur***	**sd1**	**sd2**	**total sd**	**p1**	**p2**	**p**
$H_i^{(P)}$	0.214	0.007	0.016	0.017	0.227	0.005	0.015	0.016	0.075	0.249	0.582
$H_i^{(G1)}$	0.202	0.007	0.015	0.017	0.217	0.005	0.016	0.017	0.050	0.211	0.535
$H_i^{(G2)}$	0.241	0.010	0.018	0.021	0.279	0.010	0.018	0.021	**0.008**	0.060	0.184
Salinasco Mendia	***Q. petraea***	**sd1**	**sd2**	**total sd**	***Q. robur***	**sd1**	**sd2**	**total sd**	**p1**	**p2**	**p**
$H_i^{(P)}$	0.195	0.005	0.016	0.017	0.194	0.006	0.015	0.016	0.413	0.487	0.968
$H_i^{(G1)}$	0.197	0.006	0.015	0.016	0.189	0.005	0.015	0.016	0.176	0.402	0.734
$H_i^{(G2)}$	0.264	0.008	0.019	0.021	0.260	0.008	0.018	0.020	0.377	0.459	0.881

$H_i^{(P)}$ is the phenotypic diversity; $H_i^{(G1)}$ is the G1 gene diversity; $H_i^{(G2)}$ is the G2 gene diversity; **sd1** is the standard deviation associated to the intra-locus variance; **p1** is the associated probability; **sd2** is the standard deviation associated to the inter-locus variance; **p2** is the associated probability; **total sd** is the total standard deviation associated to the total variance; **p** is the associated probability; significant values at 5% are presented in bold numbers; NC: not calculated, because bootstrap mean values differed markedly from the observed values, indicating that the bootstrap procedure is not adequate here since the sample size is low.

Table 4. – Microsatellites and AFLP markers diversity statistics at the species level.

	Quercus petraea		***Quercus robur***		
Microsatellites					
		sd1		**sd1**	**p1**
H_i	0.896	0,001	0.878	0,002	**0,000**
H_O	0.820	0,005	0.799	0,005	**0,000**
A	28.83	0,461	26.83	0,492	**0,000**
A_E	9.59	0,113	8.20	0,088	**0,000**

AFLP markers											
		sd1	**sd2**	**total sd**		**sd1**	**sd2**	**total sd**	**p1**	**p2**	**p**
$H_i^{(P)}$	0.228	0.002	0.015	0.015	0.220	0.002	0.014	0.014	**0.001**	0.370	0.697
$H_i^{(G1)}$	0.225	0.002	0.015	0.015	0.219	0.002	0.014	0.014	**0.008**	0.385	0.772
$H_i^{(G2)}$	0.252	0.004	0.015	0.016	0.256	0.003	0.016	0.016	0.202	0.380	0.857

$H_i^{(P)}$ is the phenotypic diversity; $H_i^{(G1)}$ is the G1 gene diversity; $H_i^{(G2)}$ is the G2 gene diversity; **sd1** is the standard deviation associated to the intra-locus variance and **p1** the associated probability; **sd2** is the standard deviation associated to the inter-locus variance and **p2** the associated probability; **total sd** is the total standard deviation associated to the total variance and **p** is the associated probability; a significant difference between *Q. petraea* and *Q. robur* is indicated with a **p** value in bold letters.

Q. petraea was significantly higher when the intra-locus variance was used and was never found to be significantly greater when the total sampling variance was tested.

2-AFLP markers provided a higher genetic differentiation than microsatellites, except when the G2 analysis was used

2a-Genetic differentiation between *Q. petraea* and *Q. robur* populations, performed at the same stand

The genetic differentiation (G_{ST}) measured between *Q. petraea* and *Q. robur* populations at each stand by means of microsatellites was low (*Table 5a*), ranging from 0.005 (Roudsea Wood) to 0.024 (Sigmundsherberg). For AFLP markers, much higher G_{ST} values, spanning from 0.016 to 0.096, were found, which were substantially reduced in case of the G2 method (*Table 5a*).

2b-A higher genetic differentiation among *Q. petraea* populations than among *Q. robur* populations, but not significant

When we analysed the genetic differentiation among populations within each species, G_{ST} values were found slightly higher among *Q. petraea* populations but the difference was never found significant between both species (*Table 5b*). The genetic differentiation, calculated with AFLP markers among *Q. petraea* or among *Q. robur* populations, was not significantly higher than the differentiation calculated with microsatellites, using the G2 analysis.

2c-Distribution curves of genetic differentiation for AFLP markers

Since a higher differentiation was detected using AFLP analysis in comparison with microsatellites, the variation of G_{ST} values among AFLP marker bands was further analysed. Distributions of G_{ST} values are given in *Figures 2, 3* and *4*. The three curves resemble an L-shaped distribution where more extreme values occurred among different populations of a species rather than between species. For example, only four loci exhibited a differentiation greater than 10% between *Q. petraea* and *Q. robur* populations whereas 36 loci among *Q. petraea* populations and 23 loci among *Q. robur* populations demonstrated this level of differentiation. At the species level, the null hypothesis, indicating independence between the observed frequencies and the species, was rejected for 9% (Dalkeith Old Wood) to 22% (Escherode) of the loci.

Table 5a. – Genetic differentiation between *Q. petraea* and *Q. robur* populations localised at the same stand.

	G_{ST} (microsatellites)	$G_{ST}^{(P)}$ (AFLP markers)	$G_{ST}^{(G1)}$ (AFLP markers)	$G_{ST}^{(G2)}$ (AFLP markers)
Q. petraea / Q. robur Petite Charnie	0.018[1]	0.068	0.053	0.038
Q. petraea / Q. robur Escherode	0.019[1]	0.093	0.096	0.028
Q. petraea / Q. robur Dalkeith Old Wood	0.010[1]	0.031	0.031	0.000
Q. petraea / Q. robur Meinweg	0.018[1]	0.076	0.071	0.038
Q. petraea / Q. robur Sigmundsherberg	0.024[1]	0.060	0.056	0.023
Q. petraea / Q. robur Roudsea Wood	0.005[1]	0.063	0.053	0.034
Q. petraea / Q. robur Salinasco Mendia	0.015[1]	0.051	0.040	0.021
Q. petraea / Q. robur	0.013[2]	0.037	0.030	0.016

Table 5b. – Genetic differentiation among populations within species.

	G_{ST} (microsatellites)	$G_{ST}^{(P)}$ (AFLP markers)	$G_{ST}^{(G1)}$ (AFLP markers)	$G_{ST}^{(G2)}$ (AFLP markers)
Q. petraea populations	0.023[2]	0.118	0.111	0.044
sd	0.007[2]	0.016	0.015	0.009
Q. robur populations	0.020[2]	0.114	0.111	0.030
sd	0.005[2]	0.022	0.019	0.005
p value	0.364[2]	0.482	0.592	0.126

In Tables 5a and 5b, **p** values were obtained with bootstrap samples; [1] 1st analysis of microsatellites; [2] 2nd analysis of microsatellites; $\boldsymbol{G_{ST}^{(P)}}$ is the phenotypic differentiation; $\boldsymbol{G_{ST}^{(G1)}}$ is the G1 genetic differentiation; $\boldsymbol{G_{ST}^{(G2)}}$ is the G2 genetic differentiation.

3-No correlation in levels of diversity assessed with different diversity parameters or with different markers

For microsatellites, no significant correlation was found when rankings calculated by A and H_i were compared (*Table 6*). For AFLPs, rankings given by the G2 analysis for *Q. petraea* tended to be different from the rankings given by the two other methods of analysis but the result was not significant. For *Q. robur*, the three analyses were congruent and the correlations were significant.

When H_i values for the microsatellite data were compared with H_{i1}, H_{i2} and H_{i3} values of AFLP data, no significant correlation was found.

Table 6. – SPEARMAN's rank correlation analysis among diversity values obtained with different markers.

Quercus petraea	H_i **(microsatellites)**	$H_i^{(P)}$	$H_i^{(G1)}$
A **(microsatellites)**	0.414[2]		
$H_i^{(P)}$	0.577		
$H_i^{(G1)}$	0.090	0.673	
$H_i^{(G2)}$	-0.649	-0.464	0.091
Quercus robur	H_i **(microsatellites)**	$H_i^{(P)}$	$H_i^{(G1)}$
A **(microsatellites)**	0.429[2]		
$H_i^{(P)}$	0.180		
$H_i^{(G1)}$	0.082	**0.847**	
$H_i^{(G2)}$	0.180	0.571	**0.847**

Each SPEARMAN's rank correlation is followed by the associated probability; a significant positive correlation at the 5% level is indicated in bold letters; $\boldsymbol{H_i}$: microsatellites within-population diversity; [2] 2nd analysis of microsatellites; $\boldsymbol{H_i^{(P)}}$ is the phenotypic diversity; $\boldsymbol{H_i^{(G1)}}$ is the G1 gene diversity; $\boldsymbol{H_i^{(G2)}}$ is the G2 gene diversity.

Discussion

Our experimental results confirmed the theoretical expectation: the inter-locus variance is higher than the intra-locus variance of diversity

Our experimental results confirmed NEI's prediction that a larger variance is attributable to the effect of analysing different genomic loci of a species than to the sampling of individuals within a population. The AFLP data demonstrated that the former source of variance can be up to 15 times greater than the latter. Interestingly, both microsatellites and AFLP markers provided similar data of intra-locus variance, which suggests that the sampling variance is independent of the number of alleles (*Table 2* and *Table 3*). If differences in the levels of diversity have to be assessed on the whole genome, the number of loci rather than the number of individuals should be as great as possible. This is also likely to be the case when monitoring of gene diversity is done for conservation purposes. When the attributes for which diversity is assessed are unknown, diversity should be measured at the whole genome level, by using a random set of markers distributed throughout the genome. However, a larger sampling variance would be expected, which would lead to a reduction in the power of any statistical test applied to measure diversity differences among populations. This is clearly reflected in our results. There was a trend towards higher genetic diversity in *Q. petraea*, although the difference between both species is not significant. More AFLP markers will be necessary to reveal significant differences between both species.

Microsatellite and AFLP markers revealed similar trends when measuring diversity in both oak species

Both marker technologies showed that *Q. petraea* is more variable than *Q. robur* (*Table 2* and *Table 3*), confirming results which have already been obtained in gene diversity surveys using isozymes (MÜLLER-STARCK et al., 1993, KREMER et al., 1991, ZANETTO et al., 1994) or DNA markers (MOREAU et al., 1994, BODENES et al., 1997a).

Comparable results between both types of markers were only obtained when the G2 analysis of AFLP data was not used. When the analysis was restricted to the subset of polymorphic markers only, following the recommendation by LYNCH and MILLIGAN (1994), there was an increase in the sampling variance at the intra- and inter-locus level. This increase in variance is likely to be due to the reduction in the number of loci (from 155 loci to 61 loci). For comparative analysis of levels of diversity between populations, it would therefore be preferable to use all markers, polymorphic and monomorphic.

AFLP markers revealed higher genetic differentiation than microsatellites

For genetic differentiation, contrasting results were obtained between microsatellite and AFLP markers. In general, AFLP markers exhibited higher levels of differentiation than microsatellites. There may be three explanations for these observations. First, mutation rates are higher in microsatellites and cannot be ignored when compared to migration rates. Both mutation and migration tend to decrease population differentiation (JIN and CHAKRABORTY, 1995, ROUSSET, 1996, SLATKIN, 1995). Second, high G_{ST} detected at some AFLP markers might

be due to the fact that these markers are localised in the chloroplast and/or mitochondrial genomes, that are maternally inherited in oak and therefore should display an higher genetic differentiation. This is actually the case for one RAPD marker showing a high differentiation in oak (LE CORRE et al., 1997). Third, there were more AFLP loci than microsatellites and the likelihood that some of the AFLP markers are linked to adaptative traits cannot be excluded. Oak populations are known to be highly differentiated for growth and phenological traits (DUCOUSSO et al., 1996). As a result, we might expect a high heterogeneity of G_{ST} values for different AFLP fragments. This is found to be the case and is shown by *Figures 2, 3* and *4* where the distribution of G_{ST} values follows a L-shaped curve. A few markers exhibited unusually high G_{ST} values. As a result the overall G_{ST} value for AFLP markers is higher than for microsatellites, most probably because there is a higher likelihood that some loci are linked to an adaptive trait than for microsatellites.

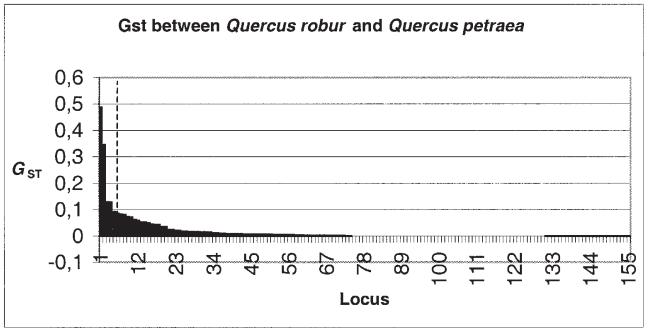

Fig. 2. – Distribution curve of G_{ST} per locus between *Q. petraea* and *Q. robur* populations.

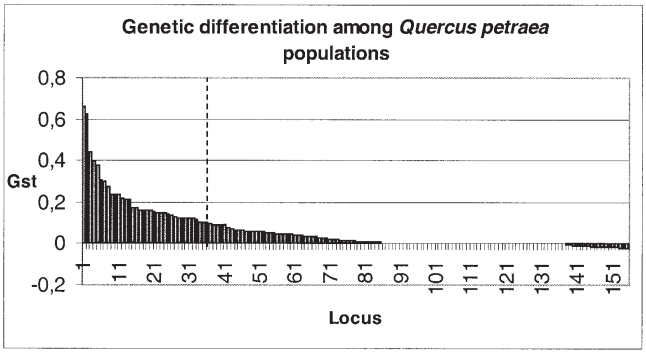

Fig. 3. – Distribution curve of G_{ST} per locus among *Q. petraea* populations.

There was also an important discrepancy between the G_{ST} values obtained from both methods of genotypic analyses with respect to the AFLP data. Differentiation was much lower when the analysis was restricted to polymorphic markers. This result was also demonstrated by ISABEL et al. (1999) who used RAPD markers and several differentiation parameters. Again, unexpected effects can be induced by restricting the analysis to polymorphic markers. For example, an AFLP fragment that is present and fixed in population A, but completely absent from population B, would be excluded by this method of analysis. However, this fragment would have a G_{ST} value of 1.

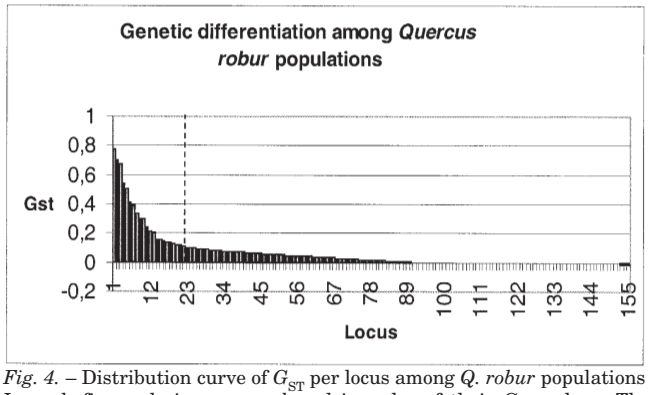

Fig. 4. – Distribution curve of G_{ST} per locus among *Q. robur* populations In each figure, loci were numbered in order of their G_{ST} values. The dotted line separated the loci showing a differentiation superior to 10% from the other loci.

Other comparative studies of different marker systems conducted in oaks provide more congruent results. For example, in a genetic study on 21 populations of *Q. petraea*, LE CORRE et al. (1997) compared the level of differentiation between 31 RAPD markers and 8 allozyme loci and found that the levels were similar (2.7% for allozymes and 2.4% for RAPDs) and comparable with the results we obtained here using microsatellites (2.3%, *Table 5b*). The congruence between these results can again be interpreted by the sampling effect within the genome. The low number of loci that are sampled by each of this method results in preferential selection of loci that are neutral and located within the tail of the L-shaped curve of G_{ST} values.

The differentiation was found somewhat higher in *Quercus petraea* than in *Quercus robur*: this observation was also true when allozyme markers were used in earlier studies (ZANETTO et al., 1994).

Comparison of levels of genetic diversity among populations of the same species

Despite the fact that microsatellites and AFLP markers provided congruent results, although not significant, in levels of diversity among both species, they did not agree in levels of intra-population genetic diversity within each species. As shown by the correlation matrix of diversity statistics (*Table 6*), there was a positive trend among H values, especially in *Q. robur*, but the correlation was never significant. This lack of congruence may be due to a contribution of different factors. Firstly, the level of diversity may be of similar magnitude in the different populations, as indicated in *Table 2* and *Table 3*. Oaks live in large populations and exhibit high migration rates (STREIFF et al., 1999). As a result, seed and pollen flow may contribute to the high homogeneity of diversity between populations. Second, the diversity statistics are estimated with an important sampling variance (*Table 4*). Again a larger number of loci would be necessary to increase the power of the statistical test to compare the level of diversity among populations.

Overall, the microsatellite and AFLP markers analysed in this study confirm earlier results obtained for *Q. petraea* and *Q. robur*. A higher level of inter-population and intra-population genetic diversity was found for *Q. petraea*. Consequently, *Q. petraea* reveals a higher genetic differentiation than *Q. robur*. However, the high inter-locus variance, detected for AFLP markers, did not allow us to significantly distinguish among populations. It also appeared from our analysis that a restriction in the number of analysed loci, as has been recommended by LYNCH and MILLIGAN (1994), leads to different rankings of populations. Even in case that the evolutionary forces

are identical for the genome parts analysed by two different types of markers, the missing information with respect to the genomic sequences analysed by AFLP markers and the limited number of available microsatellite loci could likely explain the conflicting results detected.

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