A DISCRIMINATION BETWEEN *QUERCUS ROBUR* L. AND *Q. PETRAEA* (MATT.) LIEBL. BASED ON SPECIES-INDICATIVE AFLP MARKERS

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ABSTRACT

In natural populations, *Quercus robur* L. and *Q. petraea* (Matt.) Liebl. comprise a morphological continuum due to hybridization and/or an overlap in variation between the two species. In order to obtain diagnostic markers to investigate these morphological intermediate forms, leaf morphology and AFLP were evaluated for their ability to discriminate between these two species in an autochthonous population in the Netherlands. Multivariate statistical analyses revealed a differentiation between the species based on leaf morphology data as well as AFLP data. Discriminant analysis resulted in the detection of only three out of 13 studied leaf morphology traits to be involved with species discrimination. None of the 92 polymorphic AFLP markers were diagnostic, but we found markers effective in species differentiation: there were 13 markers exhibiting significant marker band frequency differences among which there were five species-indicative marker-combinations. Regression analyses of AFLP markers on each of the 13 leaf morphology characteristics resulted in significant associations between groups of AFLP markers and leaf morphology traits. This study indicates that *Q. robur* and *Q. petraea* are closely related and probably only differ for a few genes coding for leaf morphology traits.

Keywords: AFLP, leaf morphology, species discrimination, *Quercus petraea*, *Quercus robur*

INTRODUCTION

Quercus robur L. (pedunculate oak) and *Q. petraea* (Matt.) Liebl. (sessile oak) (Fagaceae) are two closely related species occurring in most parts of Europe as sympatric species occupying different ecological niches (RUSHTON 1979; GRANDJEAN & SIGAUD 1987). Various studies have tried to discriminate between these two species based on morphological characteristics (RUSHTON 1978, 1979; GRANDJEAN & SIGAUD 1987; IETSWAART & FEIJ 1989). However, none of these studies were able to distinguish between the two species without ambiguity. The reason for this is the occurrence of intermediate types possessing morphological characteristics of either of the two species. It is assumed that these intermediate types have resulted from hybridization and backcrossing between the two species (RUSHTON 1978; IETSWAART & FEIJ 1989). However, in view of the infrequent occurrence of hybridization, a part of the intermediate types probably represents the wide range and overlap of variation of the two species (GARDINER 1970). On the other hand, hybrids and introgression products might not necessarily possess intermediate morphological characteristics, as they may also resemble one of the parents (KLEINSCHMIT *et al.* 1995).

Molecular marker techniques are promising tools for the description of species differentiation. Many molecular marker techniques (allozymes, cpDNA, RAPD, SCAR, two-dimensional gel electrophoresis of proteins, microsatellites) have been used to investigate species differences between *Q. robur* and *Q. petraea*. So far, none of these marker techniques could provide a diagnostic marker (a marker that is present in all individuals of one species, but is absent from all individuals of another species) for species identification (KREMER *et al.* 1991; PETIT *et al.* 1993; ZANETTO *et al.* 1994; MOREAU *et al.* 1994; BACILIERI *et al.* 1995; KLEINSCHMIT *et al.* 1995; SAMUEL *et al.* 1995; BARRENECHE *et al.* 1996; BODÉNÈS *et al.* 1997 a,b; STREIFF *et al.* 1998; MUIR *et al.* 2000). However, for some marker loci the species were discriminated based on significant marker band frequency differences (markers occur at a low frequency in one species and at a high frequency in another species) between the two species (ZANETTO *et al.* 1994; MOREAU *et al.* 1994; BACILIERI *et al.* 1995; BARRENECHE *et al.* 1996; BODÉNÈS *et al.* 1997 a). In none of these studies significant species-indicative markers (markers that occur in only one species, but not in all individuals, and are absent in all individuals in

another species) were found.

A new highly reproducible technique for DNA fingerprinting, AFLP, is able to efficiently generate large numbers of markers (VOS *et al.* 1995). The AFLP technique has been suitable for biosystematic studies in *Pisum* and *Solanum* as it is discriminative from the individual genotypic level to the species level (LU *et al.* 1996; KARDOLUS *et al.* 1998). AFLP was successfully used to analyze hybridization and invasion in populations of weedy *Onopordum* thistles (O'HANLON *et al.* 1999).

In this study, leaf morphology variation and AFLP polymorphisms will be compared with each other for their ability to differentiate between *Q. robur* and *Q. petraea* trees in the autochthonous population "De Meinweg" (the *Quercus* population has occurred at this location since its establishment after the last ice age). First, the subset of leaf morphology traits involved in species discrimination will be identified. This will be followed by a search for diagnostic AFLP markers that can be used to investigate trees with intermediate morphological traits. Finally, the associations between leaf morphology variation and AFLP polymorphisms will be analyzed.

MATERIALS AND METHODS

Study area

The studied population, "De Meinweg" (state survey co-ordinates *x/y* 207.5/354.8), is located in the south of the Netherlands on the slope of an old river-bed. "De Meinweg" is an autochthonous population that can be characterized as an old devastated woodland (VENNER 1985; MAES 1993; VAN DAM & DE VRIES 1998). *Q. robur* occurred in the entire area, while *Q. petraea* was only found in the higher parts. *Q. robur* and *Q. petraea* trees (N = 48 for each of both species) were sampled after an evaluation in the field based on a few species characteristics (basal shape of the lamina and the level of abaxial hairiness) and habitat characteristics (*Q. petraea* only occurs on elevated, nutrient poor soils) as described in VAN DER MEIJDEN (1990). In this way it was aimed at the exclusion of putative hybrid trees with intermediate characteristics (*Q. robur* type basal shape and *Q. petraea* type level of abaxial hairiness and vice versa). The positions of the sampled trees were recorded. The map was subsequently digitized with ArcView® GIS (Environmental Systems Research Institute Inc., Redlands, USA; Fig. 1).

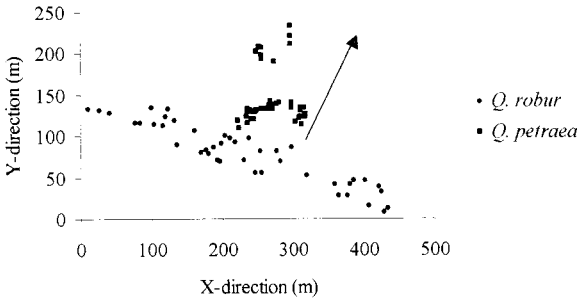

Figure 1. Geographical map of "De Meinweg" with the locations of the trees (dots are *Q. robur*, squares are *Q. petraea*). The arrow indicates the direction of the slope with a height difference of 10 m.

Leaf morphology analysis

Five fully expanded leaves were sampled from different sides from the crown of each tree. The leaves were evaluated for their level of abaxial hairiness (*HR*) on a scale from 1 (no hairs at all) to 6 (densely hairy), and subsequently dried and stored. After drying, another eight leaf morphology characteristics were scored: lamina length (*LL*); petiole length (*PL*); lobe width (*LW*); sinus width (*SW*); length of lamina from the lamina base to the widest part (*WP*); number of lobes (*NL*); number of intercalary veins (*NV*); basal shape of the lamina (*BS*; measured on a scale from 1 (wedge-shaped) to 9 (two clear lobes)). Subsequently, four derived characteristics were calculated: lamina shape, $OB = LL/WP$; petiole ratio, $PR = PL/(LL + PL)$; lobe depth ratio, $LDR = LW/(LW-SW)$; percentage venation, $PV = NV*100/NL$ (RUSHTON 1978).

AFLP analysis

DNA was extracted from fresh leaves or buds with a DNA extraction kit (Puregene®, Gentra Systems, Minneapolis, USA) including 4 % PVP-40 to remove phenolic compounds. AFLP analysis was performed according to VOS *et al.* (1995). Primers including one selective nucleotide (Eco + A and Mse + C) were used for pre-amplification of the template. The selective amplification was performed with γ^{33}P-ATP labeled primers Eco + AAG or Eco + ATA in combination with the unlabeled primer Mse + CCC. After electrophoresis the gels were vacuum dried on 3 mm Whatman paper and subsequently exposed to X-ray films (Kodak).

Reproducibility of AFLP was tested on five individuals (two *Q. robur* and three *Q. petraea*). For each of the five individuals AFLP fingerprints obtained from DNA samples from two different tissues (buds and leaves) from different years (1996 and 1997) were compared.

Associations between the two species and leaf morphology variation

The means of the leaf morphology traits per tree were plotted for each species separately in histograms. Subsequently, significant differences were tested between the means of the two species distributions per trait by means of t-tests or Mann-Whitney tests dependent on the assumption of normality of the data using Genstat 5. The data were analyzed for a sub-structuring in species based on the nine directly measured traits by means of a principal component analysis (PCA) with a correlation matrix using Genstat 5 (Release 4.1; Lawes Agricultural Trust, Rothamsted, UK). This was followed by a discriminant analysis of all 13 leaf morphology traits based on a pooled covariance matrix with a stepwise selection procedure of variables ($F_{IN} > 3.84$, $F_{OUT} < 2.71$, which is comparable with an approximate threshold value a for $F_{IN} < 0.05$, for $F_{OUT} > 0.10$) with SPSS 9.0 (SPSS Inc., Chicago, USA). Subsequently, the leaf morphology data were normalized (subtracted with the minimum and this divided by the range). Averages of the Euclidean distances between all possible pairs of trees within and between species were calculated using program Phyltools (J. Buntjer, Laboratory of Plant Breeding, Wageningen University, Wageningen, The Netherlands).

Associations between the two species and AFLP polymorphisms

The AFLP fingerprints were scored for the presence (1) and absence (0) of bands. The data were analyzed for a sub-structuring in species by means of a correspondence analysis (CA) using Genstat 5. Subsequently, a discriminant analysis based on a pooled covariance matrix with a stepwise selection procedure of variables was carried out ($F_{IN} > 3.84$, $F_{OUT} < 2\ 71$, which is comparable with an approximate threshold value a for $F_{IN} < 0.05$, for $F_{OUT} > 0.10$) using SPSS 9.0.

Average genetic distances (JACCARD 1908) between all possible pairs of trees within and between species were calculated with the program Phyltools (J. Buntjer, Laboratory of Plant Breeding, Wageningen University, Wageningen, The Netherlands). Significant band frequency differences between the two species were analyzed using the Fisher exact procedure of Genstat 5. The threshold of 5 % was adjusted according to the sequential Bonferroni test as proposed by RICE (1989).

Associations between leaf morphology variation and AFLP polymorphisms

Regression analyses of AFLP markers on each of the 13 leaf morphology traits were performed in order to analyze associations between leaf morphology variation and AFLP polymorphisms. This was done for each species separately. Associations between AFLP markers and quantitative leaf morphology characteristics were analyzed by means of multiple linear regression analyses ($F_{IN} > 3.0$, $F_{OUT} < 3.0$, which is comparable with an approximate threshold value a for $F_{IN} < 0.10$, for $F_{OUT} > 0.10$) with the STEP procedure of Genstat 5. Level of abaxial hairiness (*HR*) and basal shape (*BS*) were analyzed by means of GLM analyses assuming a poisson distribution (log as link function) of these response variables ($F_{IN} > 3.0$, $F_{OUT} < 3.0$, which is comparable with an approximate threshold value α for $F_{IN} < 0.10$, for $F_{OUT} > 0.10$) with the STEP procedure of Genstat 5.

RESULTS

AFLP analysis

A total data set was obtained with 110 different amplification products of which 16 % were monomorphic for all samples. AFLP resulted in fingerprints with an average number of 43 marker bands per sample. The reproducibility study showed that AFLP fingerprints were 100 % identical within one genotype and not affected by tissue source, year of sampling, or day-to-day variation in the lab. Identical AFLP fingerprints were obtained from different pairs of trees suggesting the presence of clones that originated due to coppicing and animal grazing (BAKKER *et al.* 2001). From each pair of trees with identical genotypes one tree was removed. To avoid an unbalanced data set of *Q. robur* and *Q. petraea* genotypes, two additional *Q. robur* genotypes were removed, thus leading to a total sample size of 43 for *Q. robur* and 43 for *Q. petraea*.

Associations between the two species and leaf morphology

All leaf morphology traits showed overlapping bimodal distributions for the two species. For leaf morphology traits *BS, HR, LDR, LL, NL, NV, PL, PR, PV*, and *SW* highly significant differences were observed between *Q. robur* and *Q. petraea* ($p < 0.005$). For leaf morphology traits *LW* and *WP* the difference observed between *Q. robur* and *Q. petraea* was less significant ($p < 0.05$). No difference between *Q. robur* and *Q. petraea* was found for *OB*. PCA based on the nine directly measured leaf morphology traits resulted in two clear groups corresponding to the previously identified species (Fig. 2). The first axis was responsible for the separation of

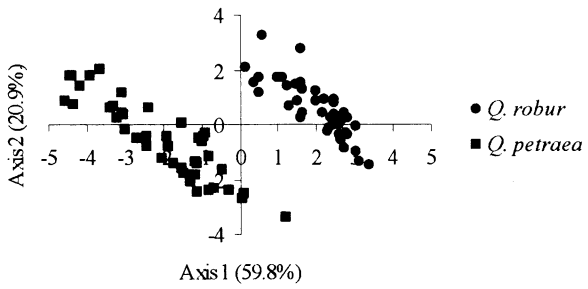

Figure 2. PCA based on nine leaf morphology traits (dots are *Q. robur*, squares are *Q. petraea*).

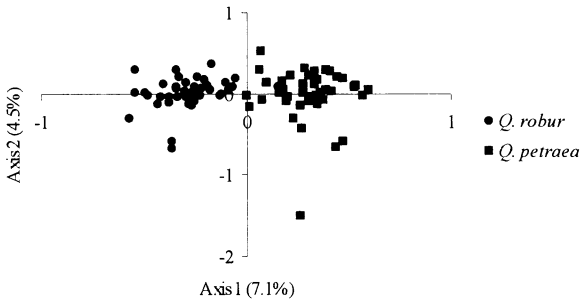

Figure 3. CA based on 110 AFLP markers (dots *are Q. robur*, squares are *Q. petraea*).

the two species. All nine variables showed high loadings. The highest loadings were observed for: *SW, HR, PL*, and *NL*. Discriminant analysis resulted in the inclusion of *HR, BS*, and *NV* in the discriminant function. The average Euclidean distance between *Q. robur* trees was significantly lower ($p < 0.05$) than between *Q. petraea* trees (Tab. 1). The average Euclidean distance between trees from different species was significantly higher ($p < 0.05$) than between trees within each of the two species (Tab. 1).

Table 1. Average Euclidean distances based on 13 leaf morphology traits (D_{EU}) and average Jaccard's (1908) genetic distances based on 110 AFLP markers (D_{JC}) within and between *Q. robur* and *Q. petraea*.

Species	D_{EU}	D_{JC}
Q. robur	0.65±0.034 a[2]	0.35±0.008 d
Q. petraea	0.84±0.041 b	0.38±0.009 e
Between [1]	1.63±0.025 c	0.41±0.005 f

[1] Between *Q. robur* and *Q. petraea*.
[2] Average Euclidean distances or Jaccard's genetic distances were marked with different letters in order to indicate that they differed significantly ($p < 0.05$).

Associations between the two species and AFLP polymorphisms

Correspondence analysis (CA) based on AFLP data resulted in a differentiation between the two species (Fig. 3). However, one individual that was previously assigned as *Q. robur* was after CA located in the *Q. petraea* cluster. The first axis was responsible for the differentiation between two groups corresponding to the two species. Discriminant analysis revealed that 19 AFLP markers were involved in the differentiation between the two species. The average JACCARD's (1908) genetic distance between *Q. robur* trees was significantly lower ($p < 0.05$) than between *Q. petraea* trees (Tab. 1). The average JACCARD's (1908) genetic distance between trees from different species was significantly higher ($p < 0.05$) than between trees within each of the two species (Tab. 1).

The Fisher exact test for association between the presence/absence of an AFLP band and a classification of *Q. robur*/*Q. petraea* resulted in the detection of 13 markers with significant differences in marker band frequency between the two species ($p < 0.05$). From among these 13 markers, five were significant species-indicative markers (Tab. 2). However, only seven out of the 19 AFLP markers that were included in the discriminant function were also observed as significant markers in the Fisher exact test. For both analyses the same marker could discriminate between the two species most significantly: Eco+AAG/Mse+CCC-235. This marker was not absent in one of the two species and present at a high frequency in the other species (species-indicative).

Table 2. Five ALFP markers which appear to be species indicative and their frequencies of occurrrence in *Q. robur* and *Q. petraea* ($N = 2 \times 43$).

Marker	*Q. robur*	*Q. petraea*
Eco+AAG/Mse+CCC-138	0	0.42
Eco+AAG/Mse+CCC-186	0.35	0
Eco+ATA/Mse+CCC-120	0.37	0
Eco+ATA/Mse+CCC-257	0	0.44
Eco+ATA/Mse+CCC-360	0.53	0

Associations between leaf morphology variation and AFLP polymorphisms

As for regression analyses the number of variables should not be higher than the number of individuals tested (MONTGOMERY & PECK 1992), the set of 76 and 72 polymorphic AFLP markers for *Q. robur* and *Q. petraea*, respectively, had to be reduced. Therefore all AFLP markers with less than five present or absent

bands were removed as these markers were expected to be less informative. It was expected that such markers would not be informative for explaining species differences, as they were only present in a small subset of each of the two species. After removing all markers with less than five present or absent bands we retained a set of 43 markers for both *Q. robur* and *Q. petraea*. Correlation analysis did not show any pairs of AFLP markers with correlation coefficients higher than 0.9. Therefore no AFLP markers were further removed from the data set. Three leaf morphology characteristics (NV, NL, and LDR) were transformed (square root, square root, and 10log, respectively) in order to obtain a homogeneous residual variation. Regression analysis of the 43 *Q. robur* and 43 *Q. petraea* AFLP markers on the 13 leaf morphology characteristics resulted in models consisting of up to 8 AFLP markers with percentages of explained variation up to 59.5 % (Tab. 3). For *Q. robur* from among the 43 analyzed AFLP markers 25 were significantly ($p < 0.05$) associated with up to four leaf morphology traits. For *Q. petraea* from among the 43 analyzed AFLP markers 27 were significantly ($p < 0.05$) associated with up to four leaf morphology traits.

Among the 19 AFLP markers that were in the discriminant function, three and seven AFLP markers were found to be significantly associated with leaf morphology traits for *Q. robur* and *Q. petraea*, respectively. However, 13 *Q. robur* and five *Q. petraea* AFLP markers that were in the discriminant function were not included in the regression analyses as these markers possessed less than five present or absent bands in the data set.

DISCUSSION

Associations between the two species and leaf morphology variation

The species distributions of the leaf morphology characteristics were all overlapping. This indicates that for a discrimination between *Q. robur* and *Q. petraea* it is necessary to take a multivariate approach. PCA based on nine leaf morphology traits could make a clear grouping between the two species. Our study shows that only three leaf morphology characteristics (HR, BS, and NV) were sufficient for a discrimination between *Q. robur* and *Q. petraea* trees. This was also observed by DUPOUEY & BADEAU (1993), however, other studies used many leaf morphology characteristics in order to define *Q. robur* and *Q. petraea* populations (RUSHTON 1978, 1979; GRANDJEAN & SIGAUD 1987; BACILIERI *et al.* 1996). It is likely that these latter studies included intermediate type trees, which makes it necessary to analyze more leaf morphology characteristics.

Associations between the two species and AFLP polymorphisms

Correspondence analysis (CA) based on 110 AFLP markers resulted in a grouping of the two species. However, one *Q. robur* individual was located in the *Q. petraea* cluster. This could be an introgression product that resembled the *Q. robur* parent as has been observed by KLEINSCHMIT *et al.* (1995).

The average JACCARD's (1908) genetic distance

Table 3. Number of ALFP markers explaining the various leaf morphology traits in regression models (for terminology see materials and methods section).

Trait	Number of ALFP markers		% explained variation	
	Q. robur	*Q. petraea*	*Q. robur*	*Q. petraea*
HR	0	0	–	–
LL	5	1	42.8	20.7
PL	4	1	32.8	11.2
LW	4	6	36.3	43.1
SW	2	4	23.4	30.0
WP	5	0	29.9	–
Sqrt *NL*	2	7	15.8	55.9
Sqrt *NV*	7	5	46.1	50.6
BS	0	0	–	–
OB	1	8	10.0	56.4
PR	3	0	29.9	–
LOGLDR	7	6	51.1	42.0
PV	5	6	50.8	59.5

within *Q. robur* was significantly lower than the average JACCARD's (1908) genetic distance within *Q. petraea*. The same result was observed after two-dimensional protein gel, RAPD, and SSR analyses of *Q. robur* and *Q. petraea* trees in natural mixed populations (MOREAU *et al.* 1994; KLEINSCHMIT *et al.* 1995; BARRENECHE *et al.* 1996; BODÉNÈS *et al.* 1997 a; STREIFF *et al.* 1998). However, allozyme analyses gave contradictory results: whereas ZANETTO *et al.* (1994), KLEINSCHMIT *et al.* (1995), and STREIFF *et al.* (1998) showed a higher genetic diversity for *Q. petraea* than for *Q. robur*, BACILIERI *et al.* (1995) and SAMUEL *et al.* (1995) showed a lower genetic diversity for *Q. petraea* than for *Q. robur*. This lower genetic diversity for *Q. petraea* was also found for SCAR analysis (BODÉNÈS *et al.* 1997b). This discrepancy in genetic variation observed for the two species is probably caused by the different characteristics of the various molecular marker methods. It appears that molecular markers that are not coding like RAPD, microsatellites, and AFLP give more consistent results than the protein and DNA markers that represent coding regions. However, as these studies used different sampling schemes, this could be another reason for the discrepancy in genetic variation observed for the two species.

Although no diagnostic markers were observed, we found significant band frequency differences (for 13 AFLP markers) between the two species. The same has been reported for allozymes (ZANETTO *et al.* 1994; BACILIERI *et al.* 1995), two dimensional gel electrophoresis of proteins (BARRENECHE *et al.* 1996), and RAPD (MOREAU *et al.* 1994; BODÉNÈS *et al.* 1997a). Like in our study, these studies excluded intermediate types. However, it is possible that the species definitions varied between these studies. Therefore, it is not possible to compare the effectiveness of AFLP to discriminate between the two species in our study with the effectiveness of other molecular marker techniques in previous studies. Although no diagnostic markers could be found, we were able to find five significant species-indicative markers. The band frequencies for these markers were high and ranged between 0.35 and 0.53. Previous studies based on other molecular markers did not detect significant species-indicative markers for *Q. robur* and *Q. petraea*. A study of RAPD conducted by BODÉNÈS et al. (1997 a), however, resulted in the same conclusions as our AFLP study. Therefore, we cannot conclude that AFLP is so far the best molecular marker method for discrimination between *Q. robur* and *Q. petraea*. Still, the band frequencies of the species-indicative markers are probably not high enough to be used for hybrid studies like the study of a hybrid zone between *Q. grisea* and *Q. gambelii* by HOWARD *et al.* (1997) where the RAPD band frequencies for species-indicative markers were all above 0.61. Moreover, hybrids can best be studied with diagnostic markers. This was done by O'HANLON *et al.* (1999) where diagnostic AFLP markers were used to investigate hybrids between *Onopordum* species.

Associations between leaf morphology variation and AFLP polymorphisms

By means of regression analyses significant associations between leaf morphology traits and AFLP markers were found for both species. For all analyzed leaf morphology traits different AFLP markers were found to be associated for *Q. robur* as compared to *Q. petraea*. This observation could be explained by the fact that for each species different markers had been excluded from the analysis. However, as these markers were rare it is not expected that this can explain our findings. It is more likely that because of the limited sample size only part of the existing associations between AFLP markers and leaf morphology traits could be detected. Other explanations are marker band frequency differences, differences in leaf morphology traits, and differences in variation of these leaf morphology traits between the two species. The percentage of explained variation varied between the leaf morphology traits and between the two species. The percentage of explained variation reached levels as high as 59.5 % for PV in *Q. petraea*. Differences in levels of explained variation can be pointing to polygenically and monogenically coded traits, respectively. Especially for the leaf morphology traits with lower levels of explained variation it is necessary to study the genetic loci involved in the inheritance of leaf morphology in order to get better models. The regression analyses presented here give a preliminary indication about linkage between leaf morphology traits and AFLP markers. However, a genetic linkage map will reveal the true linkage and the location of the traits on the genome.

In this study it was observed that species could be as well differentiated based on leaf morphology as based on AFLP. However, a clearer separation of species was obtained based on leaf morphology traits. Other studies give the opposite result: AFLP could distinguish between *Eragrostis pilosa* accessions better than morphological traits, that varied too much due to environmental fluctuations (AYELE *et al.* 1999). MACE *et al.* (1999) showed that AFLP could distinguish between taxa of the tribe Datureae more clearly than isozyme, morphology, and ITS-1 data. As *Q. robur* and *Q. petraea* are usually recognized based on leaf morphology characteristics and not based on AFLP polymorphisms, it was to be expected that therefore the groups would be better differentiated based on leaf morphology than based on

AFLP.

Our study shows that average genetic and phenotypic distances within and between species were consistent. However, for some individuals larger genetic distances might be observed as compared to phenotypic distances. This is the result of the fact that most phenotypic traits are polygenic. Polygenic traits are characterized by no clear correlation between genetic and phenotypic distances. This effect will increase when the population consists of unrelated individuals (BURSTIN & CHARCOSSET 1997).

The fact that so far no diagnostic marker has been found indicates the high relatedness between the two species. Previous studies of controlled crosses and paternity analysis have already shown that to a certain extent the species can exchange their genetic material (STEINHOFF 1993; STREIFF *et al.* 1999). Evidence about speciation is hard to obtain and therefore it is not known if the high relatedness between the two species can also be pointing to the fact that speciation is still going on. Although this study does not intend to be a taxonomic study, we have indications that *Q. robur* and *Q. petraea* are probably not pure species and therefore can better be called morphotypes (BAVERSTOCK & MORITZ 1996) or semispecies (GRANT 1971). This point of view on *Q. robur* and *Q. petraea* is shared and discussed by KLEINSCHMIT & KLEINSCHMIT (2000). A greater differentiation between the two species based on leaf morphology variation than based on AFLP polymorphisms can now be explained according to plant speciation theories: due to hybridization the two species can exchange their genetic material, but certain combinations of leaf morphology characteristics are being maintained as these combinations of characteristics are under a continuous selection pressure for adaptation to the niche of the semispecies (GRANT 1971). Certain AFLP markers are linked with these leaf morphology characteristics and have therefore been found to be associated with these characteristics in regression studies. From this study it becomes clear that in a Dutch autochthonous mixed *Q. robur* and *Q. petraea* population these two species behave as sympatric morphotypes or semispecies. For conservation purposes it is recommendable to conserve mixed *Q. robur* and *Q. petraea* populations as two sympatric morphotypes or semispecies cover more variation than only one of the two. Moreover, still only little is known about ecological processes within and between both morphotypes or semispecies. In order to enable future adaptations to changing environments it will be necessary to conserve the complete syngameon.

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