

Species status, hybridisation and geographic distribution of Irish populations of *Quercus petraea* (Matt.) Liebl. and *Q. robur* L.

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ABSTRACT

The species status of *Quercus petraea* and *Q. robur* in Ireland was investigated through morphological analysis of leaves. A number of different methods were used for the analysis. The consensus findings were that both species were readily distinguished in Ireland, although there are putative hybrids and introgressed individuals. The overall variability in leaf morphology was considerable and the percentage of distinct species based on a hybrid index was only 30% (15% *Q. petraea* and 15% *Q. robur*). The level of hybridisation was estimated at 10%, which is comparable to that reported in other studies in Europe. *Quercus robur* was found to be more distinct than *Q. petraea* as intermediate morphological forms grouped closer to *Q. petraea* in a Neighbour-Joining cluster analysis. *Quercus petraea* individuals were found mainly in peripheral mountainous regions and *Q. robur* individuals mainly in central lowland regions and these distributions roughly corresponded to typical ecological habitats for either species.

INTRODUCTION

OAK TAXONOMY

'Look at the common oak, how closely it has been studied; yet a German author makes more than a dozen species out of forms, which are very generally considered as varieties; and in this country the highest botanical authorities and practical men can be quoted to show that the sessile and pedunculated oaks are either good and distinct species or mere varieties.' (Darwin 1859)

This quote from Darwin's *'The Origin of Species'* (1859) illustrates the difficulty in agreeing on an infrageneric classification of *Quercus* (the oaks) and in particular for the species *Quercus petraea* (Matt.) Liebl. and *Q. robur* L. Although the quote may refer to tendencies in national outlook it remains true in a more fundamental and biologically meaningful way. The confusion in the identification of *Quercus* species and varieties still persists and Stace (1975) has suggested species delimitation remains to some extent a matter of opinion. A variety of identification and classification systems have been used in studies on oak, from the more orthodox systems based on morphology to less orthodox systems based on chemical characteristics (Brookes & Wigston 1979) and even classification based on biological aspects such as host specificity in insects

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(Abrahamson *et al.* 1998). There are also suggestions for alternative species concepts in oak such as multispecies and ecological species (Van Valen 1976) and in general the biological species concept has encountered some difficulty in its application to *Quercus* (Burger 1975).

Despite these difficulties in taxonomy, the largely sympatric species *Quercus petraea* and *Q. robur* (Jalas & Suominen 1976) have retained their species status because differences in morphological attributes and ecological 'preferences' are considered to be sufficiently differentiating. The main morphological differences between the species, summarised in Table 1, were compiled from a number of different sources (Aas 1993; Aas 1995; Brookes & Wigston 1979; Carlisle & Brown 1965; Cousens 1963; Jones 1959; Kelly 1996; Rushton 1976; Stace 1991; Tutin *et al.* 1964; Webb *et al.* 1996). Most of these differences are comparative rather than absolute, for example, Rushton (1976) found a significant difference in the size of pollen grains from the two species, but this was not sufficiently distinct to be useful for palynological study. The only species-diagnostic leaf character recognised is that of stellate hairs on the under-surface of the leaf in *Q. petraea* (Aas 1995). All other characters are a matter of degree. One reason for the difficulty in delimiting the species is the level of hybridisation between the species (Anderson 1949; Briggs & Walters 1997; Rushton 1993; Stace 1975; Steinhoff 1993). The dynamics of gene exchange through hybridisation can potentially hinder designating species; these must be thought of in terms of a continual evolutionary process rather than a static endpoint. Most studies of species or populations are 'snap-shots' in time.

TABLE 1. THE MAIN MORPHOLOGICAL DIFFERENCES THAT DISTINGUISH THE SPECIES *QUERCUS PETRAEA* AND *Q. ROBUR* FROM EACH OTHER

Character	<i>Q. petraea</i>	<i>Q. robur</i>
Infructescence		
Peduncle length	Short or absent (<1 cm)	Long (>1 cm)
Acorn stripe	Not striped	Striped
Peduncle pubescence	Clustered hairs	Absent
Acorn form	Less Elongated	More Elongated
Leaves		
Lamina length	Larger (approx. >10 cm)	Smaller (approx. <10 cm)
Petiole length	Long (>10 mm)	Short (<10 mm)
Auricles at leaf base	Absent	Well developed
Stellate hairs	Plentiful	Absent
Leaf shape	Ovate – broadest near middle	Obovate – broadest above middle
Veins to sinus	Absent	Present
Lobe pairs	≥6	<6
Lobe depth	Shallow	Deep
Pollen		
Pollen size	Larger	Smaller

STUDIES ON LEAF MORPHOLOGY IN OAK

One of the most striking points arising from a review of the literature on morphological studies of oak is that few authors use exactly the same characters or methods in their analyses. Studies on oak morphology have varied from studies on the taxonomic status and levels of hybridisation in individuals to studies on stands of oak and the associated ecology (Aas 1993; Aas 1995; Carlisle & Brown 1965; Cousens 1963; Cousens 1965; Kelly 1996; Kleinschmit *et al.* 1995; Minihan & Rushton 1984; Rushton 1978; Rushton 1993). A number of studies in Ireland have focused on the taxonomic integrity of the species (Cousens 1965; Kelly 1996; Kelly & Moore 1975; Minihan & Rushton 1984). Others have focused on the distribution and ecology of oak woodland types (Kelly 1981; Kelly 1996; Kelly & Fuller 1988; Kelly & Kirby 1982; Kelly & Moore 1975). Cousens (1965) provided an assessment of the status of the species in Britain and Ireland. He sampled from 12 Irish woodlands; almost half of these were confined to the eastern counties of Dublin and

Wicklow. Cousens' analysis used the concept of a Theoretical Species Type to investigate the levels of introgression and the species status. *Quercus petraea* from Ireland was shown to form, in the author's words, a 'good' species (morphologically distinct from *Q. robur*), and introgression was considered to be less extensive than that in Scottish samples (Cousens 1965). Other studies have shown that *Q. petraea* and *Q. robur* form distinct species in Ireland whilst confirming the presence of some hybrids (Carlisle & Brown 1965; Minihan & Rushton 1984; Rushton 1978; Rushton 1979; Rushton 1993). Rushton, using multivariate analysis, found the level of hybridisation in oakwoods in Northern Ireland to be 13% (Rushton 1993).

Although a number of studies on leaf morphology of oak have been completed in Ireland an adequate description of the taxonomy and distribution of the taxa is lacking. This paper aims to address this shortfall and present a firm base to our knowledge of Irish oak across its full geographical and ecological range. The Irish data are also compared to more recent studies from elsewhere in Europe. The data were analysed to investigate if distinct groups of individuals corresponding to the two species could be identified. This could indicate how distinct the species are in Ireland and what relationships, if any, there are between populations of the same species. To do this ordination, clustering analysis and hybrid index techniques were employed.

METHODS

SAMPLING

Sites were sampled from 25 locations across Ireland (Fig. 1). Sites were chosen primarily as putative ancient woodland sites. Some sites have already been recognised as ancient woodland using historic records, floristics and an assessment of woodland structure (Bohan 1997; Kelly & Fuller 1988; Rackham 1995). Where evidence was lacking, sites were chosen for their remoteness, thus lessening the possibility of in-planting from external stock.

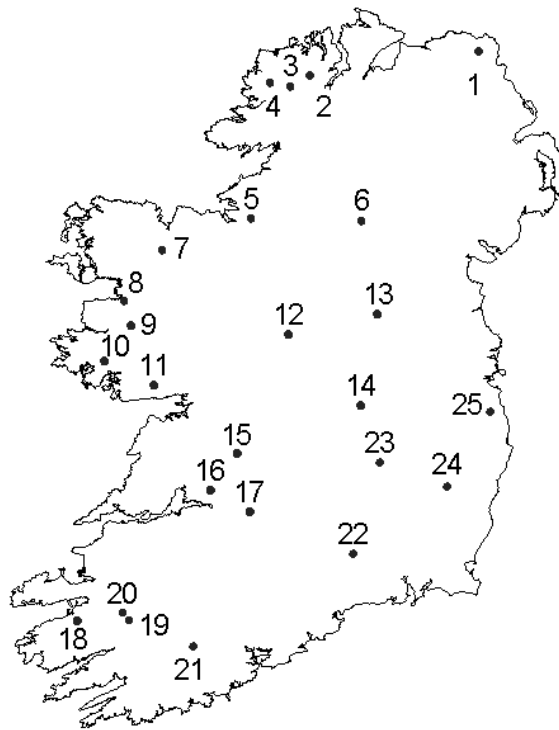


FIGURE 1. Map of sites sampled and their geographic locations. See Table 2 for site details.

TABLE 2. A LIST OF THE SITES SAMPLED, THE CODE USED FOR EACH POPULATION AND THE NUMBER OF INDIVIDUALS SAMPLED PER POPULATION (SEE FIG. 1 FOR LOCATIONS).

Number	Site (Code, Sample Size)	Number	Site (Code, Sample size)
1	Breen (N, $n = 13$)	14	Charleville (CH, $n = 10$)
2	Glenveagh (GN, $n = 10$)	15	Brian Boru Oak (BB, $n = 1$)
3	Devlin River (P, $n = 21$)	16	Garannon Wood (CT, $n = 22$)
4	Crolly (CY, $n = 6$)	17	Cappercullen Glen (GL, $n = 15$)
5	Cullentra (S, $n = 11$)	18	Glencar (GC, $n = 7$)
6	Crom (CR, $n = 13$)	19	Royal Oaks (R, $n = 7$)
7	Pontoon (M, $n = 10$)	20	Glaisín na marbh (K, $n = 19$)
8	Brackloon (B, $n = 9$)	21	The Gearagh (GH, $n = 21$)
9	Eriff (E, $n = 5$)	22	Curraghmore (W, $n = 10$)
10	Derryclare (D, $n = 10$)	23	Abbey Leix (A, $n = 33$)
11	Shannawoneen (SH, $n = 11$)	24	Coolattin (C, $n = 11$)
12	St. John's Wood (J and JR, $n = 11$)	25	Glen of the Downs (G, $n = 11$)
13	Tullyally (T, $n = 12$)		

Leaf samples were collected from open grown canopy branches to minimise variation in leaf morphology due to environmental factors such as exposure and aspect (Baranski 1975; Blue & Jensen 1988). The canopy was sampled either using a long-handle pruner or by climbing to reach the outer branches. Five well-developed leaves per tree were collected and measured. These leaves were representative samples of each tree. Fruiting shoots were also collected where available. Leaf samples were labelled and pressed.

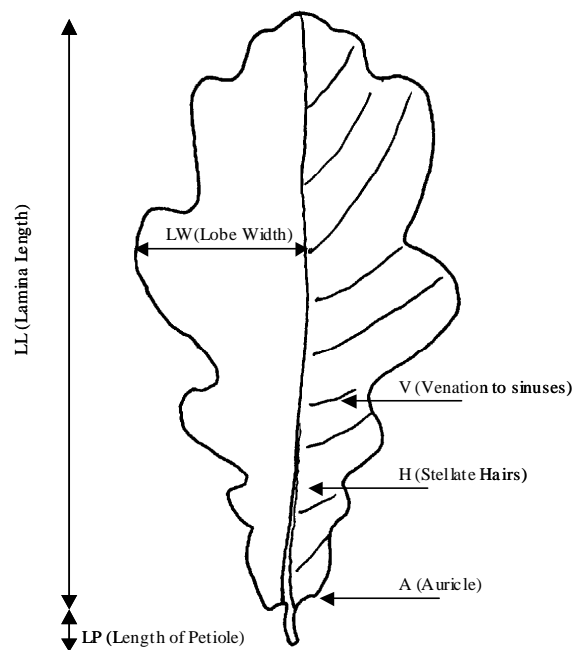


FIGURE 2. A schematic diagram of an oak leaf with illustrations of measurements and observations used in the analysis (see Table 3 also).

CHARACTER MEASUREMENTS

The analysis was limited to leaf morphology, due to the lack of consistent availability of acorn-bearing shoots. The measurements taken and the derived variables used in the analyses are given in Table 3 and represented in Fig. 2. All measurements were assessed by eye and ruler with the exception that a hand lens with a magnification of $\times 10$ was used for viewing the stellate hairs. Although some authors have expressed concern over the use of ratios in multivariate analysis (Pimentel 1979), this did not affect our analysis significantly (data not shown) and we chose to use all measurements to allow direct comparison with other studies that applied the same methodology.

TABLE 3. THE MEASUREMENTS AND CALCULATED RATIOS USED FOR THE MORPHOLOGICAL ANALYSIS OF THE LEAVES (CF. FIG. 2). LEAF MORPHOLOGY WAS ANALYSED USING CLUSTERING, ORDINATION AND HYBRID INDEX

Measurements	
1.	LL (Lamina Length). The length of the leaf lamina from the petiole to the tip of the leaf.
2.	LW (Lobe Width). The width of the lobe at the widest point of the leaf.
3.	SWa (Sinus Width above). The width of the sinus above the widest part of the leaf.
4.	SWb (Sinus Width below). The width of the sinus below the widest part of the leaf.
5.	DW (Distance to Widest part). The distance from the beginning of the lamina to widest part of the leaf.
6.	LP (Length of Petiole).
7.	Lob (Number of Lobe pairs).
8.	A (Auricles). Auricle development on a scale of 0 to 5, 0 being non-existent, 5 being very well developed with curled leaf margins.
9.	H (Stellate hairs). Stellate hairs on the under surface of the leaves. On a scale of 0 to 5, 0 being none, 5 being present in profusion and clearly visible without a lens.
10.	V (Venation to sinuses). Number of veins going to the sinuses.
Ratios	
1.	LP/LL. Ratio of petiole length to lamina length.
2.	DW/LL. Ratio of distance to widest point and lamina length – describes the obversity of the leaf.
3.	LW/LL. Ratio of lamina width to length.
4.	SWb/LW. Ratio of lobe width to sinus width – describes the level of dissection of the leaf.
5.	SWb/SWa. Ratio of Sinus width below and above the widest point. Gives a measure of leaf shape uniformity.
6.	V/Lob. Ratio between number of veins to sinuses and number of lobe pairs.

DATA HANDLING AND ANALYSIS

Principal Coordinate Analysis

The ordination technique Principal Coordinate Analysis (PCoA) (Digby & Kempton 1987; Gower 1966; Legendre & Legendre 1998) was used. Characters were standardised by z-scores to avoid bias in the analysis due to scale differences in the measured variables (Legendre & Legendre 1998; Sneath & Sokal 1973), i.e. all values were divided by the highest record to obtain a range from 0 to 1. A distance matrix was obtained using Euclidean distance in the R Package version 4.0 d1 Development Release (Casgrain *et al.* 1999). The R Package was also used for the PCoA.

Ordination methods are suitable to illustrate continuous variation but are not always statistically appropriate for assigning samples to discrete groups (Legendre & Legendre 1998), as is necessary for determining species identity. Thus, a number of methods were used and assessed for assigning individuals to a morphological grouping. A clustering analysis method and an hybrid index method (Anderson 1949) were selected.

Cluster Analysis

The form of cluster analysis used was the 'Neighbour-Joining' (NJ) method (Saitou & Nei 1987) and the distance measure was Euclidean. NJ analysis allows an objective means of clustering individuals into morphological groups and provides for classification of individuals that lie within known clusters.

TABLE 4. CHARACTERS USED IN THE DETERMINATION OF THE HYBRID INDEX AND THE SCORE ASSIGNED. SCORES OF 0 FOR *QUERCUS ROBUR* AND 1 FOR *Q. PETRAEA* WERE GIVEN TO ALL CHARACTERS EXCEPT THAT OF STELLATE HAIRS WHICH WAS DOUBLE WEIGHTED AS 0 FOR *Q. ROBUR* AND 2 FOR *Q. PETRAEA*.

Character	<i>Q. petraea</i>	<i>Q. robur</i>
Stellate hairs	Present	Absent
Auricles	≤0.9 (absent or not well developed)	>0.9 (well developed)
Lobe pairs	≥6	<6
Venation tosinuses	<1	≥1
LP/LL	≥0.1	<0.1

Hybrid Index

The hybrid index has been used in previous studies on oak to determine species identity and degree of hybridisation (Carlisle & Brown 1965; Dupouey & Badeau 1993; Kelly 1996). The hybrid index involves designating character states to one or other species and scoring them accordingly, with or without weighting of characters (Anderson 1949). Adding these scores gives a measure of the individual, ranging from extreme *Q. robur* to extreme *Q. petraea* on a scale of 0 to 6. A number of characters were chosen for the assessment of the hybrid index on the basis of their diagnostic utility and their ability to designate the species states objectively. All characters were equally weighted except stellate hairs; this character was double weighted (Table 4) as it has been shown to be species diagnostic (Aas 1995). A hybrid index was calculated for each leaf and then averaged for each individual tree.

The methodology of the hybrid index has been questioned because of the subjective nature of trait designation and thus species assignment (Dupouey & Badeau 1993). This was therefore compared with other more objective methods, such as cluster analysis.

Discriminant Analysis

The utility of NJ clustering and the hybrid index for identifying meaningful groups was assessed using 'Discriminant Analysis' in SPSS for Windows, release 10.0.5. For discriminant analysis raw data are input and groups are defined *a priori*. Discriminant analysis proceeds by first testing for differences in the variables among the predefined groups; next, if the test supports these differences, the analysis proceeds to find linear combinations (discriminant functions) of the variables that best discriminate the groups (Legendre & Legendre 1998). The discriminant analysis used was the Wilks' lambda stepwise method. This method adds traits stepwise to the analysis on the basis of the Wilks' lambda test (at each step the variable that minimizes the overall Wilks' lambda is entered). Thus it can be used to identify those traits most important in discriminating the groups. The SPSS output gives the percentage of cases that match the predefined groupings and from this the utility of these groupings can be assessed.

RESULTS

Samples were analysed from 309 trees (1545 leaves) from 25 sites. The number of samples at each site ranged from 7 to 33, except for one individual, the Brian Boru oak in Co. Clare (Table 2). Collecting was more intensive at some sites rather than others to obtain a sufficiently representative sample in more complex woodlands, i.e. woodlands with more than one species. In addition, these populations were also used for molecular genetic studies and so the sample sizes reflect simultaneous sampling for a variety of analyses.

SPECIES SEPARATION AND HYBRIDS

Neighbour-Joining Cluster Analysis

The NJ tree was rooted at the mid-point to split the data objectively at its maximum divergence. This resulted in the clustering of two groups of individuals that could be morphologically distinguished as *Q. robur* and *Q. petraea* (Fig. 3), as each group of individuals showed the leaf characteristics of the respective species (Table 1). The *Q. petraea* grouping was taken as the most

TABLE 5. THE NUMBERS AND PERCENTAGES OF THE DIFFERENT MORPHOLOGICAL TYPES ACCORDING TO THE NJ CLUSTER ANALYSIS

Morphological Group	Number of Individuals	Percentage of Total
<i>Q. robur</i>	83	27%
<i>Q. petraea</i>	195	63%
Others	31	10%

PCoA with Neighbour-joining

Principal Coordinate Analysis revealed a good separation of the individuals into groups corresponding to the two species (Fig. 4). The NJ designations are overlaid on the PCoA scatter plot (Fig. 4). Both analyses show concordance with respect to the species split, but are less concordant when the intermediate individuals are compared.

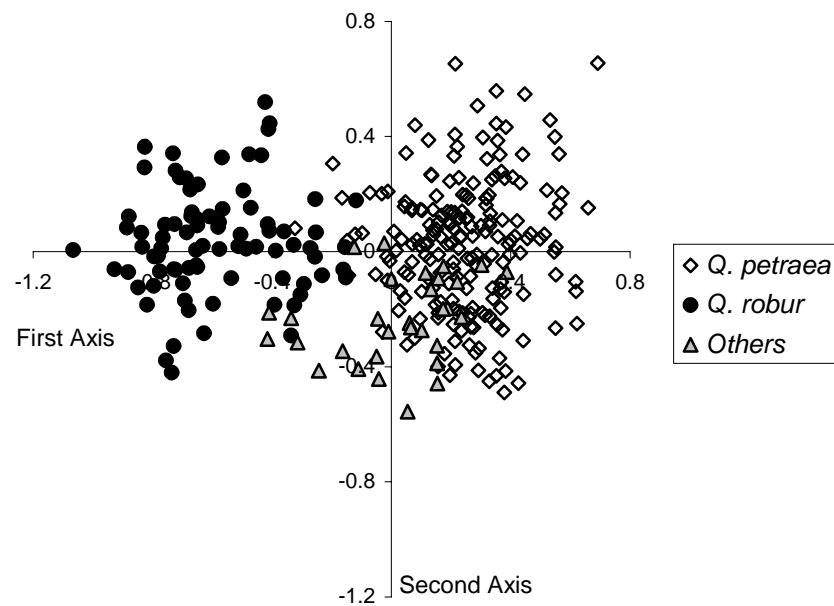


FIGURE 4. Scatter plot of the individuals on the first two principal axes of a PCoA. The first axis accounts for 48% and the second axis 15% of the variation. The overlays are the species designations according to the Neighbour-joining cluster analysis.

Hybrid Index

The hybrid index results show the diversity of the morphological groups and indicate a continuum of variation from *Q. robur* to *Q. petraea* rather than sharp divisions (Fig. 5).

A scatter plot of the first two principal coordinates with an overlay of the hybrid index for each sample shows the relationship between the PCoA and the hybrid index results (Fig. 6).

A comparison of the species designations plotted on the PCoA scatter plots (Figs 4 and 6) reveals similar salient patterns:

- There is a separation of many individuals into groups that can be recognised as *Quercus robur* and *Q. petraea*.
- There are ‘intermediate’ individuals that fall between the species groups.
- Fully intermediate individuals are relatively uncommon.

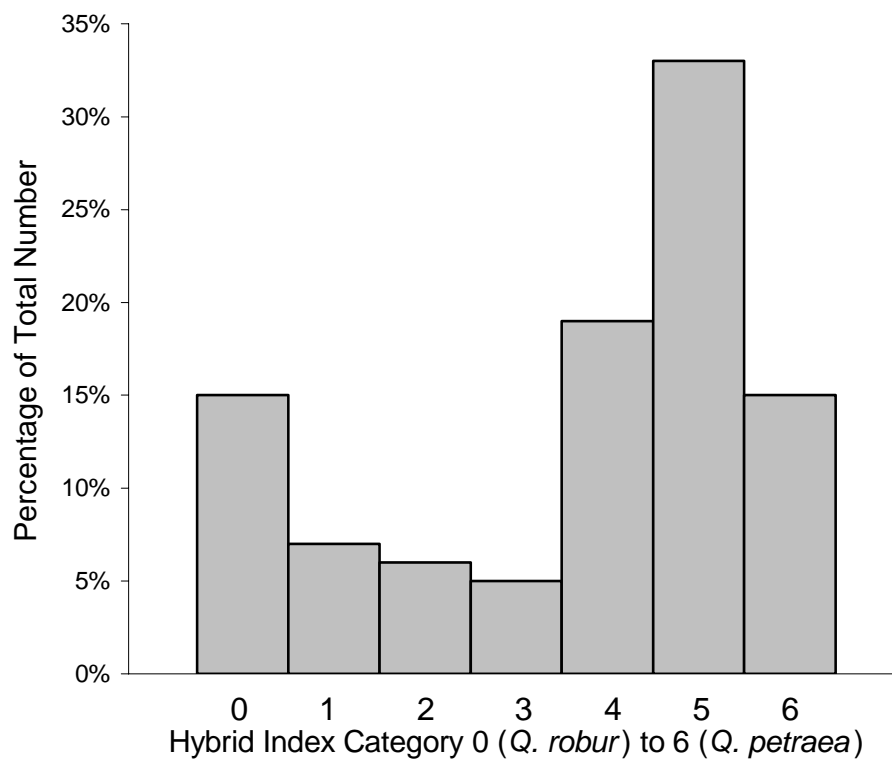


FIGURE 5. A bar chart illustrating the percentage of the total number of individuals in the different hybrid index categories. The hybrid index ranges from 0 (*Quercus robur*) to 6 (*Q. petraea*).

Discriminant Analysis

The two methods used for morphological analysis (cluster analysis and the hybrid index) were assessed for their utility in distinguishing morphological groupings. Discriminant analysis was undertaken using, first, the cluster groupings as predefined groups and then the hybrid index groupings as predefined groups. The results from the Discriminant analysis revealed the most important discriminating variables to be those listed in Table 6. From this table it appears that the groupings obtained from the NJ method are more significant than those from the hybrid index method (93.5% were correctly classified by the NJ analysis as opposed to 63.4% by the hybrid index method). The most diagnostic characters from the discriminant analysis varied according to the method used (Table 6). For the NJ analysis characters describing leaf shape, petiole length and stellate hairs were most important (LW/LL, LP/LL, DW and H – see Table 3) as they had low Wilks’ lambda values. For the Hybrid Index analysis characters for leaf shape were not as important, but length of petiole and stellate hairs were the most discriminating (LP, H).

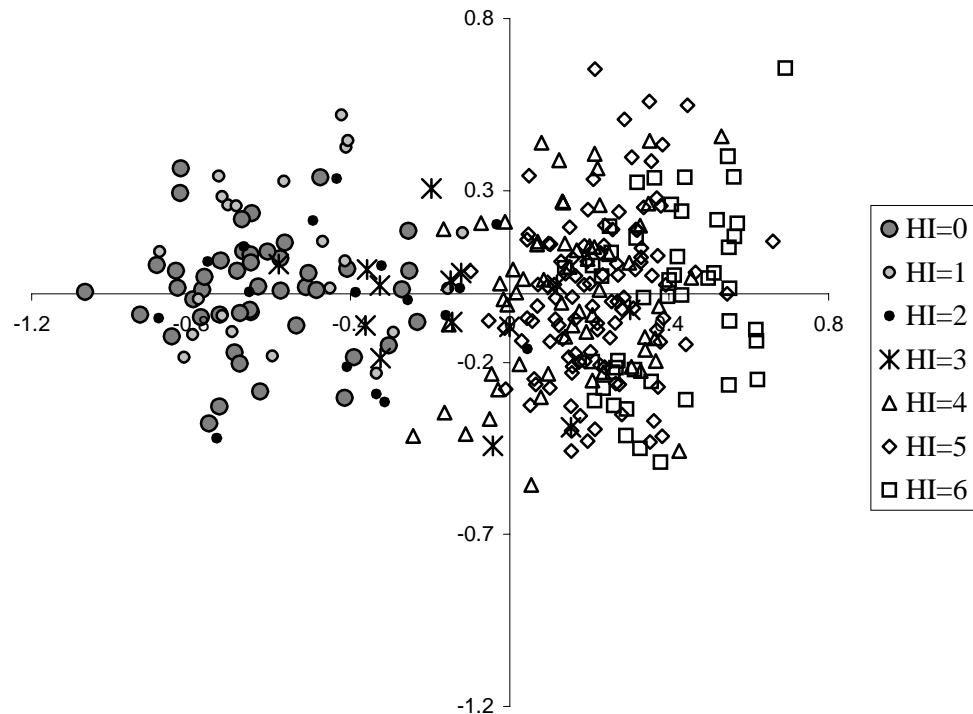


FIGURE 6. Scatter plot of the individuals on the first two principal axes of a PCoA. The first axis accounts for 48% and the second axis 15% of the variation. The overlays are the designations according to the hybrid index (HI), from 0 = *Quercus robur* to 6 = *Q. petraea*.

SPECIES DISTRIBUTION AND POPULATION DIFFERENTIATION

The geographic distribution of the morphological types as determined by the NJ analysis is presented in Fig. 7. Most populations contain a mixture of morphological groups and very few have just one species. A total of 6 populations were composed exclusively of *Q. petraea* individuals and only 1 population was composed exclusively of *Q. robur* individuals. The remaining populations (17) were composed of a mix of each species and intermediates (Fig. 7).

The individuals from each population are scattered in the analyses and do not cluster together into their respective population groupings (Fig. 3). A PCoA plot with an overlay of the populations (not shown) revealed that the populations do not form tight clusters but are dispersed within each species across the first two axes.

DISCUSSION

SPECIES IDENTITY AND SEPARATION IN IRISH OAK

Species Status

It is evident from the results that there is a morphological separation of individuals into groups recognisable as the two Irish *Quercus* species. All the techniques used showed groupings of individuals into the species *Q. petraea* and *Q. robur* and a continuum of intermediate individuals extending between the extremes (Figs 3, 4 and 6). The hybrid index revealed only 30% of the individuals with either extreme *Q. petraea* or extreme *Q. robur* characters, the remaining 70% showed different levels of intermediacy. This shows the extensive variability in leaf morphology

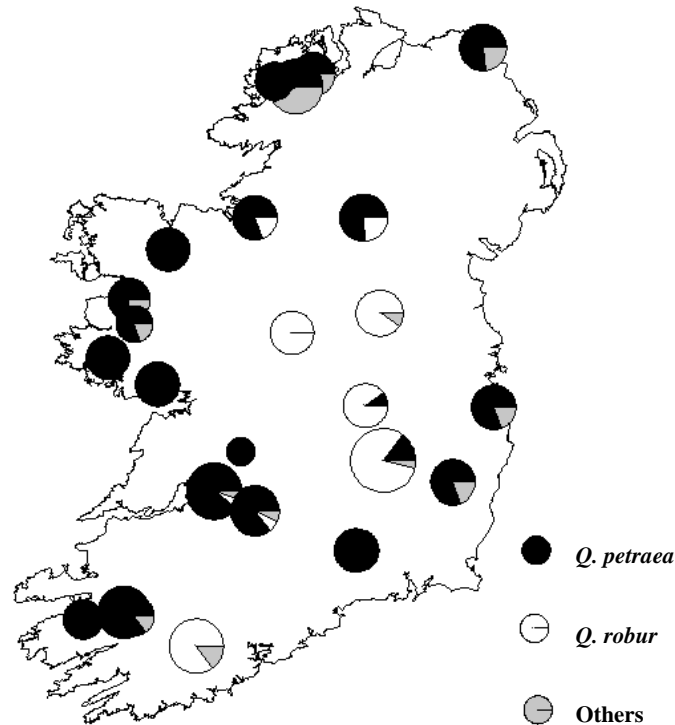


FIGURE 7. The proportions of the different morphological groups in each population sampled. Morphological groupings were designated according to the NJ analysis. Pie charts are sized according to the number of samples at each location, ranging from 1 to 33 samples. The size in the legend is equivalent to one individual.

of oak in Ireland. *Quercus petraea*-type individuals are the dominant morphological group in Ireland (Table 5). While the dominance of *Q. petraea* reflects the particular populations sampled, it is a reasonable reflection of the relative proportions of the species in Ireland as a whole. The NJ cluster analysis showed greater utility than the hybrid index in identifying morphological groups (Table 6). The discriminant analysis for the NJ analysis showed that characters for leaf shape are most important in discriminating the species. While the only species-diagnostic character identified is that of stellate hairs by Aas (1995), this was not found to be the most important discriminating character in either analysis (NJ or hybrid index).

TABLE 6. THE PERCENTAGE OF SAMPLES DETERMINED CORRECTLY IN THE *A PRIORI* CLASSIFICATION FOR EACH GROUPING METHOD

For NJ Groupings 93.5% correctly classified		For Hybrid Index Groupings 63.4% correctly classified	
Character	Wilks' lambda	Character	Wilks' lambda
V/Lob	0.397	V/Lob	0.342
A (Auricles)	0.279	V (Venation)	0.227
H (Stellate Hairs)	0.209	A (Auricles)	0.165
DW	0.176	H (Stellate Hairs)	0.123
LP/LL	0.153	LP	0.101
LW/LL	0.147		

Gives the variables used in the discriminant analysis with the Wilks' lambda statistic at each step.

TABLE 7. LEVELS OF PUTATIVE HYBRIDISATION DETERMINED FROM STUDIES IN CONTINENTAL EUROPE AND IRELAND

Region	% Hybridisation	Reference
Ireland	10	This study (Table 5)
Ireland (Northern Ireland)	13	(Rushton 1993)
France	3.5	(Dupouey & Badeau 1993)
Germany	11	(Elsner 1993)

Hybridisation and Introgression

The level of hybridisation (10%) determined in this study is comparable to that obtained in other studies in Europe (Table 7). The results from Northern Ireland and Germany agree closely with those determined in this study, but the French values are substantially lower. Dupouey & Badeau (1993) consider the level of hybridisation to be overestimated in general; however, most of the evidence suggests that hybridisation is considerable, under both natural (Bacilieri *et al.* 1996b; Rushton 1993) and artificial conditions (Kleinschmit & Kleinschmit 1996; Rushton 1977). Aas (1993) adds to the debate by arguing that the level of hybridisation will often be over-estimated because of the level of variability within the species. However, this can be a circular argument as hybridisation is likely to add to the variability observed.

The level of introgression is more difficult to determine as the probability of co-occurrence of characters becomes more uncertain with successive events of segregation and recombination in sexual reproduction. Hybrids and the resulting introgressed individuals are not mixtures of equal proportions of their parents' characteristics (Anderson 1949). They are more a mosaic of attributes of the parents (Anderson 1949; Rieseberg 1995; Wilson 1992) and can also contain characters not evident in either parent (Rieseberg 1995). The F_1 (interspecies) hybrid generation has been shown to be relatively stable and more predictable in many species (Anderson 1949; Rieseberg *et al.* 1995) compared to successive crosses. The F_2 (F_1 with F_1) generation or an F_1 individual backcrossed with either parent has less likelihood of being similar to the original parent due to further recombination events. Rather than a progressive cline of intermediates, a mosaic pattern can develop. Wilson (1992) considers that hybridisation cannot be inferred accurately from multivariate morphological intermediacy. A mosaic is evident in the analyses presented (Figs 4 and 6), since 'intermediate' individuals can be found within the grouping of either species, in particular *Q. petraea*. While there are truly intermediate individuals there are also individuals that contain a preponderance of the characters of one species but with some characters intermediate or even typical of the other species. These individuals are most likely introgressed hybrids. From the hybrid index data (Fig. 5), the numbers of individuals assigned to distinct species are low (30%). This suggests a large variability within each group, and/or a considerable amount of gene exchange between the groups.

The intermediate individuals group closer to *Q. petraea* in the NJ tree (Fig. 3). This tree was rooted at the mid-point of divergence, and suggests an asymmetry in the morphological distribution. *Quercus robur* is more clearly differentiated than *Q. petraea*; the putative hybrids and introgressed individuals group closer to *Q. petraea* than to *Q. robur*. Asymmetric hybridisation and gene exchange has been observed in other studies (Bacilieri *et al.* 1996a; Bacilieri *et al.* 1996b; Bacilieri *et al.* 1993). The fertilisation and reproductive success of inter-specific crosses has been shown to be asymmetric with *Q. robur* accepting pollen from *Q. petraea* more readily than vice-versa, and *Q. petraea* more readily accepting pollen from individuals with extreme *Q. petraea* morphology (Bacilieri *et al.* 1996b; Bacilieri *et al.* 1996c; Bacilieri *et al.* 1993; Rushton 1977). This results in the preferential pollen gene flow from *Q. petraea* to *Q. robur*. This could (if traits are inherited equally from either parental species) result in the hybrids and introgressed individuals grouping closer to *Q. robur*. However, this is not the situation in the NJ analysis (Fig. 3) in which the hybrids and introgressed individuals group closer to the *Q. petraea* grouping. This could be the result of human interference due to planting efforts in the *Q. robur* woodlands. The possibility of in-planting of *Q. robur* in some of the populations sampled (in particular in old estates) may bias the outcome of the results. If many of the *Q. robur* individuals were planted then the population dynamics will be disrupted and the gene and thus trait ratios will be skewed.

Evidence to test this hypothesis is hard to obtain because of the scarcity of records concerning planting in Ireland prior to the 19th century.

While we have shown that leaf morphological analyses reveal a distinction between the respective species, both in Ireland and elsewhere in Europe, this is not so evident when using molecular markers. Only a few studies have shown a distinction between the species in European populations based on molecular analyses (Coart *et al.* 2002; Muir *et al.* 2000). Our work has been extended to utilise a variety of molecular markers (Kelleher 2001). Thus far, using chloroplast DNA markers, we have found no species distinction in Irish populations (Kelleher *et al.* 2004) and this reflects findings elsewhere in Europe (Petit *et al.* 2002). Further analyses will help to elucidate the differences between the morphological and molecular differentiation in Irish oak.

GEOGRAPHIC DISTRIBUTION OF THE SPECIES

The geographic distribution of *Q. petraea* and *Q. robur* (Fig. 7) in Ireland confirms the pattern outlined by Kelly (1996). The topography of Ireland is described as being similar to a saucer, with lowland regions mainly in the centre and more mountainous regions mainly on the periphery. The midland regions sampled are dominated by Carboniferous limestone substrata and the peripheral regions by siliceous rock (R.I.A. 1979). *Quercus petraea* individuals were found mainly in peripheral regions and *Q. robur* individuals mainly in central lowland regions (Fig. 7). This reflects the ecological preference of each species, *Q. petraea* being more common on upland acidic soils and *Q. robur* on lowland, more lime rich soils (Jones 1959; Kelly 1996).

POPULATION STATUS

Population structure or substructure could not be determined by the leaf morphology due to the extensive variability observed (Fig. 3). While a reasonable separation of the species can be achieved by PCoA and NJ analysis, variability between individuals is too high to identify population groupings or ecotypes if they do exist.

The proportion of single-species populations in Ireland appears to be low. Out of 24 populations, 17 (71%) contain some mixture of morphological groups, while only seven populations are composed of a single species. The distribution of single species and mixed populations does not show any obvious trend (Fig. 7). Some of these mixed woods may be attributed to human influence through planting efforts, but others probably reflect the natural situation.

CONCLUSIONS

Individuals of *Q. petraea* and *Q. robur* in Ireland can be separated by morphological analysis of the leaves. However, there are putative hybrids and introgressed individuals. The level of hybridisation (10%) is comparable to that found in other studies in Europe. The different techniques used did not agree on details but the overall trend was the same. The overall morphological variability was high and the percentage of individuals allocated to distinct species based on a hybrid index was only 30% (15% *Q. petraea* and 15% *Q. robur*). *Quercus robur* was found to be more morphologically distinct than *Q. petraea* as intermediate morphological forms grouped closer to *Q. petraea* in a NJ cluster analysis. *Quercus petraea* individuals were found mainly in peripheral geographic regions and *Q. robur* individuals in more central lowland regions of Ireland. These distributions roughly corresponded to ecological preferences. Most populations contained individuals from more than one morphological group, indicating the complex and dynamic situation in Irish oak populations.

ACKNOWLEDGMENTS

This project was funded by COFORD, the National Council for Forest Research and Development. We thank Dúchas and Coillte for access to sites and helpful information; Mr David Davies, Mr Peter Fegan and Mr Edward Thornton of Abbey Leix Estate; Mr David Brickenden of Cratloe, Co. Clare; Mr William McLysaght of Tuamgraney, Co. Clare; Brother Anthony and the Abbot and Benedictine Community of Glenstal Abbey, Co. Limerick; and the Marquess of Waterford, Portlaw, Co. Waterford. We also thank Mr Thomas Pakenham for his time and assistance in Tullynally Estate, Mr Ralph Shepherd for advice on sites in Donegal and the Rangers of Glenveagh National Park for their help and hospitality.

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(Accepted June 2003)

