- *It is important to evaluate the genetic characteristics of native tree populations for the purposes of conservation and for planning future genetic improvement. The project described here was undertaken to assess genetic diversity in Irish oak.*
- *Oak woodland sites were sampled across Ireland and analysed to characterise their genetic diversity. Samples were designated as* Quercus petraea *or* Q. robur *on the basis of leaf morphology.*
- *The molecular analysis utilised chloroplast DNA (cpDNA) to assess the genetic diversity. Irish oaks were found to have low chloroplast DNA (cpDNA) diversity in comparison to mainland Europe and Britain and there was evidence of a large degree of differentiation between the populations. Five cpDNA genotypes (haplotypes) were recorded in Ireland. However, most samples were either haplotype 10 or 12. The haplotypes did not correspond to the individual species, but haplotype 12 occurs more frequently in* Q. petraea *than in* Q. robur.
- *The genetic types that dominate in Ireland correspond to those that migrated from the Iberian Peninsula glacial refugium after the end of the last glaciation about 10,000 years ago. The results position Irish oak in a European context both in terms of provenance and diversity.*

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Irish oak - genetic diversity and the Iberian connection

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Introduction

Native oak woods cover a very small proportion of the land area in Ireland (approximately 0.1 %). Coverage has diminished since 5000 BC, due primarily to human influence in the form of woodland utilisation and land conversion to livestock grazing and crop production. The woodland remnants thus represent a scarce and valuable resource. However, little is known about the genetic diversity of woodlands in Ireland. A COFORD-funded study obtained data on genetic characteristics of oak woods in Ireland (Kelleher et al. 2002). This was the first study to analyse molecular genetic characteristics of a native tree species. The study revealed an underlying geographical genetic structure in Irish oak populations and presented estimates of diversity from both nuclear and chloroplast DNA analyses (Kelleher et al. 2002, 2004a, 2005). A follow-up study funded by the National Parks and Wildlife Service sampled more populations in an attempt to further investigate geographic patterns. A synopsis of the combined analysis of these projects is presented here.

Genetic diversity can be studied at many levels from the macroscopic (gross morphology) to the sub-microscopic (using molecular markers). This project mainly utilised molecular markers (chloroplast DNA markers in particular) to investigate the diversity and distribution of oak genotypes in Ireland. Chloroplast DNA is strictly maternally inherited in oaks (Dumolin et al. 1995) and therefore can be used for investigating seed dispersal and distribution patterns. Chloroplast DNA analysis has been used to trace postglacial histories of many species such as common alder (*Alnus glutinosa*), *Plantago media, Saxifraga oppositifolia, Senecio menziesii* and *Quercus* spp. (Comes and Kadereit 1998). The PCR-RFLP (Polymerase Chain Reaction – Restriction Fragment Length Polymorphism) method used in this project was used successfully in oak populations throughout Europe to determine diversity levels and postglacial migration routes from southern glacial refugia (Petit et al. 2002b). The method is a DNA fingerprinting technique in which regions of the chloroplast DNA are amplified by PCR and digested with restriction enzymes to reveal specific fingerprints or cpDNA types

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(haplotypes). A total of 25 haplotypes have been identified in *Quercus petraea* and *Q. robur* from other European studies using this technique (Dumolin-Lapègue et al. 1997, Petit et al. 2002b).

The aims of this project were:

- To assess genetic diversity in putative native populations.
- To assess patterns of variation or distribution.
- To compare the genetic diversity of Irish oak to other European populations.

Methods

Sites and sampling

A total of 49 sites were sampled across Ireland (Table 1). Sampling involved selecting a minimum of five specimens per site (except for a few sites which had limited numbers of trees or single trees, such as Clare Island which comprised a single specimen or the Brian Boru oak in Clare). Leaf material was sampled and stored in dried silica gel to preserve the DNA for extraction. Leaves were also sampled to determine the taxonomic status of each sample by morphological analysis.

Morphological analysis and species designation

A suite of morphological characters of the leaves and fruiting structure are commonly used to designate oak species. The main fruiting structure difference is a longer peduncle in *Q. robur* (pedunculate oak) than in *Q. petraea* (sessile oak). However, the analysis in this project was limited to leaf morphology, due to the lack of fruiting material for most trees. The methods used for morphological assessment and analysis are described in detail elsewhere (Kelleher et al. 2004b). Measurements used in the analysis included leaf dimensions, lobe numbers, lobe depth, auricle development and stellate hairs (Figure 1). A hand lens with a magnification of 10 times was used for viewing the stellate hairs.

The data were analysed to assess the species status of the individuals in the woodlands sampled. Cluster analysis was used to designate species based on the morphological

Table 1. A list of the sites sampled across Ireland.

Woodland Site	County	Woodland Site	County
Breen Wood	Antrim	Adare	Limerick
Brian Boru Oak	Clare	Cappercullin Glen	Limerick
Derrymore	Clare	Ballymascanlan	Louth
Garranon Wood	Clare	Brackloon	Mayo
Mount Callan	Clare	Clare Island	Mayo
Raheen	Clare	Eriff	Mayo
Doneraile	Cork	Old Head	Mayo
Doneraile Demesne	Cork	Pontoon	Mayo
Knockomagh	Cork	Birr Demesne	Offaly
The Gearagh	Cork	Charleville Estate	Offaly
Ness Wood	Derry	Reilly's Wood	Roscommon
Crolly	Donegal	St Johns Wood	Roscommon
Devlin River	Donegal	Cullentra	Sligo
Glenveagh	Donegal	Ballydavid/Scaragh	Tipperary
Rostrevor Oakwood	Down	Cahir Park	Tipperary
Lucan Demesne	Dublin	Curragh Mor	Waterford
Crom	Fermanagh	Lismore	Waterford
Derryclare	Galway	Tullynally Estate	Westmeath
Shannawoneen	Galway	Dunganstown	Wexford
Gláisín na marbh	Kerry	Mount Garrett	Wexford
Glencar	Kerry	Coolattin	Wicklow
Royal Oak and surrounds	Kerry	Cronybyrne	Wicklow
Uragh	Kerry	Glen of the Downs	Wicklow
Garryricken	Kilkenny	Glendalough	Wicklow
Abbey Leix Estate	Laois		

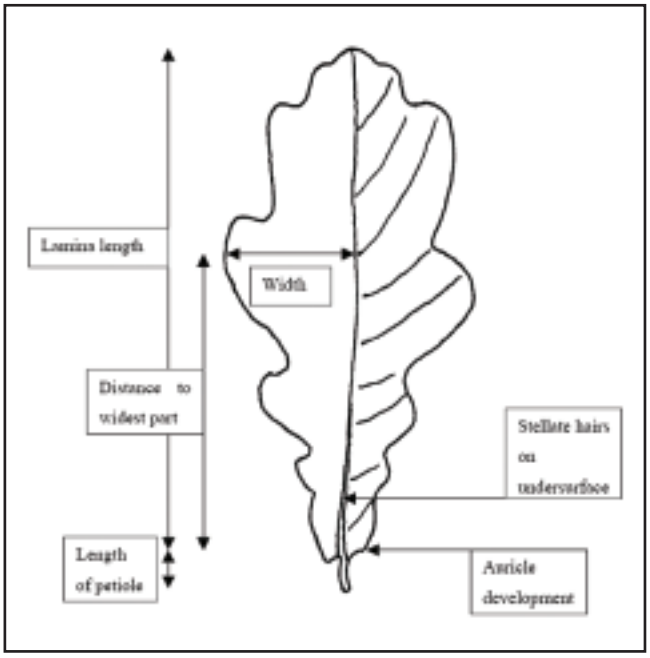

Figure 1: A schematic diagram of an oak leaf with illustration of the measurements taken and used in the analysis. For details of the analysis see Kelleher et al. 2004b.

measurements (Kelleher et al. 2004b). The form of cluster analysis used was the 'Neighbor-Joining' method (Saitou and Nei 1987) and the distance measure was Euclidean. The computer program PAUP 4 (Swofford 1999) was used for the cluster analysis. The relationship trees drawn were rooted at the mid-point of variation, thus separating at greatest divergence – the respective species. This allowed an objective method of species designation.

Laboratory work

The DNA extracted from the leaves was analysed using PCR-RFLP. This is a process whereby a gene region is assessed using restriction enzymes. The resulting banding pattern viewed on the gel can be used as a genetic fingerprint. Regions of the chloroplast genome were targeted for the analysis.

DNA extraction

DNA was extracted from the dried leaf samples using a standard hot CTAB protocol (Doyle and Doyle 1987). The DNA was cleaned using the Gibco BRL® Concert™ Rapid PCR Purification System.

PCR-RFLP analysis

Two regions of the chloroplast genome were used in the analysis, the *trn*D – *trn*T (DT) and the *trn*T – *trn*F (TF) regions (Petit et al. 2002b). Methods are described in detail elsewhere (Kelleher et al. 2004a) and are summarised here. The regions were amplified by PCR and the resulting product was digested with restriction enzymes. The restriction digestion reactions were stopped by adding loading dye (0.25% bromophenol blue, 40% sucrose) and cooling to 4°C. The reaction was loaded on an 8% non-denaturing polyacrylamide gel. The Gibco BRL® 1kb ladder was used as a sizing standard. The gels were run at 200 V for between 2 to 4.5 hours depending on the gene region being analysed. The gels were stained with ethidium bromide and viewed over a UV light box using a digital camera and Kodak 1D 2.0.2 image analysis software. The scoring and nomenclature of haplotypes follows Petit et al. (2002b).

Data analysis and mapping

Haplotype proportions in each population were calculated from the results. The computer program HaploDiv (Petit 1995) was used for calculation of the diversity within populations, h_S, total diversity, h_T, and the apportionment of diversity among the populations, G_{ST} (Pons and Petit 1995). The G_{ST} (ranging from 0 to 1) gives an estimate of the partitioning of diversity. A high G_{ST} value suggests a large degree of differentiation between populations and that the population structure is dominated by inter-population (between) differences rather than intra-population (within) differences. Conversely, a low G_{ST} value indicates a higher intra-population diversity component compared to that between populations.

ArcView GIS version 3.1 was used to map the haplotype proportions.

Results and discussion

Haplotype diversity and distribution

Only five haplotypes were recorded in Ireland out of a possible total of 25 and of these two dominate, haplotypes 10 and 12. The few trees with the other haplotypes (1 or 2, 7 and 11) were recorded in planted woodlands and it is likely that they represent stock introduced by landowners or

foresters. Haplotype 12 is the most dominant haplotype in Ireland and occurred in 40 of the sites sampled. Haplotype 10 occurred in 16 sites. While haplotype 12 is distributed throughout Ireland, haplotype 10 is focused more in the south and west (Figure 2).

The current European distribution of the various haplotypes has been used to infer the glacial refugia from which they originated (Figure 3) (Petit et al. 2002a). There are three main glacial refugias for oaks – the Iberian Peninsula, the Italian Peninsula and the Balkans. The haplotypes that dominate in Ireland are those that originated in the Iberian Peninsula. We have extremely rare occurrences of haplotypes from the Italian or the Balkan refugia and these have so far only been found in planted woodlands. The results support the pollen evidence of a postglacial migration of oak into Ireland from the south west (Mitchell 2002). A rapid postglacial colonisation of oaks containing haplotypes 10 and 12 northwards along the western coast of Europe into Ireland (Kelleher et al. 2004a) could have established populations quickly and prevented further colonisation of other haplotypes from the East. The Iberian connection of the oaks highlights questions about other 'Lusitanian' elements in the Irish flora that have connections with the Iberian Peninsula (Webb 1982). The exact provenance of many of these native plants is still unknown.

The haplotypes do not correspond to the species. The general pattern is of haplotype 12 predominating in *Q. petraea* and haplotype 10 in *Q. robur,* but there is no specific haplotype for each species. This illustrates the genetic continuum that exists between the species. While the species are morphologically distinct there are certainly hybrids and intermediates due to gene exchange. The level of hybridisation in Ireland has been estimated at 10% (Kelleher et al. 2004b).

Genetic diversity and population structure

Genetic diversity of Irish populations was lower than that found in other European populations (Table 1). A comparable country, in terms of size and distance from putative refugia is Denmark. Ireland ranks closely with Denmark in overall diversity. The expectation is to have decreasing diversity with increasing distance from the centre of a refugium (Hewitt 1999), hence Ireland has lower

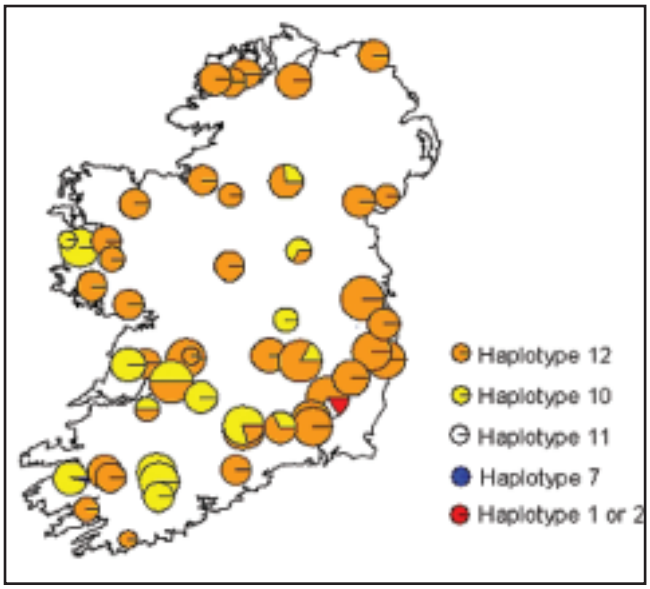

Figure 2. Proportions of the different haplotypes found in the study populations. The size of the pie chart represents the sample number, the smallest (equal to the symbol in the legend) representing one individual and the largest representing 12 individuals. Haplotype 7 is found in only one tree is the far south west of Ireland in Glengariff. Haplotypes 1 or 2 and 11 were found in single individuals in Garryricken, Kilkenny.

Figure 3. A schematic map of Europe showing the three main southern glacial refugia areas that gave rise to northern European oak populations; 1 - Iberian Peninsula, 2 - Italian Peninsula, 3 - the Balkans (adapted from Petit et al. 2002a).

Table 1. Values of intra-population diversity (h_S), total diversity (h_T) and the apportionment of diversity among the populations (G_{ST}) for oak populations in Ireland, Denmark, Britain and France.

	h_S	h_T	G_{ST}	Reference
Ireland	0.084	0.398	0.789	This study
Denmark	0.130	0.335	0.611	(Jøhnk and Siegismund 1997)
Britain	0.162	0.629	0.742	(Cottrell et al. 2002)
France	0.125	0.729	0.828	(Dumolin-Lapègue et al. 1997)

diversity (h_T) than France or Britain. In addition, being a relatively small island, Ireland has had restricted colonisation of plants and is thus less diverse. Calculations on other data have shown indications of inbreeding in Irish oak populations (Kelleher et al. 2005) and this is probably due to the current fragmented nature of the populations.

Most of the woodlands sampled are fixed for one haplotype and only a few are of mixed haplotypes (42 of the 49 populations had a fixed haplotype, Figure 2). This is reflected in the high G_{ST} value and suggests a natural distribution of the populations. It suggests that most populations were founded from a small number of pioneer parent plants rather than from a more diverse mix of seed. This does not definitively reveal a native distribution but the existence of mixed haplotypes in known planted woodlands adds weight to this argument.

Conclusion

The current study shows a genetic link between Irish oak and those that originated in the Iberian Peninsula refugium. From the results it is clear that Ireland has four main types of oak, these are *Quercus petraea* with haplotype 12, *Q. petraea* with haplotype 10, *Q. robur* with haplotype 12 and *Q. robur* with haplotype 10. The most dominant genotype is *Q. petraea* with haplotype 12. Although the haplotypes are not species specific, haplotype 12 does occur more frequently in *Q. petraea* and haplotype 10 occurs more frequently in *Q. robur*. The haplotype distribution supports pollen data for a postglacial colonisation of oak from the south, as Irish populations are shown to have a link with those from the Iberian Peninsula. The levels of genetic diversity are lower in Irish populations than that found in many other European populations. Although there is some evidence for inbreeding in Irish oak populations, it is not likely to be an important factor in their genetic fitness as they are outbreeding wind pollinated trees.

The techniques used in this project offer great potential for use in other tree species in Ireland. Many of our native species have been analysed in genetic studies across Europe and thus there is an opportunity to situate Irish populations into this framework. Work is ongoing in the National Botanic Gardens to characterise other native tree species in a European context.

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