- *Genetic characterisation is an important element in conserving and utilising natural resources.*
- *In a project to characterise genetic diversity in Irish oak, oak woodland sites were sampled across Ireland and analysed for genetic diversity at the plastid/chloroplast level.*
- *Samples were allocated to species* Quercus petraea *or* Q. robur *on the basis of multivariate clustering analysis of leaf morphological characters.*
- *Irish oaks are characterised by low chloroplast DNA (cpDNA) diversity in comparison to mainland Europe and Britain. A total of five cpDNA types (haplotypes) have been recorded in Ireland. However, most samples were either haplotype 10 or 12.*
- *The haplotypes do not correspond to the individual species, but haplotype 12 occurs more frequently in* Q. petraea *than in* Q. robur.
- *The haplotypes that dominate in Ireland correspond to those that migrated from the Iberian Peninsula glacial refugium.*
- *The haplotype shows a low level of genetic diversity in the chloroplast DNA in Irish oak.*
- *The results position Irish oak in a European context both in terms of provenance and diversity.*
- *There is an opportunity to extend this analysis to other tree species.*

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Genetic analysis reveals four Irish oak genotypes

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Introduction

Oak woods cover a very small proportion of the land area in Ireland (approximately 0.1%). The coverage has diminished since 5000 BC due primarily to human influence in the form of woodland utilisation and converting land use to livestock grazing and crop production. The woodland remnants thus represent a scarce and valuable resource. However, very little is known about the genetic diversity and composition of woodlands in Ireland. A COFORD-funded study obtained data on genetic characteristics of oak woods in Ireland (Kelleher et al. 2002). This 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, Kelleher et al. 2004a, Kelleher et al. 2005). A follow-up study funded by NPWS sampled more populations in an attempt to investigate further geographic patterns. A synopsis of the combined analysis of these projects is presented within.

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 as such is very useful for investigating seed dispersal and distribution patterns. Chloroplast genome analysis has been used in tracing postglacial histories of many species such as *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 are amplified by PCR and digested with restriction enzymes to reveal specific fingerprints or cpDNA types (haplotypes). A total of 25 haplotypes have been identified in *Quercus petraea*

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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 situate Irish oak in a European context.
- To assess genetic diversity in putative native populations.
- To assess patterns of variation or distribution.

Methods

Sites and sampling

A total of 49 sites were sampled across Ireland (Table 1). Sampling involved selecting a minimum of 5 specimens per site (except for a few sites which had limited or single champion trees, such as Clare Island which composed of a single specimen and the Brian Boru oak in Clare). Leaf material was taken and stored in dried silica gel to preserve the DNA until extraction. Leaves were taken 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 used to designate the 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 morphological analysis, due to the lack of fruiting material for each tree. 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 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

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		

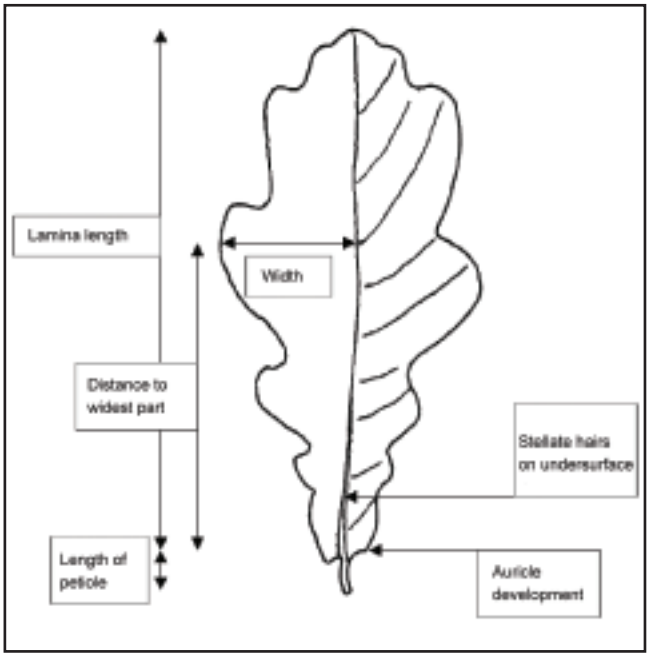

Figure 1: A schematic diagram of an oak leaf with illustration of the measurements taken and used in the analysis.

for 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. Regions of the chloroplast genome were targeted.

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.

Two regions of the chloroplast genome were used in the PCR-RFLP 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 briefly described as follows. The regions were amplified by PCR and the resulting product was digested with restriction enzymes. The DT region was subjected to restriction digestion for 2 hours at 65°C with the enzyme *Taq*I and the TF region was subjected to restriction digestion for 5 hours at 37°C with the enzyme *Hinf*I. 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 on a Gibco BRL® vertical gel rig Model V15.17. The Gibco BRL® 1kb ladder was used as a sizing standard. The polyacrylamide solution used was National Diagnostics AccuGelTM 29:1. The DT reactions were run at 200 V for approximately 4.5 hours. The TF reactions were run at 200 V for approximately 2 hours. The gels were run with standards and sized using a Gibco BRL® 1kb ladder, 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 was according to Petit et. al. (2002b).

Data analysis and mapping

From the results haplotype proportions in each population were calculated. 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} gives an estimate of the partitioning of diversity. A large G_{ST} value suggests a large degree of differentiation between populations and that the population structure is dominated by inter-population differences rather than intra-population differences. Conversely a small G_{ST} value indicates a large 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

A total of five haplotypes were recorded in Ireland. From these, two dominate, haplotypes 10 and 12. The other haplotypes were recorded in planted woodlands. Haplotype 12 is the most dominant haplotype whereas 10 is less common. While haplotype 12 is found throughout Ireland, haplotype 10 is focused in the south (Figure 2).

Looking at the European distributions of the various haplotypes that occur in Ireland we can infer where the Irish populations are derived from (Figure 3). Haplotypes 10 and 12 have a marked western distribution, whereas the other

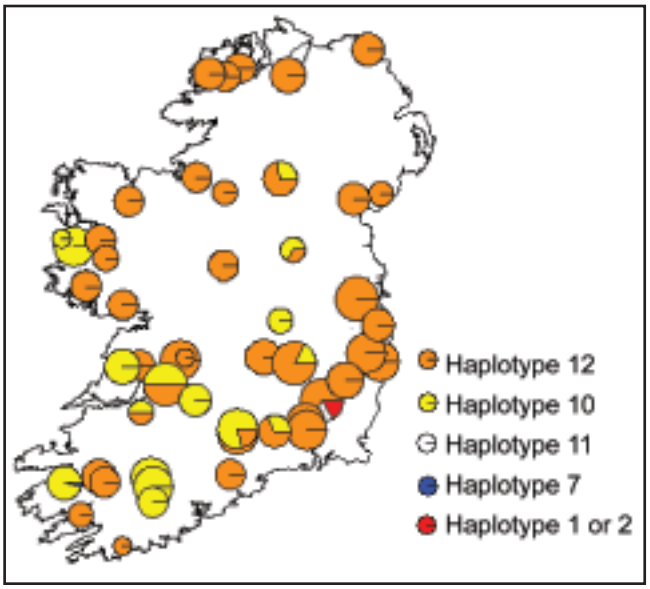

Figure 2. A map of Ireland illustrating the proportions of the different haplotypes found in the different populations. The size of the pie chart represents the sample number, the smallest (equal to the symbol in the legend) representing one individual and the largest representing 12 individuals.

haplotypes are more dominant in central and eastern Europe. A glacial refugium for haplotypes 10 and 12 is the Iberian Peninsula (Petit et al. 2002a) and it is likely that Irish populations are derived from these populations also (Kelleher et al. 2004a). This supports the pollen evidence of a postglacial migration of oak into Ireland from the south west (Mitchell 2002). There are also other "Lusitanian" elements in the Irish flora that have connections with the Iberian Peninsula (Webb 1982). The exact provenance of many of these plants is still unknown. The current study shows a genetic link between Irish oak and those of the Iberian Peninsula refugium.

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 continuum 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).

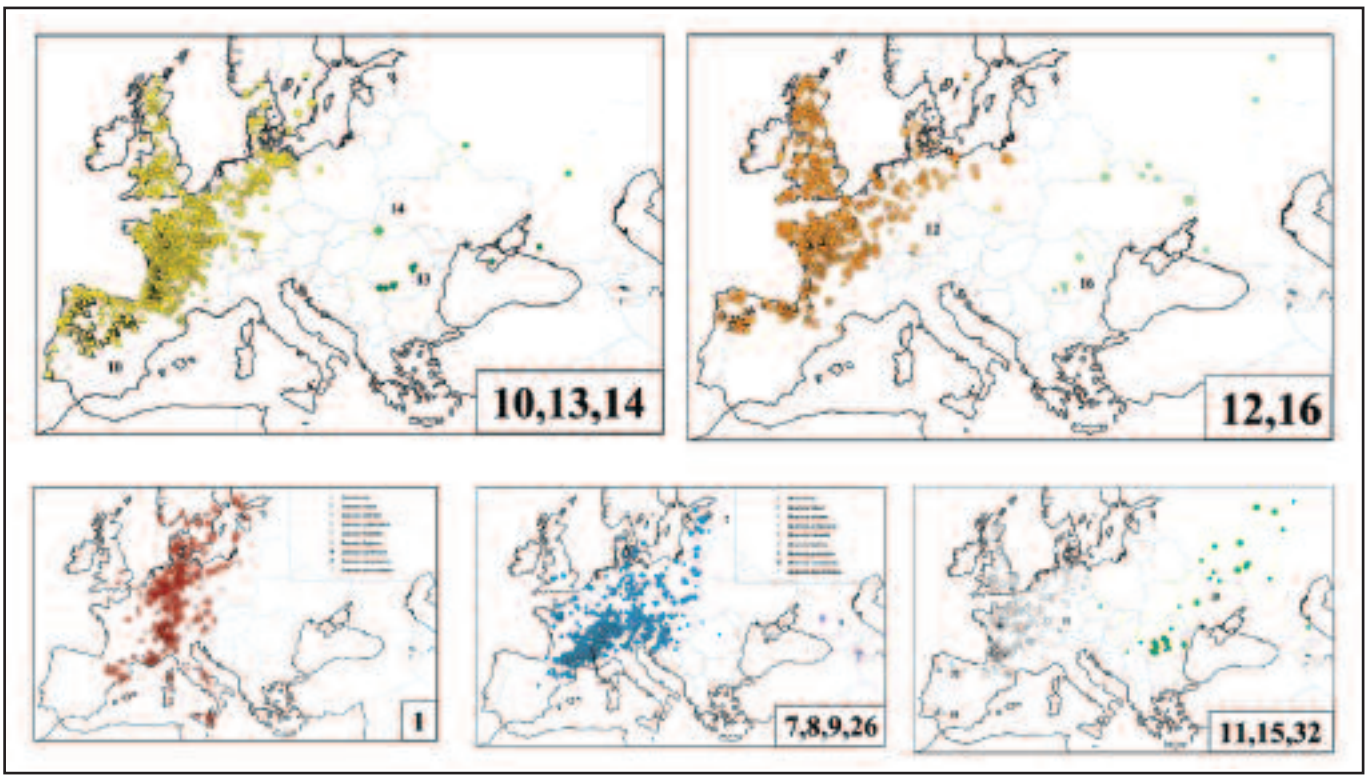

Figure 3. Maps showing the European distribution of the haplotypes found in Ireland (haplotype 2 is not shown but has a similar distribution to haplotype 1). Maps taken from (Petit et al. 2002a).

Genetic diversity and population structure

Genetic diversity was lower than that found in other European studies (Table 1). A comparable country, in terms of size and distance from refugia is Denmark. Ireland ranks closely with Denmark in overall diversity. It is expected to have decreasing diversity with increasing distance from the centre of a refugium (Hewitt 1999). In addition, being an island, Ireland has had restricted colonisation of plants and thus has less diversity. Calculations on other data have shown indications of inbreeding in Irish oak populations (Kelleher et al. 2005) and this is likely due to the fragmented nature of the populations.

Most woodlands are fixed for one haplotype and only a few are of mixed haplotypes (42 of the 49 populations had a fixed haplotype). 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 does add weight to this argument. oakgenotypes

Conclusion

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 south-westerly invasion of oak following the last glacial maximum, as Irish populations are shown to have a link with those from the Iberian Peninsula. The levels of diversity show Irish populations to be lower 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 outbreeding wind pollinated trees. The techniques used in this project offer great potential for use in other tree species in Ireland. Most of our native species have been studied in larger European projects. Work is ongoing to situate other tree species into this broader European framework.

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)

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