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High Nuclear Genetic Diversity, High Levels of Outcrossing and Low Differentiation Among Remnant Populations of *Quercus petraea* at the Margin of its Range in Ireland

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• *Background and Aims Quercus petraea* colonized Ireland after the last glaciation from refugia on mainland Europe. Deforestation, however, beginning in Neolithic times, has resulted in small, scattered forest fragments, now covering less than 12 000 ha.
• *Methods* Plastid (three fragments) and microsatellite variation (13 loci) were characterized in seven Irish populations sampled along a north–south gradient. Using Bayesian approaches and Wright's *F*-statistics, the effects of colonization and fragmentation on the genetic structure and mating patterns of extant oak populations were investigated.
• *Key Results* All populations possessed cytotypes common to the Iberian Peninsula. Despite the distance from the refugial core and the extensive deforestation in Ireland, nuclear genetic variation was high and comparable to mainland Europe. Low population differentiation was observed within Ireland and populations showed no evidence for isolation by distance. As expected of a marker with an effective population size of one-quarter relative to the nuclear genome, plastid variation indicated higher differentiation. Individual inbreeding coefficients indicated high levels of outcrossing.
• *Conclusions* Consistent with a large effective population size in the historical migrant gene pool and/or with high gene flow among populations, high within-population diversity and low population differentiation was observed within Ireland. It is proposed that native *Q. petraea* populations in Ireland share a common phylogeographic history and that the present genetic structure does not reflect founder effects.

Key words: *Quercus petraea*, microsatellites, plastid DNA, population differentiation, outbreeding.

INTRODUCTION

Remnants of the formerly extensive Atlantic woodland that covered up to 80 % of the island of Ireland are now fragmented, covering less than 0·2 % (Mitchell, 1995; Rackham, 1995). *Pinus sylvestris* L., formerly an abundant taxon in this woodland, is thought to have declined to extinction by a combination of climatic change and, more recently, exploitation (Mitchell, 2003). One of the predominant species in scattered woodland remnants is *Quercus petraea* (Matt.) Liebl., now covering less than 12 000 ha (Keogh, 1987). The history of woodland exploitation in Ireland has parallels with the decline of Caledonian native pinewoods in Scotland. Both have been exploited for industrial purposes, particularly during the last 400 years, whereas only more recently has attention turned to their management and conservation. Surveys in Caledonian pinewoods indicate that despite the reduction in size of some stands to only hundreds of individuals, genetic variation remains high (Provan *et al*., 1998). In addition, increased selfing rates have not been detected as a result of the reduction in tree density (Helgason and Ennos, 1991).

Counts of radiocarbon-dated pollen indicate that Ireland was colonized by *Q. petraea* from south to north (Mitchell, 2003). Maternally inherited plastid markers support this migration route and indicate that Irish populations of *Q. petraea* were derived from glacial refugia located on the Iberian Peninsula (Dumolin-Lapègue *et al*., 1997; Petit *et al*., 2002). Nevertheless, sample sizes in these studies were small in Ireland, and Irish oaks have thus not been well characterized with molecular markers compared with British or mainland European populations.

Radiocarbon-dated pollen also indicates a reduction in forest cover from a maximum of 80 % (approx. 7000 years ago), with a decline across all woody taxa beginning in Neolithic times (Mitchell, 1988). While climate influenced the postglacial dynamics of colonization at this time as well, the impact of human activity was sufficiently intense to register on the pollen record by the Bronze Age, approx. 3000 years ago (Mitchell, 1995). By the 1600s, census data indicate that woodland cover had declined to 2·1 % (Rackham, 1995). The effects of this exploitation on levels

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of genetic variation are unclear, but the value of these remnants as part of the natural heritage of Ireland is high.

Remnants of Irish oak represent populations at the north-western margin of the range of *Q. petraea* in Europe. Irish populations are far from the refugial core and may have been exposed to (multiple) founding events. Latterly, fragmentation and reduced population sizes may also have led to a reduction in gene flow among previously connected populations and to an increase in mating between closely related individuals. To evaluate the impact of genetic drift, populations were sampled along a north–south gradient in close geographical proximity, as well as at more distant locations. If drift was a significant force then isolation by distance would be expected under colonization from south to north. Bayesian statistics and a model-based clustering algorithm were also used to estimate the most probable population genetic structure in Ireland. Using a common European haplotype nomenclature (Dumolin-Lapègue *et al*., 1997), plastid markers were used to establish if Irish oaks share a common postglacial colonization history with British oaks (Cottrell *et al*., 2002). In addition, nuclear microsatellites were used to compare the level of genetic diversity in Ireland with mainland European populations.

Genetic bottlenecks can lead to an increase in mating between closely related individuals within populations and to purging of deleterious alleles. In turn, this may lead to a change in the mating system (Vogl *et al*., 2002). In common with other trees, *Q. petraea* is a highly outcrossing species and maintains a high genetic load (Ledig, 1986). Given the contraction of ancient woodland in Ireland to current levels of less than 0·2 % and the persistence of only small and disjunct populations, past mating between closely related individuals may have influenced the mating system of *Q. petraea.* To infer the degree of reproductive isolation among remnant individuals, a recently developed Bayesian approach was used to place posterior confidence intervals on individual inbreeding coefficients. Rather than estimate a population average that may mask differences among individuals, individual inbreeding coefficients were estimated based on microsatellites (13 loci) to provide a higher resolution of mating patterns.

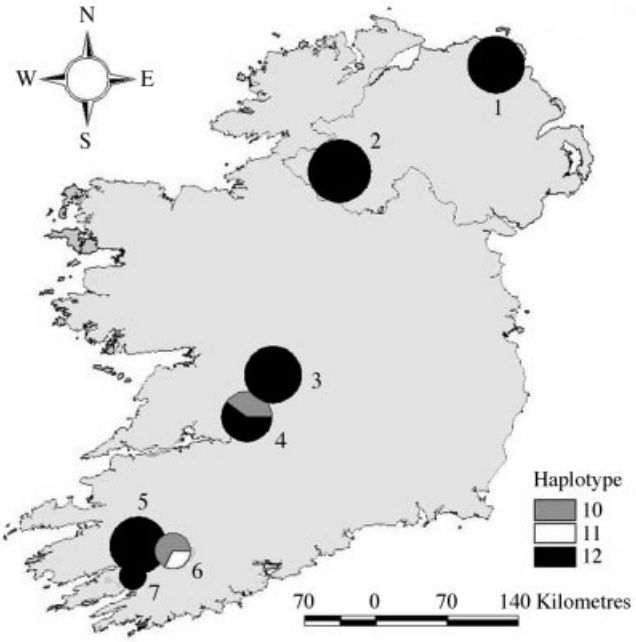

FIG. 1. Frequency of three plastid haplotypes detected in seven Irish populations of *Quercus petraea*. The size of the circle is proportional to the number of individuals sampled (n = 2–6). Population numbers correspond to those given in Table 3. Haplotype nomenclature follows Dumolin-Lapègue *et al.* (1997).

MATERIALS AND METHODS

Plant material

To obtain a latitudinal representation of the genetic variation present in Irish oakwoods, *Q. petraea* individuals (n = 25–33; see Table 3) were sampled from each of seven woods on a north–south gradient (see Fig. 1). All samples came from mature, even-aged woods (125–175 years old) that form disjunct populations and have a density of approx. 300–500 stems ha^{-1}. The two woods from the Killarney Valley (Tomies and Derrycunihy), however, were separated by only 6 km. Leaves were sampled from: Breen, Co. Antrim 55°7′N 6°20′W, Correl Glen, Co. Fermanagh 54°25′N 7°47′W, Garranon, Co. Clare 52°41′N 8°38′W, Pollnaknockaun, Co. Clare 53°3′N 8°24′W, Tomies, Co. Kerry 52°1′N 9°30′W, Derrycunihy, Co. Kerry 51°58′N 9°29′W and Glengarriff, Co. Cork 51°45′N 9°33′W. Latitudinal and longitudinal co-ordinates were obtained from http://www.getty.edu/research/tools/vocabulary/tgn/index.html. Derrycunihy belongs to a cluster of woods in the Killarney Valley, thought to represent the largest ancient woodlands in Ireland (Kelly, 1981; Mitchell, 1988). Counts of radiocarbon-dated pollen from Derrycunihy indicate a continuous *Quercus* pollen record for at least the last 5000 years and, most probably, direct continuity with post-glacial primary forest (Mitchell, 1988). Nevertheless, historical records indicate that Tomies wood, also in the Killarney Valley, was felled and replanted at the beginning of the 19th century (Mitchell, 1988). It is likely that regrowth of this wood occurred via natural regeneration or locally sourced seeds. However, forest management in these woods cannot be discounted. Oakwoods are referred to as populations in the text.

As a mainland European reference, *Q. petraea* individuals (n = 10–16; see Table 3) were genotyped from three locations in Spain and France using DNA kindly provided by C. Bodénès and A. Kremer. These locations were in northern Spain 41°07′N 3°30′W, south-western France 43°11′N 0°40′E and eastern France 48°18′N 4°28′E.

DNA extraction and PCR application

Genomic DNA was extracted from leaves using the Nucleon Phytopure Kit (Amersham) according to the manufacturer's instructions. PCR reaction, amplification and electrophoresis conditions were as described in

Schlötterer (1998). In total, ten populations (248 individuals) were genotyped using 13 microsatellite loci: 1/5, 1/2, 7, 9, 15, 110, 119 (Steinkellner *et al*., 1997) and 7, 87, 96, 101, 108, 112 (Kampfer *et al*., 1998). To establish the affinity of Irish oaks with plastid lineages from the European mainland, we determined the cytotypes for approx. five individuals from each of the seven Irish populations (n = 40). The restriction fragment length polymorphism analysis of plastid PCR was as described in Cottrell *et al*., (2002) and scoring of these fragments followed a widely used nomenclature (Dumolin-Lapègue *et al*., 1997).

Data analysis

Microsatellite analyses. Diversity indices and general statistics were calculated using MICROSATELLITE ANALYZER (MSA; Dieringer and Schlötterer, 2003). Estimates of allelic richness were corrected using the rarefaction option in FSTAT (Goudet, 2001). The MSA program was also used to determine Θ, an unbiased estimate of Wright's Fixation Index (Weir and Cockerham, 1984), to characterize the extent of population differentiation. The significance of pairwise Θ values (referred to as F_{ST} in the text) was tested by performing permutations of genotypes among groups. This method of permutation does not rely on Hardy–Weinberg assumptions (Goudet *et al*., 1996). A Bonferroni correction was applied to account for multiple testing.

To test for a significant correlation between genetic and geographic distances, we compared an $F_{ST}/(1 - F_{ST})$ matrix with a geographical distance matrix (ln km) using a Mantel test (10 000 permutations) in GENEPOP (Raymond and Rousset, 1995). A web-based distance calculator was used to determine the surface distance between two sampling points (http://www.wcrl.ars.usda.gov/cec/java/lat-long.htm).

A model-based clustering method implemented in the program STRUCTURE (Pritchard *et al*., 2000) was used to assign individuals to homogeneous clusters (populations) without consideration of sampling localities. Estimated posterior probabilities were calculated assuming a uniform prior for K, where $1 \leqslant K \leqslant 4$. To minimize the effect of the starting configuration during the Monte Carlo simulation we simulated 50×10^3 updates of the Markov chain before data for the parameter estimation were collected from another 10^6 iterations. Three independent runs of the Markov chain, each of at least 10^6 updates were performed to assure convergence of the chain and homogeneity between runs for each prior of K. The posterior probabilities of K were then calculated using Bayes' rule. The program was run without population identifiers (USEPOPINFO = 0) and in the admixture mode (NOADMIX = 0).

Inbreeding coefficients were estimated for the 209 Irish individuals using MUSTATS (Vogl *et al*., 2002). The program uses Gibbs sampling to estimate the posterior distribution of inbreeding coefficients by cyclically updating the conditional distribution of three sets of key variables. These are: (1) a set of variables that indicates identity by descent for each individual–locus combination; (2) the inbreeding coefficient for each individual; and (3) the population allelic proportion for each locus. From the marginal posterior distribution, the mean inbreeding coefficient and the lower 0·05 and upper 0·95 posterior interval were then calculated for each individual.

Plastid analyses. Restriction fragment polymorphisms were scored as unordered binary characters. Total diversity (h_T) and total differentiation as measured by G_{ST} were estimated using HAPLODIV (Pons and Petit 1995). Glengarriff was omitted from the latter because of the low number of individuals assayed (n = 2).

RESULTS

Colonization and population structure

All 40 individuals analysed for plastid DNA variation possessed one of three cytotypes, 10 (10 %), 11 (5 %) and 12 (85 %), all three belong to the west European lineage B (Dumolin-Lapègue *et al*., 1997). Hence, in common with British oaks, native populations of *Q. petraea* in Ireland represent lineal descendants of refugial ancestors from the Iberian Peninsula (Cottrell *et al*., 2002). Five of the seven populations were monomorphic and fixed for cytotype 12. The remainder, located in the south (Fig. 1), were polymorphic, possessing at least two cytotypes. G_{ST} was relatively high among Irish populations (0·501) but h_T was considerably lower (0·384) than published estimates for *Q. petraea* in Britain (h_T = 0·569; Cottrell *et al*., 2002), and the Iberian Peninsula (h_T = 0·804; Olalde *et al*., 2002).

Low population substructure both in Ireland and mainland Europe was observed for microsatellites by F_{ST}-analysis (Table 1). There was no statistically significant difference between average pairwise F_{ST}-values in Ireland ($\bar{F}_{ST}$ = 0·008) and mainland Europe ($\bar{F}_{ST}$ = 0·020), respectively (Mann–Whitney U-test, P = 0·0795). With two exceptions (from a total of 21), values for pairwise comparisons within Ireland were not statistically significant.

Values for the highest pairwise F_{ST} comparisons within Ireland were confined to the two most northerly populations. One of these populations (Correl Glen) was significantly different from both populations located in central Ireland (Pollnaknockaun, Garranon). Nevertheless, consistent with the single west European cytotype (12) detected in this population and a shared colonization history, Correl Glen was not significantly different from its northern neighbour (Breen).

In the model-based clustering approach, individuals from seven locations in Ireland were assigned to populations (K) on the basis of their microsatellite genotype, ignoring their actual geographic origins within Ireland. This mimics a scenario in which there is no *a priori* information as to the geographic origin of individuals. Most of the posterior probability was on K = 1 (Table 2). Hence, despite the substructure observed in Correl Glen based on the F_{ST}-analysis, Irish *Q. petraea* as a whole can be regarded as one genetic cluster.

As the level of genetic differentiation depends on gene flow and genetic drift, the analyses above suggest high rates

TABLE 1. *Genetic differentiation among populations of* Quercus petraea *measured by pairwise* F_{ST} *(below diagonal): significance values based on the permutation of microsatellite genotypes among populations (above diagonal)*

	North		Central		South					
	Breen	Correl Glen	Pollnaknockaun	Garranon	Tomies	Derrycunihy	Glengarriff	Eastern France	SW France	Spain
Breen		ns	ns	ns	ns	ns	ns	*	ns	ns
Correl Glen	0·013		**	**	ns	ns	ns	**	ns	**
Pollnaknockaun	0·008	0·020		ns	ns	ns	ns	**	ns	ns
Garranon	0·011	0·024	0·004		ns	ns	ns	**	ns	ns
Tomies	0·002	0·006	0·001	0·006		ns	ns	**	ns	ns
Derrycunihy	0·006	0·012	0·004	0·008	0·002		ns	**	ns	ns
Glengarriff	0·007	0·019	0·003	0·008	0·004	0·004		**	ns	ns
Eastern France	0·028	0·036	0·033	0·029	0·031	0·027	0·033		ns	ns
SW France	0·005	0·016	0·018	–0·001	0·007	0·008	0·010	0·010		ns
Spain	0·014	0·032	0·015	0·010	0·017	0·016	0·009	0·040	0·011	

P-values determined using 10 000 replicates. **, $P \leqslant 0{\cdot}01$, *, $P \leqslant 0{\cdot}05$, ns, not significant (after Bonferroni correction); SW, south-western.

TABLE 2. *Estimated posterior probabilities of* K *assuming four genetic clusters*

*K**	ln *P*(*X*|*K*)	*P*(*K*|*X*)**
1	–11172·8	~1
2	–11214·2	~0
3	–11290·4	~0
4	–11289·3	~0

Calculations based on seven locations (209 individuals of *Q. petraea* from Ireland).

* number of clusters assumed using STRUCTURE; ** assuming a uniform prior for *K*, where $1 \leqslant K \leqslant 4$ (for explanation, see text).

of historical gene flow between populations and/or large effective population sizes. If genetic drift and low gene flow were significant forces, then adjacent populations along the north–south gradient in Ireland should show more genetic similarity than distantly located ones. However, the Mantel test detected no significant correlation between pairwise genetic distance and geographic distance in Irish *Q. petraea*.

Levels of genetic variation

We observed substantial levels of microsatellite variation ($\bar{H}_E > 0{\cdot}7$ and $\bar{V} > 20$, Table 3), comparable with those obtained using the same 13 loci (plus seven) in a larger survey of 20 microsatellite loci, scored in five populations of *Q. petraea* from mainland Europe (G. Muir and C. Schlötterer, ITG, Vienna, Austria, unpubl. res.). Hence, observed levels of variation in Ireland cannot be an artefact of a higher sampling variance associated with fewer loci. Irish populations also showed comparable levels of variation to putative source populations in mainland Europe. We compared diversity indices (in Table 3) for each of the 13 loci surveyed among the two groups: Ireland and mainland Europe. With the exception of allelic richness (*A*), there was no statistically significant difference among the diversity indices (Table 3) between populations from mainland Europe and Ireland (Wilcoxon sign test).

Mating system

The majority of individual inbreeding coefficients were close to zero with average inbreeding coefficients per population between 0·010 and 0·031 (Table 4). On average, only one individual per population possessed coefficients above 0·12, and no individual in the whole data set possessed coefficients above 0.38 (Fig. 2; see also supplementary material). Hence, there is little evidence for mature selfed individuals, or for a change in mating system as a result of population fragmentation in Ireland.

DISCUSSION

Low genetic differentiation among populations

Approximately 100 generations separate the current cohort of trees from the first post-refugial colonizers to Ireland. Assuming a mutation rate of 10^{-4} per generation for plant microsatellites (Thuillet *et al*., 2002), the number of mutations that have arisen in this time-frame can be considered negligible. Hence, new mutations are unlikely to influence the genetic variation observed at microsatellite loci between Ireland and the mainland or within and among Irish populations. As such, the present population structure in Ireland either reflects historical levels of differentiation or, as an alternative, high gene flow among populations.

The F_{ST} analysis indicated that population substructure within Ireland was limited to Correl Glen. Nevertheless, the model-based clustering algorithm indicated that Irish *Q. petraea* as a whole can be regarded as one genetic cluster. The difference in the detection level between these analyses may stem from the *a priori* groupings of individuals made for the F_{ST} analysis. The clustering approach uses the genotypes of single individuals rather than *a priori* groupings to infer population substructure. Together with a relatively small number of loci, this may have provided

TABLE 3. *Diversity indices by population across 13 microsatellite loci in* Quercus petraea

No.	Population	H_O	H_E	V	A	n
1	Breen	0·70	0·81	20	7	25
2	Correl Glen	0·66	0·79	20	7	33
3	Pollnaknockaun	0·68	0·79	24	7	29
4	Garranon	0·67	0·80	21	7	29
5	Tomies	0·68	0·79	17	7	32
6	Derrycunihy	0·71	0·79	19	7	29
7	Glengarriff	0·72	0·81	24	7	32
	Eastern France	0·64	0·82	21	7	13
	SW France	0·66	0·77	16	6	10
	Spain	0·61	0·76	19	6	16

No., population numbers in Fig. 1; H_O, observed heterozygosity; H_E, expected heterozygosity; V, variance in microsatellite repeat number; A, allelic richness after rarefaction to 16 chromosomes; and n, number of analysed individuals.

TABLE 4. *Estimates of average inbreeding coefficients,* $E(\bar{F}_I)$*, and lower (0·05) and upper (0·95) posterior intervals for seven Irish* Quercus petraea *populations (estimated using 13 microsatellite loci)*

Population	$E(\bar{F}_I)$	0·05	0·95
Breen	0·031	0·005	0·060
Correl Glen	0·018	0·001	0·046
Pollnaknockaun	0·014	0	0·042
Garranon	0·021	0·001	0·045
Tomies	0·024	0·003	0·053
Derrycunihy	0·020	0·001	0·046
Glengarriff	0·010	0	0·026

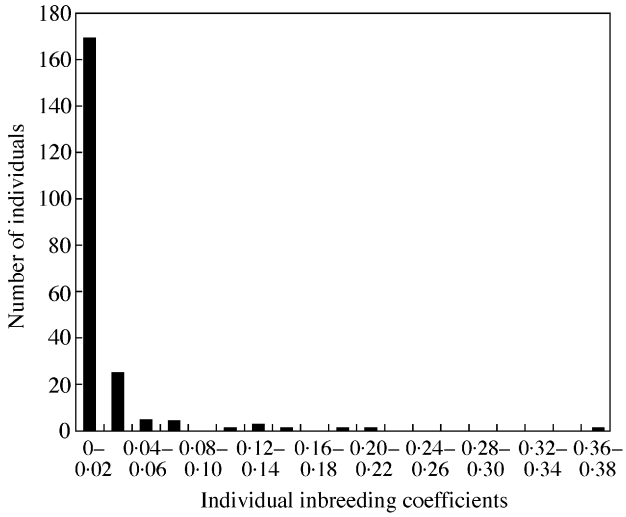

FIG. 2. Frequency distribution of inbreeding coefficients estimated for 209 *Quercus petraea* individuals from seven Irish populations. Corresponding posterior intervals can be found at http://i122server.vu-wien.ac.at.

insufficient power to detect the population substructure in Correl Glen.

Pairwise F_{ST} comparisons of Irish populations with those in mainland Europe indicated no statistical difference between most Irish populations and those from Spain and south-western France. All Irish populations, however, were statistically different from the population in eastern France (Table 1); a result which is consistent with a colonization route along the north-western seaboard of Spain and France (Olalde *et al*., 2002; Petit *et al*., 2002). Surveys of additional populations would be required to support this claim.

High levels of genetic variation within populations

Despite the peripheral location and the large reduction in forest cover, *Q. petraea* populations in Ireland have similar allelic proportions and comparable levels of microsatellite variation with those in mainland Europe. This pattern contrasts with the decrease in variation expected with distance from a refugial source, but is supported by other comparisons of diversity indices from the margins and core distribution of the species (e.g. Cottrell *et al*., 2003). The lack of genetic differentiation between the above two regions may reflect admixture (at mid-latitudes) of populations derived from independent glacial refugia (Petit *et al*., 2003).

Additional information on the demography of oaks can be obtained from the maternally inherited plastid genome (Dumolin *et al*. 1995). With an effective population size of one-quarter relative to nuclear markers under neutrality, plastid DNA is likely to reveal higher levels of differentiation among populations. Cytotype differentiation and diversity were higher in the south of Ireland, as has been observed within Britain (Cottrell *et al*., (2002). Whereas variability of cytotypes in Ireland was reduced compared with mainland Europe (see also Cottrell *et al*., 2002), this was not indicative of a severe genetic bottleneck. Hence, the observed difference in differentiation among nuclear and cytoplasmic markers can be explained by the differences in effective population size of the two genomes. In the remainder of this paper, several scenarios that may account for the level of genetic variation observed in Ireland are discussed.

High rates of gene flow

Founder events during the colonization of Ireland may have resulted in stochastic loss of genetic variation and divergence among populations. On the other hand, gene flow may have compensated for these processes. Populations may formerly have been continuously connected by gene flow (via pollen and seed). This is supported by the analysis of populations sampled along the north–south gradient in Ireland. With nuclear markers, we detected low and (mostly) statistically insignificant population differentiation and an absence of a distance effect (no isolation by distance) expected under colonization from south to north. Additionally, no evidence for population

substructure was detected using the model-based clustering algorithm. These observations all suggest extensive (at least historical) gene flow among populations or colonization in large numbers or both.

Recent exploitation

Extensive pollen flow may have maintained historically large effective population sizes despite recent negative demographic effects. However, levels of forest cover in Ireland are documented only for the last 400 years, when pressures were intense. Since this period corresponds to less than five oak generations, the effects of exploitation may not yet be detectable. Nevertheless, deforestation did occur prior to this period, at least since the Bronze Age (approx. 30 generations ago). To consider the potential for drift within populations, we assumed a standard model of population divergence with migration, and estimated the evolutionary divergence of subpopulations within Ireland. Given a single panmictic population split t generations ago into subpopulations of size N_e, population differentiation between subpopulations over time can be estimated by $F_{ST} = 1 - e^{-t/N_e}$ (Crow and Kimura, 1970). Thus, the change in effective population size expected over five generations (assuming an effective population size of 1000) is 0·005. Over 30 generations, the expected change is 0·03. Hence, given a relatively small number of generations exposed to drift and a large effective population size (maintained by gene flow and/or many colonizers), it is possible that more recent demographic effects have not yet manifested themselves.

The ameliorating effects of a large colonization pool and/or gene flow notwithstanding, observed levels of genetic differentiation in Ireland are also consistent with theoretical predictions that show an absence of founder effects when the long juvenile phase in woody plants is incorporated into migration models (Austerlitz *et al*., 2000; Austerlitz and Garnier-Géré, 2003).

Admixture

Irish populations of *Q. petraea* may have gained additional genetic variation by admixture from the closely related pedunculate oak (*Q. robur*). The sharing of cytotypes within the natural distribution of these species has been interpreted as evidence for hybridization between the two taxa within glacial refugia (Ferris *et al*., 1993; Dumolin-Lapègue *et al*., 1997). Cytotypes observed in *Q. petraea* in this study were also observed in one *Q. robur* population from central Ireland (results not shown). Hence, the two species may share common colonization routes. Both species share substantial amounts of genetic variation (Bodénès *et al*., 1997; Muir *et al*., 2000; Gömöry *et al*., 2001; Coart *et al*., 2002). This may result from shared ancestral polymorphism rather than recurrent interspecific gene flow. But even with low rates of introgression, the introduction of 'new' genetic variants may result in the rapid spread of advantageous alleles in heterospecific genetic backgrounds (Barton, 2001). Hence, interspecific hybridization cannot be discounted as a source of additional genetic variation.

CONCLUSIONS

Consistent with a large effective population size in the historical migrant gene pool and/or with high gene flow among populations, low population differentiation and high levels of genetic variation were observed in Ireland. Extensive pollen flow has been documented in *Q. petraea*, e.g. by Streiff *et al*. (1999). Deforestation at the level recorded in Ireland, however, can affect the number of migrants and can result in a decline in the number of effective mating partners. Studies on the effects of deforestation among insect-pollinated trees indicate an inverse correlation between pollen flow and tree density (Dick, 2001; White *et al*., 2002). However, among wind-pollinated trees, such as oak, the effective number of pollen donors, as defined by Smouse *et al*. (2001), can vary positively with tree density (Sork *et al*., 2002). Hence pollen may travel further on average among fragmented forest-trees pollinated by insects. Nevertheless, Sork *et al*. (2002) infer a decline in the effective number of pollen donors in *Quercus lobata* (Californian valley oak) over a relatively short (55-year) period.

Our results do not indicate historical genetic isolation of native oaks in Ireland. However, they continue to be threatened because the present tree structure is even-aged and natural regeneration is unprotected from browsing. Recognition of an ageing demography has resulted in the initiation of restoration programs. As seed collected from native/autochthonous populations is unlikely to be inbred, as shown by the present study, this material may be used to expand the present resource. Nevertheless, the transfer of genetic material for planting should attempt to match differences in adaptive genetic variation (Ennos *et al*., 1998).

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