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Chloroplast DNA variation within the Nordic countries

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Abstract

Chloroplast DNA (cpDNA) variation was studied in the white oak species, *Quercus robur* and *Quercus petraea*, from Denmark, Norway, Sweden and Finland. A total of 474 trees from 91 populations were screened within the region for a set of previously characterised cpDNA haplotypes. Within the sample, haplotype 1, known to have originated from an Italian Pleistocene forest refugium, was the most common (55%), and was well-distributed across all Scandinavian countries and dominated in Sweden. We propose that material of Italian origin was part of the first wave of post-glacial migration of oak into Scandinavia, and that the Italian haplotype migrated into Finland from Sweden across the Baltic Sea. In Finland, a sharply defined transition zone between the haplotype of Italian origin and haplotype 5, originating from an eastern European refugium in the Balkans, was observed in southern Finland, and confirms the findings of a previous study [Heredity 80 (1998) 584]. Evidence from the study of Csaikl et al. [For. Ecol. Manage., this issue] confirms that this haplotype migrated into Finland via an eastern route through the Baltic States. Another haplotype of Balkan origin (haplotype 7) was only common in southern Denmark and known to be present throughout the native German forests [For. Ecol. Manage., this issue], from where we believe it migrated into Denmark. It appears probable that this haplotype reached Denmark later than the Italian lineage as its progress towards north appears to have been hindered by a bottlenecking in central Denmark. Finally, three haplotypes originally of Iberian origin (haplotypes 10–12) were also found at intermediate frequency throughout the Nordic countries sampled, except Finland. This pattern of distribution suggests that migration occurred concurrently or just behind that of the Italian lineage. One of the Iberian haplotypes (haplotype 12) is rare in autochthonous stands, but found at a frequency of 24% in planted non-autochthonous stands in Denmark. Overall, the proportion of populations fixed for a single haplotype was high in natural, autochthonous stands (77%) and lower in artificial stands (54%). In addition, population subdivision was higher ($G_{ST} = 0.87$) and diversity lower ($h_S = 0.075$ and $h_T = 0.6$) in autochthonous stands than in artificial stands ($G_{ST} = 0.69, h_S = 0.22, h_T = 0.72$). The pattern of haplotype distribution across Scandinavia and Finland appears fairly clumped and is discussed in relation to natural gene flow, leptokurtic post-glacial dispersal and human influences. Finally, no relation between cpDNA haplotype and six quantitative traits measured on autochthonous stands in Denmark was found. The potential for cpDNA haplotype screening for setting gene conservation and seed certification priorities is discussed.

Keywords: Chloroplast DNA; *Quercus robur*; *Quercus petraea*; PCR–RFLP; Phylogeography; Post-glacial re-colonisation

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1. Introduction

Cytoplasmic markers have proven to be an effective tool for demonstrating the evolutionary genetics and post-glacial migration patterns of many species of plants and animals (e.g. Taberlet et al., 1998). One of the best studied examples is that of European white oaks, for which molecular variation in the maternally inherited chloroplast genome (cpDNA) has been successfully applied to determine the patterns of post-glacial re-colonisation and seed-mediated gene flow of oak species across Europe (Ferris et al., 1993; Petit et al., 1993; Dumolin-Lapègue et al., 1997). Using a fine scale molecular assay that utilises the numerous cpDNA mutations so far characterised for oak species (Dumolin-Lapègue et al., 1997), it may be possible to assist practical gene conservation and breeding programs for oak species in Scandinavia. In addition, it should be possible to establish the post-glacial re-colonisation pathway of oaks through Scandinavia and determine the origin of material, and will be useful for the management of forest reproductive material.

Both *Quercus robur* L. and *Quercus petraea* (Matt.) Liebl. are common in southern Scandinavia. Oak grows throughout the coastal areas of Norway, and *Q. robur* is found at its northern limit close to Trondheim (63°V18′N), Dalälven in Sweden and North of Turku in Finland (approximately 61°N). The northern limit of distribution for *Q. petraea* is approximately 100–200 km south of that for *Q. robur*, and *Q. petraea* does not grow north of Östergötland län, Skaraborg län or in Finland and the Baltic republics in general.

Pollen analysis of cores dating from the early Pleistocene indicate that when conditions were too cold for oak to grow in northern Europe, the species was maintained in refugia in central Spain, central Italy and the Balkans (Huntley and Birks, 1983; Bennet et al., 1991; Brewer et al., 2002). The pollen core data suggest that oak colonised Scandinavia from south to north after conditions had became warmer and the ice retreated. Oak pollen was first recorded in Scandinavia around 8500 BP, and was found in Finland from 8000 BP (Huntley and Birks, 1983). At 6000 BP the North Sea was largely dry land between Denmark and the British Isles, as was the Baltic Sea between Denmark, Germany and Sweden (Schou, 1969). More recently, the sea level rose creating a natural water barrier between the regions. Between 6000 and 2500 BP, oak was far more common in Scandinavia than it is today, and had a more northerly distribution (Gløersen et al., 1957; Ferris et al., 1998; Brewer et al., 2002) and constituted 10–25% of the total pollen record in certain areas of the Danish region (Huntley and Birks, 1983).

Ex situ and in situ conservation programmes have been implemented at varying intensities in the different Scandinavian countries, and tree breeding initiatives have been carried out in southern Sweden and Denmark. Many new areas of oak have been established in southern Scandinavia and oaks are considered a very important species for new plantations.

The high adaptive variation and quality of oaks has been widely recognised by foresters, and knowledge of provenances and their origin is a central issue for conservation and utilisation. Thus, there is a serious need for useful and practicable tools for certification of stands and seed. The ecophysiological demands and growth patterns of oak species are highly variable (Kleinschmit, 1993) and whilst utilisation is important, protection of existing oak stands is also of prime importance. This motive naturally led to establishment of provenance trials, and later on, as additional methods became available, e.g. molecular markers, a variety of different techniques were used to describe the genetic variability and population dynamics of oak stands.

In Denmark, many oak provenance trials have been established, and measurements from these trials have been used to define ecogeographic gradients (Jensen, 2000). Data from Swedish, Finnish and Norwegian provenances in Danish provenance trials also prove the existence of specific, adapted ecogeographic provenances (Jensen, 1993, 2000). Such ecogeographic patterning is probably naturally caused by the strong climatic gradient across Scandinavia that ranges from a typically coastal climate at the North Sea coast of Denmark and Norway to a continental climate in Sweden and Finland. Significant variation in day length and growth period also occur in oak forest growing between 54°N, in southern Denmark, and 61°N in Finland.

A number of studies have also been carried out to examine the genetic variation of Scandinavian oaks using biochemical and molecular markers. An isozyme study undertaken by Siegismund and Jensen (2001) showed no geographic pattern of allelic variation

within the Danish region, and generally this region showed no significant deviation in diversity measures (F_{ST} and G_{ST}) from the overall pattern observed for Europe (Zanetto et al., 1995; Kremer and Petit, 1993; Müller-Starck et al., 1992, 1993). This was also found to be the case for a more restricted analysis of Danish, Swedish and Norwegian stands (Zanetto et al., 1995). Finally, an isozyme study undertaken in Finland by Mattila et al. (1994) revealed less heterozygosity than expected compared to European oak populations (Zanetto et al., 1995), possibly indicating a marginal effect at the extreme range of this species in Scandinavia. Studies of oak cpDNA variation and reconstruction of possible routes of post-glacial recolonisation across Scandinavia have been described by several authors. However, so far only a few Scandinavian locations have been subjected to fine scale investigations using many haplotypes (Dumolin-Lapègue et al., 1997). Using point mutations in the $tRNA^{leu}$ intron of cpDNA that distinguish oaks of eastern and western European origin (Ferris et al., 1993), Jøhnk and Siegismund (1997) revealed that provenances from Jutland were nearly all homogeneous for western type cpDNA. In contrast, both western and eastern cpDNA types were present in eastern Denmark, and these populations exhibited a higher degree of intra- and inter-population diversity compared to those from Jutland. The authors proposed that Denmark was a possible transition zone between eastern and western European lineages. A more recent study by Ferris et al. (1998) on 166 trees at 48 sites across Sweden, the Åland Islands, Finland and Russia using the mutation in the TF^{cfo} intron detected a contact zone between eastern and western haplotypes in the Salpausselkä ridges in southern Finland. On the strength of these data, Ferris and co-workers proposed independent colonisation of the Finnish region from both east and west.

The recent fine scale discrimination of European oak cpDNA (Dumolin-Lapègue et al., 1997) offers the opportunity to further distinguish material of refugial origin. Using these markers this study aims to: (i) undertake a fine scale mapping of cpDNA variation in putative autochthonous stands in Scandinavia and Finland; (ii) analyse putative artificial stands of unknown or non-autochthonous origin; (iii) contribute to the deduction of natural migration routes for European oak tree species since the last glacial maximum.

2. Material and methods

2.1. Plant material

The plant material used in this study comprises samples collected by the Arboretum, Royal Agricultural and Veterinary University (55 putative autochthonous provenances from the Scandinavian region), and two Danish provenance trials established by the Danish Forest and Landscape Research Institute (36 provenances). Table 1 describes the total number of provenances collected and analysed for cpDNA variation in this study. In summary, a total of 474 individuals were analysed from 91 provenances and of these 51 samples were from Danish stands, 21 samples from Swedish stands, 10 stands from Finland and nine stands from Norway. Most of the stands were putative *Q. robur* stands, and only 10 putative *Q. petraea* stands.

Table 1
List of the collections (number of provenances) included in the cpDNA analysis. The material includes in situ collections and collections in existing provenance trials

Trials	Number of provenances	Native natural	Native planted	Non-native	Imported
1042	24	1	18	4	1
P-8	12	6	4		2
Collection DK	20	18	2		
Collection SF	10	9	1		
Collection S	18	16	2		
Collection N	7	7			
Total	91	57	27	4	3

2.2. Field collection

Of the 55 autochthonous Scandinavian populations, most are believed to be of natural origin and 29 were collected in situ, and 26 ex situ from field collected seed grown in the Arboretum nursery. Danish collections comprised 15 stands corresponding mainly converted shrubs and coppice with standards or very old stands/trees (>300 years; Oppermann, 1932) and five stand collections from the nursery at the Tree Improvement Station, Denmark. Swedish material was located with the help of SkogForsk, Sweden and Lennart Ackzell (Skogsstyrelsen). Fifteen of the Swedish samples were collected in forest reserves and in the archives of the Swedish Forest Gene Conservation Programme (Ackzell, 1996) and samples from three stands were collected at the nursery of the Tree Improvement Station, Denmark. Several samples were collected in landscape or forests believed to be of local origin. However, stands at Visingsö and probably Vanås Gods are planted. Norwegian and Finnish stands are most likely established from local seed sources, since oak plantations in these countries are uncommon. Norwegian material (seven stands) was sampled from a small provenance trial at the former Institute for Landscape Plants at Hornum in Denmark, and at the nursery of the Tree Improvement Station. Finnish material was collected by the Finnish Foundation for Forest Tree Breeding. One Finnish stand (Ekenäs, Brötet) is probably planted and its origin is unknown (Vakkari, pers. comm.).

2.3. Provenance trial

Two provenance trials, each comprising predominantly Danish material, were chosen for analysis. The first field trial (No. 1042 in Børsted forest, Bregentved forest district, Zealand), was established in 1967 and mainly represents east Danish stands consisting of trees from 24 provenances. Of the provenances, 22 were selected as Danish oak seed stands that have been approved for forestry production (Dansk Skovforenings Frøudvalg, 1969; Statens Herkomstkontrol med Skovfrø og-planter, 1982). The second field trial (No. P-8, C.E. Flensborg plantation, northern Jutland), was also established in 1967, and represents mainly west Danish oak stands from a total of 12 provenances. Eight of the provenances are west Danish scrub stands, one provenance (Wedellsborg, F.51.d) is an east Danish forest seed stand that has been approved for forestry production, one (Marselisborg) is an east Danish non-selected forestry stand, one (Kalmar län) is Swedish, and another (Spoordonk) is Dutch. Some of the scrub stands (i.e. Hald ege, Løjten enge near Langå and Tvis) have been recognised as seed sources for establishment of west Danish shelterbelts and other landscape purposes (Norrie and Brander, 1997).

In addition, the haplotypes of two Swedish provenances (Öglunda and Markaryd) are known from Ferris et al. (1997), and the haplotype of two provenances from each of Norway, Sweden and Denmark (Tjore, Birkeland, Hørbylunde, Løndal Næs, Uppsala, Stockholm, Uppsala) are known from the work by Dumolin-Lapègue et al. (1997). Three of the provenances were collected from the same population (Hald Ege, Kærgaard plantation and F.51.d Wedellsborg).

2.3.1. Collection, transport and storage of the material

Where possible, five trees were selected at random from each provenance/population. When sampling in situ, a minimum distance of 50 m was observed between trees to reduce the possibility of sampling closely related individuals. Twigs with at least five live buds or young leaves were cut from the top of the crown and labelled with trial number, provenance name and tree number. The base of the twigs was wrapped in damp newspaper, placed in plastic bags and stored cool to keep them fresh during transportation to the laboratory for molecular analysis where they were either used directly or stored at −20 or −80 °C.

2.3.2. Experimental procedures

Samples from the two Danish provenance trials were processed in the molecular laboratory at the Institute of Terrestrial Ecology, Edinburgh, Scotland, and Arboretum collected samples were analysed at the Austrian Research Centre Seibersdorf (ARCS) after DNA was extracted at the Danish Forest and Landscape Research Institute (DFLRI), Denmark.

2.3.2.1. DNA extraction and PCR–RFLP conditions. DNA was extracted from peeled, fresh bud material following the small scale CTAB extraction method of

Harris (1995) (for details, see Cottrell et al., 2002) or from ground frozen leaf material using the QIAGEN DNeasy plant extraction Minikit (Quiagen, 1996) following the instructions of the manufacturer.

Four cpDNA primer pairs DT (Taberlet et al., 1991), CD, AS and TF (Demesure et al., 1995) were selected to detect mutations previously identified by Dumolin-Lapègue et al. (1997). PCR amplification took place in 0.5 ml microfuge tubes, in a 25 μl volume reaction mix which contained: 10 ng DNA, 250 μM of each of dATP, dTTP, dCTP and dGTP (GIBCO BRL), $\frac{1}{10}$ volume 10× PCR buffer (supplied with *Taq* polymerase), 1 unit of *Taq* DNA polymerase (Amersham Pharmacia Biotech or Boehringer Mannheim), 2 mM $MgCl_2$ and 2 μM of each of the primers (Tables 2 and 3), with sterile, distilled water to make up the final volume. The reaction mixture was overlaid with mineral oil, to prevent fluid evaporation, and tubes were placed inside the heating plate of a DNA Thermal Cycler. Optimal amplification conditions are as previously determined by Dumolin-Lapègue et al. (1997) although optimal annealing temperature for primer AS was increased to 62 °C. Amplified products were digested with one of the three different restriction endonucleases (i.e. fragments DT and CD with *Taq*I, AS with *Hin*fI, and TF with *Alu*I, supplied by Amersham Pharmacia Biotech) according to manufacturer's instructions.

Digested fragments were separated under electrophoresis, using 8% native polyacrylamide gels (acrylamide: bisacrylamide, 39:1, BioRad) and TBE buffer (0.09 M Tris–HCl, pH 8.0, 0.09 M Boric acid, 2 mM EDTA, Na_2). An electric current (constant voltage 300 V, current 30–50 mA per gel) was passed through the gels for 3.25 h, after which they were stained with ethidium bromide and restriction patterns were visualised by UV transillumination and recorded.

2.3.3. Molecular data analysis

Variation in the restriction patterns was interpreted as length and site mutations in the cpDNA genome. Mutations were recorded as characters, and interpreted as haplotypes (Tables 4 and 5), as described by Dumolin-Lapègue et al. (1997). The description of the electrophoretic profiles of the haplotypes is in Annexes 1 and 2 of Petit et al. (2002).

Haplotype frequency was analysed in two ways; tree by tree and stand by stand. The tree by tree analysis revealed the frequency of trees possessing each of the haplotypes, while the stand by stand analysis revealed the frequency of populations that were homogeneous for each haplotype. Genetic diversity within and between populations (h_S and h_T) and

Table 2
Relative percentage of trees possessing different haplotype lineages. The Danish stands have been divided in autochthonous stands and planted stands. All stands in other countries are considered native

Country	Number of trees	Haplotype 1 (%)	Haplotype 5 (%)	Haplotype 7 (%)	Haplotype 10 (%)	Haplotype 11 (%)	Haplotype 12 (%)
Denmark—auto	145	52	0	12	19	17	0
Denmark—planted	124	42	1	18	16	1	24
Finland	44	75	25	0	0	0	0
Norway	33	36	0	0	33	31	0
Sweden	128	69	0	9	16	3	3
Total	474	55	2	11	17	8	7

Table 3
Relative number of monomorphic and polymorphic stands

	Number of provenances	Natural	Planted (import + native)	Unknown
Monomorphic	63	77%	54%	67%
Polymorphic	28	23%	46%	33%
Number of stands	91	57 stands	28 stands	6 stands

Table 4
Estimates of diversity and differentiations[a]

	Number of haplotypes	Number of populations	h_S	h_T	G_{ST}
Denmark	4	32	0.085	0.61	0.86
Sweden	5	20	0.083	0.47	0.83
Finland	2	10	0.13	0.45	0.72
Norway	3	9	0.10	0.74	0.86
Baltic republics	5	22	0.025	0.69	0.98
Natural stands	6	54	0.075	0.60	0.87
Planted stands	6	27	0.222	0.72	0.69

[a] h_S: within population diversity; h_T: total gene diversity; G_{ST}: gene differentiation over all populations. Data from Baltic republics is given for comparison (Csaikl et al., 2002b).

Table 5
Details of the haplotypes identified during this study (described in more detail by Dumolin-Lapègue et al., 1997)

Haplotype number	Fragment[a]								
	DT *Taq*I	DT *Taq*II	DT *Taq*III	AS *Hin*fI	AS *Hin*fII	AS *Hin*fIV	TF *Alu*I	CD *Taq*I	CD *Taq*II
1	9	1	2	3	2	2	2	1	3
5	1	1	2	1	3	1	3	1	2
7	2	1	5	1	3	2	3	1	2
10	1	2	3	1	2	1	3	1	2
11	1	2	3	1	2	1	1	1	2
12	1	2	4	1	2	1	3	1	2

[a] Notation is as follows: each fragment is identified by the primer/enzyme combination that has produced it, followed by a number indicating its position on the gel, in decreasing size. A second number, given in the main body of the table, identifies which particular mutation is present in each of these fragments (1–5 are indels and 9 is a site mutation). Again, numbers correspond to fragments of decreasing molecular weight.

population structuring (G_{ST}) was calculated by the methods described by Pons and Petit (1995).

2.3.4. Quantitative trait analysis

Correlation between haplotype and four quantitative traits (height, time of flushing, stem form, and epicormics) for trees from the provenance trial was assessed using an regression analysis of variance. Calculations were performed based on mean plot values and, consequently, only populations homogeneous for one haplotype were included. In total, 15 of the east Danish provenances, eight of the west Danish provenances and the single Swedish provenance were included. Mean trait values for the populations were obtained from Jensen (1993). The following model was used in the analysis:

$$\text{trait}_i = \mu + b\,\text{haplotype}_i + \varepsilon_i$$

where trait_i is the mean value of quantitative trait value for provenance i, μ the general level, b the coefficient, haplotype the haplotype of provenance i, and ε the residual value for provenance i. Data from the two trials were pooled in order to investigate interaction between haplotype and trial.

3. Results

Fig. 1 and Table 2 illustrate the geographic distribution of the six different haplotypes identified in the Scandinavian oak stands. The refugial origin of surveyed haplotypes is known based on previous work by Dumolin-Lapègue et al. (1997). The composition of the different lineages A–F in Europe is indicated in Figs. 1 and 2 of Petit et al. (2002).

The only haplotype of Italian origin, haplotype 1 (lineage C), dominates Scandinavia (55% of the trees) and is found throughout the region. Of the three haplotypes of Iberian origin found in Scandinavia, haplotype 10 (lineage B) is found at intermediate

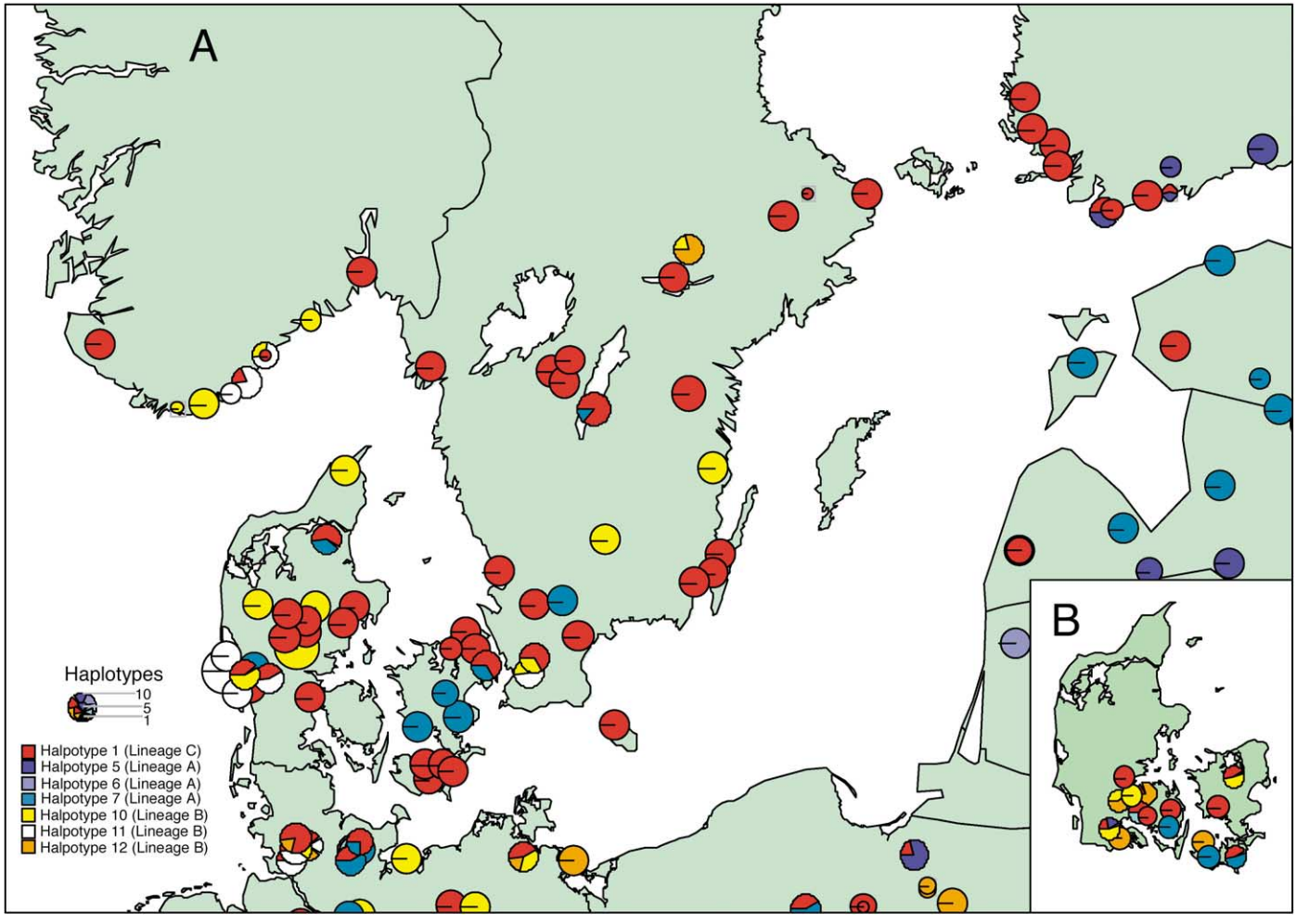

Fig. 1. Distribution of cp-DNA haplotypes (1–12) in Scandinavia: (A) presumed autochthonous stands; (B) planted stands of foreign or unknown origin (allochthonous). Haplotypes: Italian (I), Balkan (B) and Iberian (S). Adjacent data from neighbouring countries are presented by courtesy of König et al. (2002) and Csaikl et al. (2002b).

frequency (17%), but is scattered in patches throughout Scandinavia. For example, in Denmark, haplotype 10 was only found in Jutland. Haplotype 11 (lineage B) is rather uncommon (8% of the trees) and only found close to the coast in Jutland, at two locations in southern Norway, and in an old stand at Dalby Söderskog in southern Sweden. Haplotype 12 (lineage B) was found in some planted stands in Denmark (24% of planted trees in Denmark). This haplotype is particularly common within the provenances originating from Germany and the Netherlands (Zevenaar, Pederstrup, Stenderup, and Gavnø). It was also found in Danish planted populations, which are considered to be autochthonous (Graasten F.315, Wedellsborg F.51.d, F.51.f, Petersgaard F.96.i). Haplotypes of eastern lineage, originating from a Balkan refuge, were also found. Haplotype 7 (lineage A) is common in eastern Denmark but uncommon at other locations (11% of trees in Scandinavia). Haplotype 5 (lineage A) was found in four populations from eastern Finland but absent from the rest of Scandinavia except for a single tree found in a non-autochthonous stand in southern Denmark (Graasten F.316). Both *Q. petraea* and *Q. robur* shared the most common haplotypes at approximately equal frequency and there was no concordance between haplotype and species distribution across Scandinavia.

The majority of autochthonous populations are monomorphic (77%, Table 3), although a few old forests, for example Dalby Söderskog and Charlottenlund, are polymorphic. Of the high forest stands, which are almost certainly artificially established, only 54% are monomorphic. However, between them, these planted stands contain all the haplotypes found in Denmark. Of the four stands known to be of German or Dutch origin, two (Pederstrup and Gavnø) are fixed for haplotype 12, and the remaining two (Stenderup and Bidstrup) are polymorphic consisting of a mixture of haplotypes 10, 12 and 1. These results are also reflected in the estimates of genetic diversity and population subdivision (Table 4). Approximately, half of the individuals from Norway possess haplotypes of Iberian origin (haplotypes 10 and 11) the other half exhibit Italian haplotypes (haplotype 1). A similar split is also observed for Finnish material with half possessing the Italian haplotype (haplotype 1) and the other half one of Balkan origin (haplotype 5). Total genetic diversity (h_S) is higher in Denmark (0.085) than Sweden (0.038) and reflects the predominance of haplotype 1 in Sweden. The highest diversity estimates were recorded for Norway (0.10) and Finland (0.13). Genetic differentiation (G_{ST}) across all populations was high for natural populations (0.87), but lower for planted stands (0.69). However, within populations diversity and total gene diversity are higher for the planted stands (0.22 and 0.72, respectively) than for natural populations (0.075 and 0.6, respectively).

Significant correlation ($P < 0.001$) were found between haplotype and height and stem form for the east Danish field trial material (Table 6). However, differences in flushing time and epicormics were not significant. Overall, trees with haplotype 1 were the smallest (7.67 m), while those with haplotypes 7 (7.70 m), 10 (8.00 m) and 12 (8.63 m) exhibited increasing height order. In addition to being the tallest,

Table 6
Mean growth data (LSMEANS) for populations with homogeneous cpDNA haplotypes in experiment No. 1042, Børsted forest, Bregentved forest district

Haplotype	Number of populations	Height (dm)[a]	Flushing[b]	Stem form[c]	Epicormics[d]
1	17	76.7	4.1	2.8	2.4
7	5	77.0	4.0	2.9	2.6
10	6	80.0	4.3	2.8	2.6
12	9	86.3	3.7	2.5	2.3
Significance[e]		**	–	**	–

[a] Measured in 1990.
[b] Measured in 1980; 3: buds elongated; 4: leaves developing.
[c] Measured in 1990; 2: straight, one plane; 3: not straight, any plane.
[d] Measured in 1990; 2: 2–4 significant; 3: 5–15 significant.
[e] Significance level: $^{**}0.001 < P \leq 0.01$.

Table 7
Mean growth data (LSMEANS) for populations with homogeneous cpDNA haplotypes in experiment No. P-8, C.E. Flensborg plantation

Haplotype	Number of populations	Height (dm)[a]	Flushing[b]	Stem form[c]
1	4	65.3	3.3	2.6
7	2	67.0	2.6	2.6
10	5	69.0	2.7	2.7
11	4	70.0	1.9	2.5
12	2	65.5	2.5	2.6
Significance		–	–	–

[a] Measured in 1990.
[b] Measured in 1980; 3: buds elongated; 4: leaves developing.
[c] Measured in 1990; 2: straight, one plane; 3: not straight, any plane.

trees with haplotype 12 also displayed the best stem form (scale value 2.5), while those with haplotypes 1, 7, and 10 (scale values 2.8–2.9) were significantly less straight ($P < 0.005$). However, similar findings were not evident for material from the west Danish experiment (Table 7). A pooled analysis of height data from the two trials showed a significant interaction between haplotype and trial ($P < 0.005$), but there was no significant effect of haplotype alone. Results of a pooled analysis of stem form data were not significant.

4. Discussion

4.1. Post-glacial re-colonisation of Scandinavia and Finland

The post-glacial migration history of European flora and fauna has been studied and discussed by several workers. However, comparative analyses of results show quite different patterns and pathways of re-colonisation for different species both geographically and temporarily, which may reflect species vagility and successional stage (Taberlet et al., 1998). Across Scandinavia, a migration route following from south to north may appear on first inspection to be most probable, but a number of alternate pathways are possible for boreal species. For example, Lagercrantz and Ryman (1990) speculated that Norway spruce migrated from Finland to Sweden and Norway via the Baltic States (Lagercrantz and Ryman, 1990).

In this analysis of Scandinavian white oak, cpDNA haplotypes from all three southern refugia (Spain, Italy and the Balkans) were found in our sample of Nordic material. The overall pattern of post-glacial re-colonisation suggested from these data, is that oak migrated through Denmark to Norway, Sweden and Finland and such a model is in accordance with the pollen records of Huntley and Birks (1983) and Brewer et al. (2002). However, it also appears that a second route of re-colonisation proceeded through the Balkan states and into southern Finland, where the two migration routes met. Our results also suggest that material from the Appenine refugia (lineage C) was the fastest to migrate into Scandinavia having moved northwards out of the refuge through a narrow passage in Switzerland (Fineschi et al., 2002). CpDNA haplotypes from the Iberian peninsula (10–12) are scattered throughout western Scandinavia and intermixed with the Italian lineage. The Iberian lineages were not found in autochthonous stands at Zealand and therefore migration from Denmark to Sweden and Norway may have gone through Jutland or possibly from Germany and Poland across the Baltic Sea. It is possible that the Iberian lineages (B) may have been amongst the first wave of re-colonisation together with material of Italian lineage. However, the domination of Sweden by lineage C strongly suggests that only this lineage was successful in the primary colonisation of northern Scandinavia. Oak originating from the Balkans exhibits a split migration path. In the west, haplotype 7 does not extend any further north into Scandinavia than southern Denmark (the exception being one autochthonous population in southern Sweden). However, in the east, haplotype 5 has reached eastern Finland where it forms a contact zone with the Italian lineage.

4.2. Denmark

The distribution of the Italian (haplotype 1) and Balkan (haplotype 7) lineages in Denmark agrees well with a previous analysis of 25 Danish populations using the TF^{leu} primers (Jøhnk and Siegismund, 1997). There is a comparable measure of stand polymorphism between the two studies, where Jøhnk and Siegismund (1997) found that seven out of 22 (31.8%) putative native stands were polymorphic (10 trees per population analysed). Jøhnk and Siegismund (1997) also

found that a haplotype of eastern origin, was more common in southern Zealand, Funen and eastern Denmark than western Denmark and proposed an east–west transition zone in Denmark. Studies of the Danish pollen record suggest that oak migrated into Denmark by two pathways, from a western route via Holstein and via an eastern route from eastern Germany (Iversen, 1967). If this is so, then the Balkan lineage would have had to migrate into the southern Danish islands directly from eastern Germany. However, cpDNA data from Poland (Csaikl et al., 2002b), Germany (König et al., 2002) and Sweden, do not support this theory. Haplotypes of lineage A do not dominate the northern German coast, which would be the expected pattern for such a migration route. In contrast, the German map of cpDNA variation (König et al., 2002) suggests that oak probably migrated into Denmark entirely through Holstein comprising both lineages A and C. In northern Germany, haplotype 7 was found between Hamburg and Travemünde, and there is practically a continuum of oak forests possessing haplotype 7 in Holstein close to Denmark (König et al., 2002).

On the coast of western Denmark, a well-defined cluster of populations containing haplotype 11 can be found. This haplotype is also found in many stands in East Anglia in England (Cottrell et al., 2002). These populations could be part of a remnant of a North Sea population containing haplotype 11, with other remnant populations surviving in eastern England (Cottrell et al., 2002), northern France (Petit et al., this issue) the Netherlands and Belgium (König et al., 2002). However, human mediated introduction cannot be ruled out.

4.3. Norway

Haplotypes of Italian (haplotype 1) and Spanish origin (haplotypes 10 and 11) were found in Norway and migration into this region could have occurred either straight from Jutland or first via Sweden. The frequency of haplotypes in Norway is more similar to that found in Jutland than compared to Sweden (N.B. haplotype 11 is almost absent from Sweden). It is most likely that re-colonisation of Norway proceeded directly from Jutland across the channel between Norway and Denmark (Skagerrack) which would have been much reduced in extent at that time. However, to further investigate this possibility more forests should be sampled from the regions of Oslofjord in Norway and Bohuslän in Sweden.

4.4. Sweden

Haplotype 1 (lineage C) is by far the most common type in Sweden and is widespread across the whole country. These findings fit well with the preliminary results of Dumolin-Lapègue et al. (1997) and Ferris et al. (1998). In Sweden, haplotype 7 (lineage A) was very rare and only found in six samples. Apparently, lineage A did not co-migrate into Sweden along with the lineages B and C, and may indicate a later arrival of haplotype 7 in Zealand, Denmark. The within population diversity of Swedish stands is low compared to Denmark and may reflect a lower human impact on Swedish oak forests.

4.5. Finland

The junction of two different haplotype lineages in Finland gives reason to discuss the migration routes into Finland in more detail. Previously, Cajander (1921) and Skult (1965) suggested that oak had colonised Finland from Sweden. However, in the Nordic countries haplotype 5 is only found in Finland and has evidently migrated though Russia or the Baltic regions, since it originates in the Balkans (Csaikl et al., 2002a). Haplotype 7, which is also of Balkan origin, was not found in Finland, although it does occur on the northern coast of Estonia. Acorns could theoretically have crossed into southern Finland from Estonia by bird or water, as acorns are still germinable after floating for several days in saltwater (Ording, 1933). However, considering the haplotype composition of Estonia (Csaikl et al., 2002b) it appears more likely that haplotype 5 was introduced into Finland via Russia. The transition between lineages A and C in southern Finland is very distinct and was also described previously by Ferris et al. (1998). In a more detailed mapping of cpDNA variation within the region, Ferris et al. (1998) located this sharply defined contact zone in the region of the Salpausselkä ridges in southern Finland. Recent analysis of material collected from four of the same locations gave identical results, and indicate that haplotype 1 migrated into Finland from Sweden.

However, haplotype 1 was also found in the eastern part of Estonia and Latvia, and migration of this haplotype from the Baltic States cannot be excluded. Ferris et al. (1998) further discuss the stability of regional haplotypes, particularly considering the distinctness of the contact zone. Between 6000 and 3000 BP, the climate was warmer, and oaks would have been distributed 2–300 km further north than present. As the climate cooled, the limit of distribution would have moved south and so Ferris et al. (1998) conclude that even if the separation between Italian and Balkan types is well defined at present, this line of separation may not always have been located in its present day position.

4.6. Pattern of re-colonisation

Despite the fact that Denmark has a long history of oak exploitation, a number of key sites and trees are very old, suggesting that the contemporary pattern of cpDNA variation reflects natural processes. For example, the oaks of Jægerspris include the oldest oak (''King Oak'') in northern Europe with an estimated age of 1400–1900 years, and in Charlottenlund the ''Foresters Oak'' is approximately 800 years old (Holten, 1998). Both these oaks possess haplotype 1. The oaks of Skjoldenæsholm only 50 km from Jægerspris are several hundred years old as well and are all haplotype 7 and the old stand of Hald in Jutland haplotype 10. As observed by Petit et al. (1997), oak populations are often fixed for a single haplotype (77% of autochthonous stands), and occur in geographically patchy distributions. Within the limited Danish region, there are distinct groupings of haplotypes. There is a cluster of populations possessing haplotype 7 in southern Zealand, with clusters of haplotype 1 in northern Zealand and eastern Jutland and a small cluster of haplotype 11 in western Jutland. Both of these patterns of distribution of cpDNA variation probably reflect the particular mechanism of dispersal of oak which is most likely to be by single long distance migrants becoming well established in an area ahead of the main advancing front of colonisation. The genetic integrity of such mature blocks is then difficult to penetrate even after the main advancing front has passed and these genetic footprints of the leptokurtic dispersal mechanism remain for many generations.

4.7. Human influence

Many stands in the Nordic countries are of foreign origin, and this investigation shows clearly different patterns in allelic polymorphism for autochthonous than for allochthonous stands. The pattern also reflect the human influence through history.

Oak forests have been felled by man since at least 6200 BP, when he began to raise animals and cultivate crops. In addition, oaks were exploited for house and ship building in Norway, Sweden and Denmark. By the 17th century many oak forests had been seriously exploited, and much land was permanently transformed into agricultural area. Laws and regulations were introduced in Denmark, Norway and Sweden (Gløersen et al., 1957; Krahl-Urban, 1959; Fritzbøger, 1992) to protect existing oak forest. However, deforestation still continued and reached its peak around 200 years ago, when only 2% of Denmark remained forested (Danmarks Statistik et al., 1994). Nordic oaks suitable for construction became particularly rare between 1700 and 1800. In response to the timber shortage crisis, over the next 20 years new oak forest were established in Denmark and Sweden specifically for the purpose of providing wood for the naval construction as was common in other European countries between 1600 and 1900 (Krahl-Urban, 1959).

The establishment of planted forest has been systematically practised in Denmark since the 17th century but with limited success (Fritzbøger, 1992). However, oak trees from various origins may have been established as a consequence of human practices. Acorns were a valuable food source for livestock, particularly pigs, and could have been moved between countries. It is also known that acorns were intentionally imported from southern Europe for the purpose of establishing oak plantations. Acorns were imported from Poland to Sweden by Karl XII as early as 1700 (Krahl-Urban, 1959), and within the past 200 years, large quantities of acorns were imported into southern Sweden and eastern Denmark (Jensen, 1993). The establishment of some of these stands has been well documented, but documentation is not available for most of them. Consequently, the origin of most approved Danish seed stands older than 100 years must surely be dubious, and are most probably of mixed origin from different stands and countries. Most of the stands defined as Danish in the list of stands

approved for seed production (Dansk Skovforenings Frøudvalg, 1969) are most likely of Danish, Dutch or northern German origin. The findings of this investigation supports the existence of stands of dubious origins.

The origin of some of provenances investigated here may be brought into question as haplotypes found in these stands do not match with those of surrounding populations. This is the case for the Nørholm populations in western Denmark which possess haplotype 7, whereas trees from surrounding populations only possess haplotypes 1 and 11. Indeed, Oppermann (1932) previously suspected that some of the stands from this area were artificial. Two trees from Vinala in central Sweden are the only trees possessing haplotype 12 in our survey of Swedish material. Throughout the study of Scandinavian trees, haplotype 12 was mainly evident in planted imports and thus could be a useful indicator of foreign imports. The sample from Dalby Söderskog in Sweden includes four trees possessing haplotype 11, which is very rare in this part of Europe and again the only trees from Sweden in this survey found to possess this haplotype. Finally, on the coast of western Denmark is a well-defined cluster of populations containing haplotype 11, which is not found in neighbouring stands. This haplotype also occurs at high frequency in East Anglia in England (Cottrell et al., 2002) from where it could have been introduced fairly recently (less than 1000 years ago) by human transplantation. Alternatively, it could be a remnant of a North Sea population containing this haplotype (it is also found in northern France, Belgium and the Netherlands).

Many shrub and less intensively managed oak forests still exist in Sweden and Denmark. They are frequently located in remote regions and on poor soils, and many are remnants of shrubs or areas of coppice. Theoretically, these forests are extremely old, and normally considered to be autochthonous.

However, several of the stands in this current study have been shown to consist of several different haplotypes (e.g. Skindbjerglund and Petersgaard), or include rare, probably introduced haplotypes (e.g. Wedellsborg and Graasten F.316). It is possible that stands of mixed haplotype may be found at the contact zone between areas dominated by different types, but ancient natural stands are expected to be fixed for a single haplotype (Petit et al., 1997). The occurrence of more than one haplotype and/or the detection of rare haplotypes could therefore be a valuable indication that some or all of a stand is artificial and probably of mixed origin.

4.8. Gene conservation and tree breeding/seed certification

Several stands analysed within this investigation are important for breeding and gene conservation purposes. Analysis of cpDNA variation within these stands may provide a tool for the future management of oak genetic resources. The largest source of oak seed in Denmark is Hald Ege, however, it has come under scrutiny in the past, due to the possibility of illegal mixing of seeds from other oak populations. Analysis of cpDNA variation offers the potential to examine such problems, although the results are not always unambiguous. However, it is hoped that the development of a more sensitive detection system for cpDNA variation marker system will help to resolve the situation. Csaikl (pers. comm.) have already made the first steps towards this goal and through the use long-plate PAGE have found that several of the haplotypes screened for in this study are comprised of sub-types (e.g. haplotypes 1, 5, 7, 10 and 11). However, a reliable system for differentiating these sub-types was not developed in time to process all the samples in this study, and so the variation reported here is that detectable in standard PAGE systems.

Nevertheless, assessment of haplotypes is an efficient tool for identifying possible forest genetic resources and reproductive material. However, cpDNA analysis is restricted in the sense that it does not reflect quantitative traits or the general genetic properties of the nuclear genome. The relation between quantitative traits and cpDNA markers is further discussed by Kremer et al. (2002). Despite the fact that cpDNA type is neutral, provenances possessing haplotype 12 and growing in the eastern Danish provenance trial, 1042, grew better than material from other sources. However, these provenances are all of Dutch or German origin and their growth pattern is more likely to be explained by their adaptive properties (i.e. longer growing season) rather than the possession of a certain cpDNA haplotype (Jensen, 1993). Also a strong site effect was detected in the quantitative assessment of these provenances, and no significant correlation

between cpDNA type and performance was detected across sites. Ecogeographic zones for breeding and gene conservation should not be established based only on extra-nuclear markers, but other markers such as nuclear markers and adaptive traits are central to the issue of gene conservation (Graudal et al., 1995) and the use of a number of complementary marker and assessment systems for the management of oak genetic resources is stressed and encouraged.

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