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Chloroplast DNA variation of white oak in the Baltic countries and Poland

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Abstract

In this study we were interested how recolonisation of oak in the Baltic region occurred after the last ice-age. To analyse the chloroplast DNA (cpDNA) variation for white oak species at the more northerly limit of their distribution, a total of 394 samples from 54 locations from Estonia, Latvia, Lithuania and Poland were assessed for previously characterised as well as newly found cpDNA variation. Of the oak samples taken, *Quercus robur* was most frequently found throughout the entire Baltic area. *Quercus petraea* was only found and characterised in Poland.

A total of 13 different haplotypes were found within the Baltic area. Most frequent (75% of all samples) are members of lineage A (common in the Balkan refugial area) while 16% belong to oak from the Italian Pleistocene forest refuge and only a total of 9% originate in the Spanish refuge the majority of which is likely to be also allochtonous. Haplotype 7 of lineage A is found in 33% of the sample set in the entire Baltic region. Haplotype 6 is found at the more northern limit of the area. Haplotype 5 a more eastern member of the lineage is found at low frequency in the east of the region. Haplotype 4, rather sparse in Europe, is mainly scattered throughout Poland. Lineage C is represented by haplotypes 1 and 2 the former found close to the Baltic Sea while the latter, being an Eastern member of the lineage originating in Italy, is found at low frequency to the east of the region. Oak from refugial areas in Spain has limited impact on the area and is found exclusively in Poland. Such genotypes are found mainly close to the Baltic Sea in Poland and only 2% show up in uniform stands. Therefore members of lineage B could either have their most north-easterly limit of distribution in Poland or most likely they have been transferred there from possible German locations. In spite of the historical anthropogenic influences in the Baltic States as well as in Poland clear tracks of recolonisation could be identified.

Keywords: Chloroplast DNA (cpDNA); *Quercus robur*; *Quercus petraea*; PCR–RFLP; Phylogeography; Post-glacial recolonisation; Haplotype

1. Introduction

1.1. History

During the last glacial period many plant species, including oak, became restricted to the southern part

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of their present distribution, where climatic conditions allowed survival of the species. Those species that could not find appropriate conditions died during the prolonged ice-age period (Tallis, 1991). For those that survived, a recolonisation process started when the climate improved. This recolonisation process can be traced, in part, by analysis of pollen profiles in the layers of bogs, moors and cave floors; post-glacial pollen distribution maps have been established from these data (e.g. Firbas, 1949; Huntley and Birks, 1983; Brewer et al., 2002). On the basis of pollen core data oak refugia located on the Iberian peninsula, the Apennine peninsula and in the Balkans have been proposed (Huntley and Birks, 1983). Refugia west of the Black Sea and secondary refugia (regions colonised during the late glacial which have persisted during the climatic deterioration of the Younger Dryas, before the Holocene) have been proposed in more recent investigations (Brewer et al., 2002).

From knowledge on pollen deposits it is proposed that oak had migrated to Lithuanian territory earlier than 8000 BP. At the beginning of the Atlantic period (7500–5000 BP) oak was present in about 1–2.5% of the area studied. Climatic conditions for the growth of broad-leaved trees including *Quercus robur* were probably optimal about 5–4000 BP (Kaibailiene, 1990).

1.2. Botany

Two species of oaks, *Q. robur* and *Quercus petraea*, grow naturally in the region. *Q. robur* is the most abundant and grows throughout the region and even further north in Finland (Jensen et al., 2002) whereas *Q. petraea* is at the extreme of its northerly distribution limit in the border regions between Poland and Lithuania and between Poland, Byelorussia and Ukraine. It is relatively abundant in Poland but its distribution elsewhere in the region is confined to a single woods up to 70 ha in the southern part of Lithuania since the eastern distribution of *Q. petraea* is restricted by low winter temperatures (Dengler, 1992). It is not clear how such small *Q. petraea* 'islands' have appeared and can persist far from the limits of the major natural distribution range of the species. A possible explanation is that after the last ice-age (which had stopped in Southeastern Lithuania) *Q. petraea* specimen reimmigrated during the warmer (in comparison to nowadays) climate periods and survived on the hilly landscape as it is more competitive on these sites in comparison to *Q. robur*. Investigations on the degree of hybridisation in that area provide evidence for extensive introgression between the two oak species, which may be an indication for the natural occurrence of *Q. petraea* in this region. More recent introduction of *Q. petraea* would indeed result in more clear-cut differences between the two taxa (Baliuckas, unpublished results).

1.3. Ecology and economic background

Estonia, Latvia and Lithuania, located at the eastern, and northern Poland at the southern coast of the Baltic Sea, share unique environmental conditions. The climate in the Baltic countries is of a temperate maritime type, which represents a transition between the East European continental and the West European maritime climate.

Geomorphologically, the Baltic countries are within the western part of the East European plain showing hilly lowlands with podzolic soils in Lithuania, which turn into flat lowlands in Latvia. The prevailing natural vegetation is that of the forest zone of mixed coniferous and deciduous trees. Most broad-leaved forest tree species have declined due to various natural and anthropogenic reasons (Karazija, 1995; Baliuckas et al., 1996; Baumanis et al., 1996; Gailis and Smaukstelis, 1998). During the 16th century, oak accounted for about 15–20% of the total Lithuanian forest area (Lukinas, 1967). Severe felling of oak for the shipbuilding industry of the Russian Empire, without subsequent regeneration during the 18th and 19th century, reduced the oak forest to 2–3% of its original range by 1895. Currently, infestations of pests, such as the oak roller moth and pressure from grazing mammals such as deer, are resulting in further reductions of oak. Natural regeneration is also restricted by grazing mammals and by the fact that seed production is low because the larger, older trees are infested by wood rotting fungi (Karazija, 1995; Baliuckas et al., 1996; Baumanis et al., 1996; Gailis and Smaukstelis, 1998).

Forest coverage in Estonia has doubled since 1940 and now accounts for 48% of the total land area (Leemet and Karoles, 1995). Due to forest management, forest cover has also increased from 25% in 1923 to approximately 44% of the country boundary in Latvia in 1994 (Baumanis et al., 1996). Meanwhile,

in neighbouring Ukraine, the average proportion of forest coverage is 14%, with higher forest densities (up to 30 or 40%) only in certain regions (Mazhula et al., 1998). In Lithuania, forest coverage is 30% (Baliuckas and Danusevicius, 1998) and in Poland it is 28% (Korczyk, 1998).

In Estonia there is a long tradition in silviculture and 30% of the forests are established artificially. The frequency of oak is rather low with approximately 0.25% (Leemet and Karoles, 1995). In Latvia artificial plantations, frequently with reproductive material of unknown origin, were established by the end of the 19th century. In particular, *Q. petraea* trees of unknown origin were introduced mostly in the western part of the country (Gailis and Smaukstelis, 1998). In Poland, in addition to these two species, *Q. pubescens* is also found as a single small outlier in the westernmost part of Poland (Dygasiewicz et al., 1988). As told by local foresters during collection in Poland as well as in the Baltic countries, in the past forests have been traditionally managed by Germans or German-educated foresters. Therefore probably many oak stands have been established from imported material. Thus genetic variability might to a certain degree resemble the changeable history of the entire area.

In Latvia in spite of the high coverage of land with forests oak frequency is rather low as well. Forestry favouring the growth of coniferous species and neglecting broad-leaved species left forests with oak dominance at only 0.35% of total forests (Gailis and Smaukstelis, 1998). In Lithuania oak has a share of 15% in natural forest populations. Elsewhere oak is found at a frequency of 1.7% of forestland areas and mixed in whenever site conditions allow (Baliuckas, unpublished results). All over the Baltic countries, the silvicultural regime is favouring now birch, oak and ash instead of spruce and pine, depending on site conditions (Leemet and Karoles, 1995).

In the Baltic countries, boundaries of the range of distribution of different deciduous forest species cross or stop close by e.g. *Q. petraea* which grows in scattered outliers in the southernmost parts of Lithuania. The trend to replace coniferous and hard broadleaf species by softwood deciduous trees has been a distinct feature of the forests of the Baltic countries (Karazija, 1995). Past trends in silviculture as well as the problem of moist and wet sites on lowlands with a humid and cool climate have promoted the development of land covered with softwood and brush. This trend causes significant economic problems (Baumanis and Lipins, 1995).

Currently, the social, ecological and economic importance of forestry in Latvia is increasing. It is therefore essential that scientifically based forest management and utilisation systems are developed to ensure effective forest regeneration with appropriate material (Baumanis and Lipins, 1995). Concerns have been expressed regarding the health status of forests in Poland and there are reports that up to 50% of oak trees are damaged (Korczyk, 1998).

Oak decline in European forests could threaten the genetic resources of these species, given the small size of many stands. Additionally, with global warming the potential range of oak should increase at the expense of coniferous species, resulting in the need to describe the available genetic resources (Oszako, 1998). Detailed knowledge on available genetic resources and autochthony of oak in Europe will be indispensable to silviculture in the future.

1.4. cpDNA haplotyping

The chloroplast DNA (cpDNA) consists of a circular molecule which has been intensively studied in several plant species (e.g. Curtis and Clegg, 1984; Sugiura, 1992). In angiosperms, the cpDNA molecule is approximately 150 kb and contains a high proportion of coding regions (e.g. Zurawski et al., 1984). The highly conserved nature of the genome, along with the fact that it is maternally inherited makes it an ideal marker for population studies (Dumolin et al., 1995; Petit et al., 1993). In spite of the slow rate of cpDNA evolution, molecular analysis has revealed a complex pattern of mutational changes (Clegg et al., 1994). Insertions, deletions and point mutations all result in detectable variation using PCR/RLFP based methods. Analysis of approximately 10% of the chloroplast genome in the AS, CD, DT and TF regions (as described in Petit et al., 2002) is sufficient to distinguish the haplotypes belonging to the different lineages found in the major refugial areas. Previous studies in oak have shown that the cpDNA variation is largely geographically based and is independent of oak species (e.g. Petit et al., 1997).

Prior to the current study, cpDNA variation in oak in this region had only been examined in a few Polish

stands and no material from Estonia, Latvia or Lithuania had been tested. The current study harnessed the knowledge of local specialists to organise a collection of oak material from what was considered to be autochthonous populations in the Baltic area as well as southern Poland. This study completed the sampling of the northern area of Europe with Germany to the west (König et al., 2002), eastern central Europe to the south (Bordács et al., 2002) and Finland to the north (Jensen et al., 2002). Information from this region was required to determine the migration routes to Scandinavia more accurately.

Assessment of oak in different Baltic countries should enable autochthonous stands to be identified and thus will form a sound scientific basis for decision making when it comes to conservation and reforestation of oak in the Baltic region.

2. Material and methods

A collection trip through Estonia, Latvia, Lithuania and Poland was organised by the Danish partner and with the help of the local specialists the most important stands in the respective countries were sampled. The material was then analysed in the Austrian laboratory. The results obtained from this collection and analysis were pooled with those of the German partner in the EU project (A. König, Grosshansdorf) who collected and analysed material from Poland, which was growing in German provenance trials. Additional Polish material was analysed by the Polish co-author during her visit to the laboratory in France. The procedures used for samples processed in Austria are described below. The procedures used in France and Germany to process the Polish samples have been described elsewhere (Petit et al., 2002; König et al., 2002).

2.1. Collection, transport and storage of the material

Twigs with leaves from an average of five oak trees standing approximately 50 m apart were collected in each population. The bases of the twigs were wrapped in damp newspaper, then the whole twig was put in plastic bags, labelled with information regarding the location. The leaves were stored cool to keep them fresh and shipped as soon as possible to the laboratory for molecular analysis and either used directly or stored at −20 or −80 °C. Collections at a minimum distance of 50 km between locations were attempted, but in some cases it was impossible due to lack of oak stands.

2.2. Oak species determination

The determination of the species has been performed according to a standard method based on leaf morphology, which was developed during this project and used by all participants.

2.3. DNA extraction

At ARCS about 1 cm^2 of frozen leaf material was ground as described by Csaikl et al. (1998). DNA extraction was done using the QIAGEN DNeasy Plant DNA Extraction (1996) following the instructions of the manufacturer (Qiagen Minikit Handbook) except that after the second washing step with Buffer AW an additional spin step was included to dry the column completely. Two DNA fractions of 100 μl each were collected and the second fraction was used in a 1 + 4 dilution for the PCR amplification.

2.4. PCR primers

Four cpDNA primer pairs DT, CD, AS and TF were selected to detect mutations and to identify the different haplotypes described by Dumolin-Lapègue et al. (1997). The primers were designed by Taberlet et al. (1991) and Demesure et al. (1995).

2.5. PCR conditions

A modified PCR-protocol (in comparison to the standard method of Petit et al. (2002) was used. For one sample mix: 2× buffer 12.5 μl, Primer 1 (2 μM) 2.4 μl, Primer 2 (2 μM) 2.4 μl, H_2O 2.55 μl, expand long template PCR system (Boehringer Mannheim) enzyme 0.15 μl (i.e. ∼0.5 U by sample). To prepare 2× buffer (1 ml) mix 200 μl of 10× buffer 2 (provided with the kit, 2.25 mM $MgCl_2$), 10 μl dNTP (20 mM for each dNTP), dH_2O 790 μl. Additionally we changed the annealing temperature for primer pair AS from 57.5 to 62 °C. The amplified PCR products were either used immediately or stored at 4 °C.

2.6. Digestion of PCR products

All of the amplified fragments DT and CD were digested with *Taq*I at 65 °C for 3 h, AS with *Hin*fI, and TF with *Alu*I both at 37 °C for 5 h to overnight (restriction endonucleases supplied by Amersham Pharmacia Biotech). For a subset of approximately 100 samples TF fragments additionally have been digested with *Hin*fI. The digested PCR products were either used immediately or stored at −20 °C.

2.7. Separation of PCR products

Samples were separated on a 0.5 mm thick 8% PAGE gel in 1× TBE using "Rotiphorese Gel 30" (Roth, 30% acrylamide with 0.8% bisacrylamide).

2.8. Molecular data analysis

The haplotype nomenclatures as defined by Petit et al. (2002) were followed. The description of the electrophoretic profiles of the haplotypes is found in Annexes 1 and 2 of Petit et al. (2002). The composition of the different lineages is indicated in Figs. 1 and 2 of Petit et al. (2002). Several uncommon (referred to as 'rare') haplotypes have been found in the area and will be described together with those found in other areas in more detail by Csaikl and Burg (in preparation).

For *Taq*I digests of DT and CD the three biggest fragments each (No. 1–3) were considered, for AS *Hin*fI four fragments out of the five biggest (AS1, AS2, AS4, AS5; while AS3—the third biggest—is uniform in all oak samples investigated so far and was therefore omitted). At ARCS fragments TF *Alu*I have been investigated for all samples and variation at the second largest fragment recorded. In the subset where TF *Hin*fI patterns were additionally analysed variation at fragment 8 was recorded. Alleles '1' at the respective TF *Alu*I and TF *Hin*fI sites, characteristic for haplotype 11, have been found to coincide (data not shown). Variation in the restriction patterns was interpreted as length and site mutations in the cpDNA molecule. Mutations were recorded as characters. Different combinations of these characters within individuals correspond to a number of cytotypes, as described by Dumolin-Lapègue et al. (1998). The frequency of haplotypes was analysed in two ways:

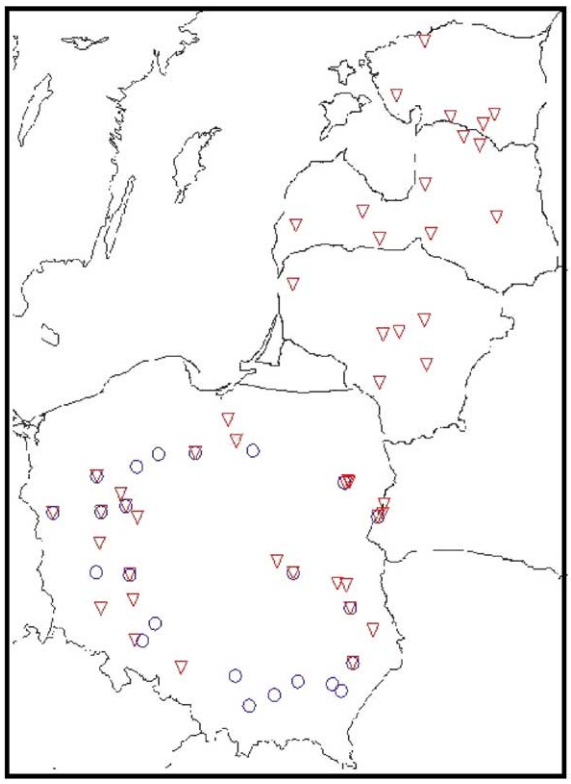

Fig. 1. Distribution of European white oak, *Q. robur* (△) and *Q. petraea* (○) in the Baltic countries.

tree by tree and stand by stand. Tree by tree analysis revealed the frequency of trees possessing each of the cytotypes, while the stand by stand analysis revealed the frequency of populations that were homogeneous for each cytotype.

The phylogenetic relationship of rare haplotypes E8, E11, E22 and E50 and their relationship to the standard haplotypes have been examined. Haplotypes E8 and E11 are new members to lineage C, while E22 and E50 are new members to lineage A. E8 has characteristics of both haplotypes 1 and 32. Haplotype E11 is identical to haplotype 1 only the characteristics found for AS are more commonly found in lineage B. Haplotype E22 is an intermediate between haplotype 6 and 7, fragment DT1 is identical to haplotype 6, fragment DT3 is identical to haplotype 7. Haplotype E50 is identical to haplotype 5, only fragment CD3 shows a new allele (see also Csaikl and Burg, in preparation). Other than at the seashore of the Baltic Sea haplotype E8 is found in quite a few of the

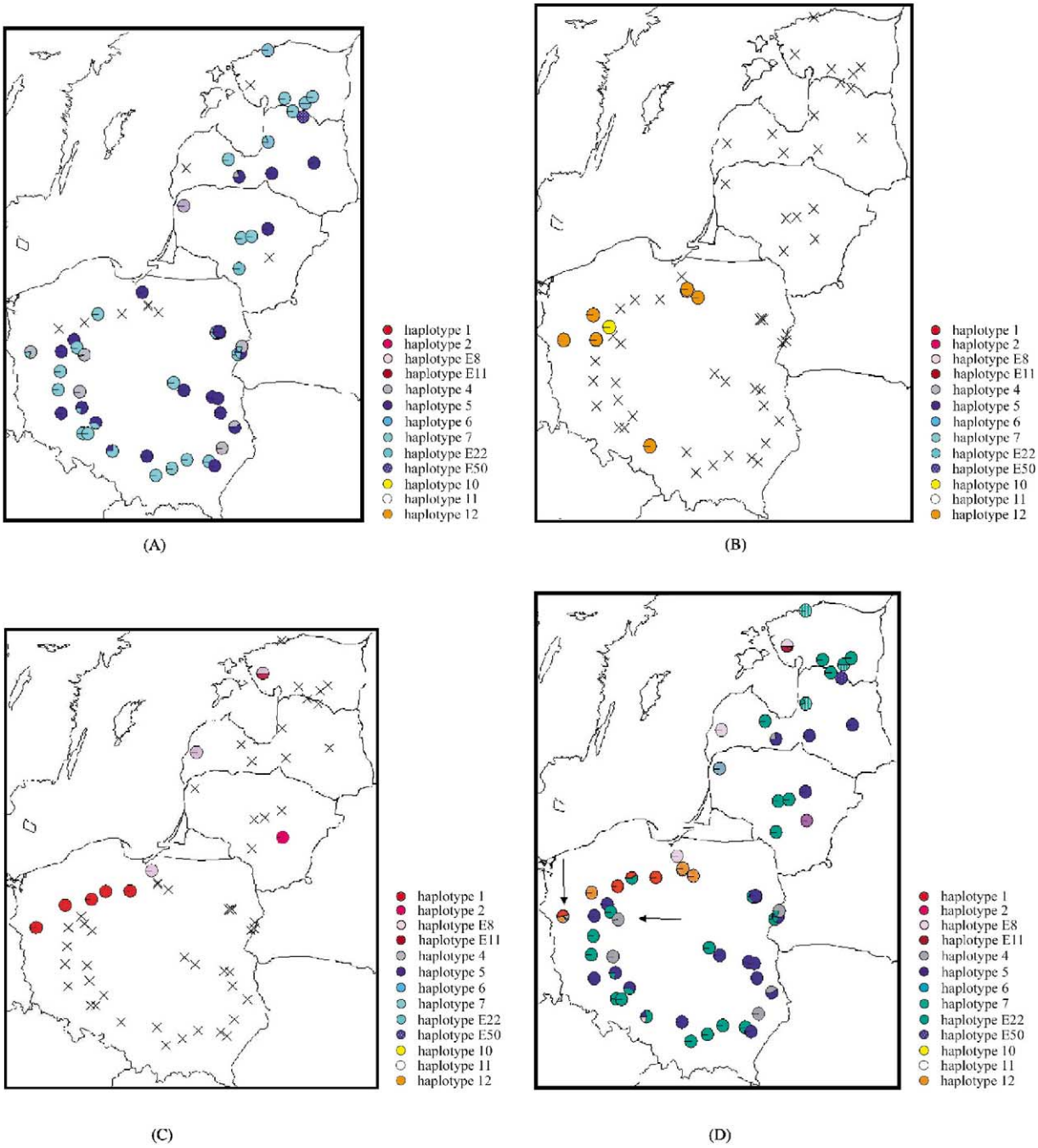

Fig. 2. Haplotype distribution of European white oak in the Baltic States and Poland. Equally sized circles show distribution of the 13 different haplotypes found in populations. The colour codes for the different haplotypes are indicated on the graph. X indicates population from a different lineage, arrows indicate populations with more than 30 samples investigated: (A) haplotypes of lineage A; (B) haplotypes of lineage B; (C) haplotypes of lineage C; (D) all haplotypes found in the area.

Austrian populations in the Danube valley (Csaikl and König, 2000; König et al., 2002) as well as at the east flank of the Alps (see Csaikl et al., 2002).

The geographical presentation has been done with the aid of MapInfo Professional Version 5.01 (MapInfo Corporation).

3. Results

3.1. Sampling achieved and species distribution

A total of 394 samples from 68 populations from the Baltic States and Poland has been included in the study. We have investigated 37 samples from seven Estonian, 40 samples from eight Latvian and 30 samples from six Lithuanian populations. All 21 populations consisted of *Q. robur* only. In Poland 287 samples were investigated from a total of 47 populations. Nine of the Polish populations included both *Q. robur* and *Q. petraea*, 14 comprised *Q. petraea* and 24 *Q. robur* only (see Table 1, Fig. 1). *Q. pubescens* from Poland, and *Q. petraea* growing naturally in Southern Lithuania were not included in the study. *Q. petraea* introduced to Latvia (see Section 1.2) was also omitted. Some of the Polish samples originate from a stand where the historical background was not clear but which could originate from reforestation with German material (A. König, personal communication). We kept the samples in the survey even though they turned out probably not to be autochthonous. Despite the relative rarity of oak in the region, which resulted in a relatively low sample size, this survey indicates the level and distribution of cpDNA diversity in the region.

3.2. Haplotype distribution

A total of 13 haplotypes were detected in the Baltic States and Poland (Fig. 2D). Most frequent (75% of all samples) are haplotypes of lineage A (haplotypes 4–7), as well as the rare haplotypes E22 (found in Estonia and Latvia) equally related to haplotypes 6 and 7, and E50 which is closely related to haplotype 5 (Table 1, Fig. 2A). To lineage C belong 16% of the samples with haplotype 1 found in Poland and haplotype 2 found in Lithuania. The rare haplotypes E8 and E11—both closely related to haplotype 1—were found in Estonia, in Latvia and as well as Poland (Table 1, Fig. 2C). The remaining samples (9%) belong to lineage B (haplotypes 10–12) and are exclusively found in Poland, some of them in mixed populations (Table 1, Fig. 2B).

3.3. Frequency of haplotypes

3.3.1. Lineage A

Haplotype 7 is the most frequent haplotype shared by 33% of all samples throughout all the four countries. It is the most common haplotype in Estonia, Lithuania and Poland where it occurs in 51, 50 and 31% of the samples from each country, respectively (see Table 1). It is not the most common haplotype in Latvia where it represents only 15% of the samples.

Haplotype 6 is found exclusively in one Lithuanian population (17% of the Lithuanian sample set). Haplotype E22 is found in one Latvian and two Estonian populations (13 and 22% of the countries sample sets, respectively). In total these represent 5% of the total Baltic sample set.

Haplotype 5 is found in 20% of the whole sample set of the region. It is the most frequent haplotype in Latvia where it is present in 35% of the samples. It is present in 21 and 17% of the Polish and Lithuanian samples respectively but is entirely absent from Estonia. The closely related haplotype E50 is found only in one population in Estonia (14% of Estonian sample set).

Haplotype 4 with an overall frequency of 16% is found mostly in Poland, whereas it is rare in Latvia and absent in the two other countries.

3.3.2. Lineage B

Haplotypes of this lineage are confined to Poland and account for 10% of the total and 13% of the Polish sample set. The B lineage samples consist of 29, 3 and 68% of haplotypes 10, 11 and 12, respectively.

3.3.3. Lineage C

Haplotype 1 represents 9% of the total sample set and is found exclusively in Poland (where it comprises 13% of the samples).

Haplotype 2 and the related rare haplotypes E8 and E11 make up 6% of the Baltic sample set. We find 1% each of haplotype 2 in Lithuania (17% of the local samples) and E11 (10% of the Latvian samples) while E8 is found in Estonia and Latvia (13.5 and 25%, respectively) and 1 sample in Poland (see Table 1).

Table 1
Distribution of haplotypes, lineages and species in different Baltic States and Poland. The number of individuals as well as the percentage in the respective countries and the entire sample set are given

		Lineage C					Lineage A							Lineage B				Total	*Q. robur*[a]	*Q. petraea*[b]	Total populations	Mixed population
		Haplotype 1	Haplotype 2	Haplotype E8	Haplotype E11	Sum lineage C	Haplotype 4	Haplotype 5	Haplotype 6	Haplotype 7	Haplotype E22	Haplotype E50	Sum lineage A	Haplotype 10	Haplotype 11	Haplotype 12	Sum lineage B					
Estonia	No. of individuals	0	0	5	0	5	0	0	0	19	8	5	32	0	0	0	0	37	7	0	7	0
	% of total Baltic sample set	0	0	1.3	0	1.3	0	0	0	4.8	2	1.3	8.1	0	0	0	0	9.4				
	% of sample set Estonia	0	0	13.5	0	13.5	0	0	0	51.4	21.6	13.5	86.5	0	0	0	0	100				
Latvia	No. of individuals	0	0	10	4	14	1	14	0	6	5	0	26	0	0	0	0	40	8	0	8	0
	% of total Baltic sample set	0	0	2.5	1	3.6	0.3	3.6	0	1.5	1.3	0	6.6	0	0	0	0	10.2				
	% of sample set Latvia	0	0	25	10	35	2.5	35	0	15	12.5	0	65	0	0	0	0	100				
Lithuania	No. of individuals	0	5	0	0	5	0	5	5	15	0	0	25	0	0	0	0	30	6	0	6	0
	% of total Baltic sample set	0	1.3	0	0	1.3	0	1.3	1.3	3.8	0	0	6.3	0	0	0	0	7.6				
	% of sample set Lithuania	0	16.7	0	0	16.7	0	16.7	16.7	50	0	0	83.3	0	0	0	0	100				
Poland	No. of individuals	37	0	1	0	38	62	60	0	89	0	0	211	11	1	26	38	287	33	23	47	9
	% of total Baltic sample set	9.4	0	0.3	0	9.6	15.7	15.2	0	22.6	0	0	53.6	2.8	0.3	6.6	9.6	72.8				
	% of sample set Poland	12.9	0	0.3	0	13.2	21.6	20.9	0	31	0	0	73.5	3.8	0.3	9.1	13.2	100				
Total area	No. of individuals	37	5	16	4	62	63	79	5	129	13	5	294	11	1	26	38	394	54	23	68	9
	% of total Baltic sample set	9.4	1.3	4.1	1	15.7	16	20.1	1.3	32.7	3.3	1.3	74.6	2.8	0.3	6.6	9.6	100				
	% of lineage	59.7	8.1	25.8	6.5	100	21.4	26.9	1.7	43.9	4.4	1.7	100	28.9	2.6	68.4	100	100				

[a] Occurrence of *Q. robur* in No. of populations.
[b] Occurrence of *Q. petraea* in No. of populations.

3.4. Haplotype distribution across species

All the haplotypes from lineages A and C, as well as the most frequent of the haplotypes of lineage B (haplotype 12) found in Poland are shared by both oak species. On the other hand, haplotypes 10 and 11 are found only in *Q. robur*. In the other three countries, only *Q. robur* samples have been investigated.

4. Discussion

Recolonisation of oak in central and northern Europe from the southern refugia proposes interesting questions like which refugia contributed to the recolonisation process, which migration routes have been taken and if new oak cpDNA haplotypes can be dectected. Evolution of new oak cpDNA haplotypes so far has been described as a characteristic of refugial areas (Dumolin-Lapègue et al., 1997). Information regarding the distribution of oak haplotypes in Poland and the Baltic States forms a crucial link between the Scandinavian investigation (Jensen et al., 2002) to the north, the central European study (König et al., 2002) to the west and the northern Balkan survey (Bordács et al., 2002) to the southeast. Oak migrated to the Baltic Sea as well as to Byelorussia around 9000 BP while oak in parts of Poland as well as the main part of the Baltic States arrived about 1000 years later (Brewer et al., 2002). On the other hand it cannot be excluded that existing oak stands are of rather recent origin and established during the phase of traditional European forest management using imported seed material. Therefore special care was taken in the Baltic States to collect from old oak stands. In the Baltic States out of 21 populations only one has shown different haplotypes (4 and 5), three have shown different but highly related haplotypes E8 and E11 and haplotypes 7 and E22, respectively. In Poland 10 populations with different haplotypes at one location out of a total of 47 have been described, being an indication of probably not autochthonous populations.

4.1. Species distribution in the Baltic region

Of the oak samples taken, *Q. robur* was most frequently found throughout the entire Baltic area. *Q. petraea* was only found and characterised in Poland where *Q. pubescens* was also found at a low frequency (Korczyk, 1998). *Q. petraea* found in the other countries are suspected to be allochthonous and thus have been excluded from the study. Overall, the proportion of populations fixed for a single haplotype was high in natural, probably autochthonous stands (75%) with uniform species and lower in stands with mixed species (40%). The overall amount of oak sampled in the entire region has been rather low. The sample size we have analysed in this survey is only about 40% in comparison to Spain, which covers about the same area as the entire Baltic region.

4.2. Genetic diversity in the Baltic region

For comparative analysis with the remainder of Europe the Baltic region had to be pooled with Scandinavia to form a 'northern European region' (Petit et al., 2002). Even then the number of specimen collected has been the lowest in any of the European regions. G_{ST} and N_{ST} values in the region have been described as surprisingly high with only marginal differences for the two species. For details see Petit et al. (2002). In the Baltic region a total of 13 different haplotypes of three lineages were detected. Four out of the 13 haplotypes identified were rare haplotypes. The two predominant haplotypes (5 and 7) were found in more than half of the entire Baltic sample set. Haplotype 7 is also the most frequent haplotype in Europe and has a distribution ranging from the Pyrenees to the Baltic countries and to the western part of Romania (Petit et al., 2002). Haplotype 5 is also quite widespread in Europe with distribution ranging from southern Italy in the South to Finland in the north, Romania in the east and at least scattered in Germany. Most frequent are the different members of lineage A (common in the Balkan refugial area) with 75% of all samples while 16% belong to oak from the Italian refuge and only a total of 9% originate in the Spanish refuge. The majority of populations showing Spanish types also contain haplotypes of a different origin and therefore are not likely to be autochthonous. No members of lineages E and F, quite frequently found in the eastern part of Europe (E as close as the Ukraine) could be found in the entire Baltic region.

4.3. Putative migration routes

Haplotype 1 from lineage C is found all around the Baltic Sea (see also Jensen et al., 2002; König et al., 2002). In the southeast haplotype 1 and the related haplotype E8 is found only very close to the seashore while haplotype 2 is found at a low frequency further to the east. Oak of the later haplotype is rather thinly spread from Italy all the way up to Lithuania (Petit et al., 2002). Further investigations in some of the eastern countries could clarify this situation. Another member of lineage C, rare haplotype E8 is found quite widespread in the entire Scandinavia as well as in Estonia and Latvia. Other than that it is found in 15 different populations in Austria as well (Csaikl et al., 2002; Csaikl and Burg, in preparation). The origin of E8 will have to be analysed with more refined analysis methods. A second rare haplotype (E11) of the lineage is found so far only in Latvia. Both types share most characteristics with Haplotype 1. E8 and E11 are phylogenetically very closely related and in one group with haplotype 32 which has been found in Hungary (Bordács et al., 2002). Haplotype 1 has most likely reached Poland through Germany while haplotype 2 probably moved north from Eastern Austria and West Hungary between the Sudetean Mountains to the west and the Carpathian Mountains to the east. It is thought that oak of haplotype 1 could have reached the southern coast of the Baltic Sea in Poland as a side branch to the proposed first wave of post-glacial migration of oak into Scandinavia (Jensen et al., 2002), and migrated further to Sweden either over the Danish islands or further east across the Baltic Sea (Jensen et al., 2002; Petit et al., 2002). Finland was probably colonised with haplotype 1 from the west (Jensen et al., 2002).

Finland additionally was inhabited by oak of haplotype 5, originating from an eastern European refuge in the Balkans. Migration north of haplotype 5 most likely lead through the Carpathian Mountains and from the south through the Baltic countries (Petit et al., 2002). On the other hand the isochron maps of pollen show oak arriving from the Balkan refugial area in Byelorussia at the same time as that from more western refugia at the Baltic Sea (Brewer et al., 2002). So haplotype 5 might as well have migrated into the Baltic region from the west. Intensified sampling in the area further east will have to clarify this point. The occurrence of haplotype 5 throughout the Baltic countries supports that the samples found in Finland (Jensen et al., 2002) are probably of natural origin. Haplotype 6 is rather sparse throughout Europe, the main occurrence is in western Romania and eastern Hungary. Haplotype 6 is found as far west as eastern Austria and in northern Croatia. Migration to the north has possibly taken place through the Carpathian Mountains. A few findings in Ukraine and Byelorussia mark the path up to the Baltic countries. Oak of haplotype 6 in Lithuania is the most northerly location yet discovered for this haplotype. Further north we have so far detected only the rare haplotype E22 which is an intermediate between haplotype 6 and 7. More intense sampling in Lithuania and in the countries further to the north and east might demonstrate a more northerly limit of haplotype 6 or will prove E22 to be the northern variant of this haplotype. In Latvia haplotype 4 has been found in a population together with oak of haplotype 5. Haplotype 4, rather sparse in Europe, is mainly scattered throughout Poland where it occurs in populations of uniform haplotypes as well as together with haplotypes 5 and 7. In the western-most population haplotype 4 is found together with haplotypes 1, 5 and 7 in a population which is mixed for *Q. robur* and *Q. petraea* as well. More intense sampling in northern Poland and Lithuania will have to confirm whether the finding of haplotypes 4 and 5 in Latvia is artificial or not. Another haplotype of lineage A (haplotype 7) probably arrived to Poland from Germany as well as through the Carpathian Mountains and found its way up to Estonia but it does not seem to reach Finland. Haplotype 7 spreads from more southern parts of Europe all the way up to the Baltic Sea, on the western side up to Sweden and on the Eastern flanks up to Estonia but it does not seem to reach Finland (Jensen et al., 2002; König et al., 2002; Petit et al., 2002). Haplotype 7 of lineage A is found in 33% of the sample set in the entire Baltic region. Haplotypes 6 and E22 (an intermediate between haplotypes 6 and 7) are found at the more northern limit of the area. The pattern of distribution suggests that migration of the two lineages occurred concurrently. In accordance with the data from Scandinavia (Jensen et al., 2002), the Italian lineage could have arrived somewhat earlier.

Members of lineage B are found mainly close to the Baltic Sea and only 2% show up in uniform stands.

Haplotype 10 of lineage B is found in two Polish populations, both of which show different additional haplotypes. So they are either not autochthonous or they are on the borders of their limit of distribution. The same holds true for haplotypes 11 and 12. Thus probably none of the Atlantic haplotypes of lineage B found in Poland are autochthonous. This would mean that the Atlantic types naturally reach as far east as the eastern part of Germany. Investigations with more refined technology will have to prove that point.

5. Conclusions

The pattern of haplotype distribution throughout the Baltic States and Poland appears fairly structured. In spite of the historical anthropogenic influence in the Baltic States as well as in Poland clear tracks of recolonisation described by this analysis blend in with analysis in the neighbouring regions. Oak from refugial areas in Spain has only limited impact on Poland. The pattern of recolonisation is in accordance with the pollen records of Huntley and Birks (1983). The finding of new rare haplotypes, members to the Balkan as well as the Italian lineage, is somewhat surprising suggesting that new genotypes can appear also in non-refugial areas. Information on the haplotype distribution can be used in the future for gene conservation and seed certification purposes.

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