

How Far to the East Was the Migration of White Oaks from the Iberian Refugium?

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The goal of this study was to investigate the postglacial recolonization pathways of the white oaks *Quercus robur* and *Quercus petraea* in Poland, and especially to evaluate the impact of Iberian refugium in this part of Europe. Chloroplast DNA polymorphism of 310 individuals older than 200 years was analyzed. Six haplotypes in total were found to differentiate three maternal lineages: the Balkan (haplotypes 4, 5, and 7), the Apennine (haplotypes 1 and 2), and the Iberian (haplotype 12). The most abundant were members of the Balkan (71.5% of all samples) and the Apennine lineage (23.1%), and only 5.4% of individuals were of Iberian origin. The geographic distribution of the three lineages is clearly structured. The northernmost territories of Poland are occupied by Apennine (haplotype 1) and Iberian (haplotype 12) lineages, whereas samples in central and southern Poland represents the Balkan lineage. The population structure might be the result of competitive colonization among lineages after migration from different refugia. It is likely that colonization of northernmost parts of Poland by the Balkan lineage was halted or at least hampered due to the arrival of the Apennine populations. The most significant result of this study concerns the presence and status of the Iberian lineage in Poland, which is most likely of natural origin.

Keywords oaks, postglacial recolonization, *Quercus robur*, *Quercus petraea*, refugia

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1 Introduction

During the last glacial period, vast areas of Europe were covered by ice for approximately 100 000 years. Distribution of living organisms, including oak species, was restricted to the southern areas, where favorable climatic conditions allowed their

survival. Glacial refugia were first defined by analyzing beetle exoskeletons and fossil pollen profiles in the layers of bogs, moors, and cave floors. On the basis of paleobotanical and genetic studies, three main Vistulian refugia for *Quercus* and other species in Europe have been proposed (Taberlet et al. 1998, Petit et al. 2002). They were

located on three Mediterranean peninsulas: the Iberian, the Apennine, and the Balkan. Recent investigations have revealed also additional secondary refugia established in Younger Dryas (Brewer et al. 2002).

Recently, the study of a genetic and evolutionary consequences of glaciation for plant species has become a highly important subject (Konnert and Bergman 1995, Demesure et al. 1996, Taberlet et al. 1998, Newton et al. 1999, Hewitt 1999, 2000, Cottrell et al. 2002, Palme and Vendramin 2002). Population genetic structure is the result of both present processes and past events (Taberlet et al. 1998, Comes and Kadereit 1998, Hewitt 1999). Pleistocene ice ages has shaped the patterns of genetic diversity seen in contemporary plant/forest trees populations (Jaramillo-Correa et al. 2004, Acheré et al. 2005, Ran et al. 2006, Pyhäjärvi et al. 2007). However, population structure may be seriously influenced by intensification of forestry and seeds and plants transfer over the long distances. Thus, artificial reforestations which might have disturbed the genetic structure of natural stands should be considered in reconstruction of species postglacial history

At present, molecular techniques are useful tools for the analysis of the impact of ice ages on plant distributions and genetics. Several investigations have verified the value of molecular markers for tracing the recolonization routes of different species. These techniques have been successfully used to test hypotheses regarding the existence of isolated glacial populations and refugia during prolonged glacial periods (Sinclair et al. 1999, Palme and Vendramin 2002, Petit et al. 2002, Godbout et al. 2005). Recently, analysis of geographic distribution of *cpDNA* or *mtDNA* haplotypes has provided insight into tree species postglacial history (Soranzo et al. 2000, Liepelt et al. 2002, Petit et al. 2002, Palme et al. 2003, Gömory et al. 2004).

In this study we aim to investigate in more detail the postglacial history of white oaks in Poland. A previous survey done by Csaikl et al. (2002) in Central and Eastern Europe did not clarify the impact of the Iberian lineage and the possibility that it is not autochthonous in that region. Our specific questions were: 1) what is the status of Iberian lineage in Poland? 2) is the lineage native to Poland? or 3) is there any evidence

that the lineage was transferred with seeds material from western Europe? This study attempted to describe the recolonization routes in Poland based on the most reliable and comprehensive material available. Hence, only individuals more than 200 years old were used which most likely reflect past native stands.

2 Materials and Methods

2.1 Sampling

A total of 297 trees were sampled from 65 oak stands throughout Poland. Two to twelve individuals were chosen from each stand selected based on the age and distributions of oak species. Only individuals older than 200 years were studied. As the forests in the northern and northeastern regions of Poland have been managed by foresters during the past 200 years, the age criterion was used to ensure an autochthonous population. Detailed age assessments of the trees were done using dendrochronological analysis. Tree ring samples were taken from at least two individuals in each stand. The samples were taken at breast height with a Pressler drill. Ages were calculated by counting the number of growth rings and adding 10 years, the period of time required to reach breast height. Very often, the wood core did not include the pith, so the distance to the pith had to be estimated. In case of protected trees age determination was not possible so only individuals that measured more than 300 cm perimeter at breast height were included for genetic analysis. The age of the protected trees was estimated based on the perimeter and average annual radial growth rates. In open areas the growth rate is about 2.5 mm per year and in forested areas it is about 1.8 mm per year (K. Ufnalski, unpublished study). In addition, 13 single trees were included in the study that are defined as natural monuments and thus may represent the oldest oaks in Poland. The single trees were located in cities, along the roads, in private areas, and also in the forests. Information about stands and plant material used is summarized in Table 1.

2.2 PCR-RFLP Methods

About 1 cm² of frozen leaf material was used for total DNA extraction following the method described by Dumolin et al. (1995). Total DNA was used as a template in the PCR reaction using three primer pairs for amplification of *trnD/trnT*, *trnC/trnD*, and *psaA/trnS cpDNA* regions (Demesure et al. 1995). These were used to distinguish polymorphisms as described by Dumolin-Lapegue et al. (1997). In most cases, polymorphisms in the DT region were enough to denote haplotypes found in Poland. Addition regions (CD and AS) were only necessary for haplotype identification in a few instances.

PCR amplification was carried out in a total volume of 25 µl containing about 20 ng of template DNA, 2.5 mM MgCl₂, 0.5 mg of BSA, 100 µM of each of dNTP, 0.2 µM of each of primer and 0.25U *Taq* polymerase, with the respective 1×PCR buffer (*Taq* polymerase and 10× PCR buffer were provided by Novazym, Poland), and it followed the cycle profile and primers described by Dumolin-Lapegue et al. (1997).

Amplified fragments of DT, and CD regions were digested with *TaqI* at 65°C overnight. Digestion was conducted in a total volume of 20 µl with 3 U of restriction enzyme and 15 µl of PCR product. AS fragments were digested in 20 µl reactions with 3 U *HinfI* at 37°C for 5 h (restriction endonucleases supplied by EurX, Poland).

Restriction fragments were separated in 8% polyacrylamide gels, using Tris Borate EDTA buffer (1×) at 300 V for 3–4 h. Vertical electrophoresis was conducted with a Hoefer SE 600 (Amersham Biosciences) under cooling conditions (+4°C). After electrophoresis, gels were stained with 0.5 µg/ml ethidium bromide. Results were visualized under UV light and documented with a BioCaptMw documentary system (Vilber Lourmat).

Variation in the restriction patterns was interpreted as a haplotype and the haplotype nomenclature was used as described in Petit et al. (2002). In the study by Petit et al. (2002) a total of 32 haplotypes pooled into six maternal lineages were described and mapped across Europe. For *TaqI*/DT/CD, the three biggest fragments were considered. Four fragments were scored for *HinfI*/AS.

3 Results

3.1 Maternal Lineages

Six haplotypes representing three maternal lineages were identified in this study: haplotypes 1 and 2 (Apennine lineage), 4, 5, and 7 (Balkan lineage), and 12 (Iberian lineage). The geographic distribution of these lineages is shown on Fig. 1. The composition of maternal lineages and frequencies of haplotypes are presented in Tables 1 and 2. Clear geographical structuring of maternal lineages in Poland can be seen (Fig. 1). The Balkan maternal lineage is dominant and it occurs mainly in the southern and central regions of the country. The northernmost regions contain lineages B (Iberian) and C (Apennine), with some individuals from Balkan lineage.

3.2 Frequency of Haplotypes

3.2.1 Balkan Lineage (A)

The most common Balkan maternal lineage was represented by 71.4% of samples (Fig. 1.). Haplotypes 4 and 7 were the most common, with equal frequencies 28.1% and haplotype 5 was 15.2%. The majority of Balkan stands were fixed for one haplotype (84%). In four stands, Balkan haplotypes were found together with Apennine (haplotype 1 or 2). This lineage included the oldest trees in this study. Of the Balkan individuals, over 35% were up to 300 years old, 56% were up to 400 years old, and 8.9% (5 individuals) were over 400 years old. The oldest individual was 748 years and it is one of the oldest oak trees in Poland.

3.2.2 Iberian Lineage (B)

Out of six Iberian haplotypes described by Petit et al. (2002) only haplotype 12 was detected in this study (Fig. 1). It was noted in 17 individuals (5.4% of the total sample set) and was confined to the northernmost part of Poland. All stands were monotypic. Youngest trees were 250 and 273 years old. Ten individuals were more than 300 years old. However, the age of the largest individual, estimated based on perimeter (1015 cm at

Table 1. Age of studied oak individuals and detected *cpDNA* haplotypes.

Sites	Number of trees	Age Counted	Age Estimated	Apennine lineage(C)		Balkan Lineage (A)		Iberian Lineage (B)	
				H1	H 2	H 4	H 5	H 7	H 12
1. Wiselka	5	433		5					
2. Smoldzino	5	230		5					
3. Bychowo	5		450				4	1	
4. Darzewo	3	300		2		1			
5. Mateblewo	5	250				2		3	
6. Jagodno	3		300, 400					3	
7. Kadyny	5	273							5
8. Kadyny 2	1		706						1
9. Frombork	3		497*, 300			1		2	
10. Laczki	2	340, 285				1		1	
11. Kruklanki	5	300						5	
12. Mikolajki	2		300					2	
13. Lipowo	5	360		2		1		2	
14. Nowy Las	5	390						5	
15. Jedwabno	5	390							5
16. Gasiory	5	312							5
17. Kruki	6		300				6		
18. Malbork	1		250						1
19. Sarna	2		300				2		
20. Nogat	2		613*, 230	2					
21. Gaj	5	250		5					
22. Lutowo	5	300		5					
23. Boroszewo	4	264						4	
24. Szubin	5	310		5					
25. Runowo	5	296		5					
26. Zlotow	5	320		5					
27. Kaczory	2		350	2					
28. Debno	5	460		5					
29. Radecin	5	475		5					
30. Prusin	1		270				1		
31. Ploty	2		300				2		
32. Konarzewo	2		340				2		
33. Kielpino	2		350				2		
34. Maszkowo	2		270, 400				2		
35. Choszczno	5	240		5					
36. Lichen	1		370	1					
37. Barlinek	7	300				7			
38. Rzepin	5	210				5			
39. Torzym	5	400						5	
40. Sulechow	5	300				5			
41. Sierakow	5	210				5			
42. Lopuchowko	5	302					5		
43. Zielonka	6	280					6		
44. Kornik	5	250				5			
45. Rogalin	6		300			6			
46. J.Male	1		300			1			
47. Zaniemysl	3		350			3			
48. Piotrowice	1		730*					1	
49. Sobolice	1		380				1		
50. Zabor	1		660				1		

Table 1. Continued.

Sites	Number of trees	Age Counted	Age Estimated	Apennine lineage(C)		Balkan Lineage (A)		Iberian Lineage (B)	
				H1	H 2	H 4	H 5	H 7	H 12
51. Milicz	2		320				1		1
52. Brzostowo	2		320				2		
53. Drołtowiec	7	250							7
54. Olesnica	5		300				2		3
55. Jugow	5	219				5			
56. Prudnik	5	222				2			3
57. Bierdzany	1		300			1			
58. Kłobuck	5	205			5				
59. Pszczyna	4		250						4
60. Walew	1		350						1
61. Zamczysko	5	243				5			
62. Tuchlin	5	210				5			
63. Warszawa	5	350				5			
64. Jezyska	5	250				3			2
65. Siemiony	6	320					6		
66. Bialowieza	5	360				5			
67. Hajnówka	12	360							12
68. Korczew	4	250			2	2			
69. Sawin	5	210							5
70. Nagłowice	5	280			5				
71. Suchedniow	1		300						1
72. Chelmowa G.	5	240							5
73. Dudkow	1		270						1
74. Pila	6		370			1	2		3
75. Barycz	5	213				1			4
76. Zagnansk	1		670*						1
77. Dydnia	5	217				5			
78. Chorkow	5	260			1	4			

* Age data from Pacyniak (1992)

Table 2. Frequency of *cpDNA* haplotypes and maternal lineages detected in white oaks in Poland.

	Apeninie lineage		Balkan lineage		Iberian lineage	
	Haplotype1	Haplotype 2	Haplotype 4	Haplotype 5	Haplotype 7	Haplotype 12
	19%	4.2%	28.1%	15.2%	28.1%	5.4%
Sum	23.2%		71.4%		5.4%	

breast height), was 706 years. It was the second oldest tree in the entire sample set.

3.2.3 Apennine Lineage (C)

The Apennine (C) lineage represents 23.2% of the total sample set and consists of haplotypes 1 and 2 (Fig. 1). The dominant was haplotype 1 detected in 19% of the trees. The haplotypes have exclud-

ing geographic distributions. Individuals carrying haplotype 1 were present in the north of Poland, whereas haplotype 2 was found in the south. The age of the oldest individual was estimated at 613 years and it had haplotype 1. Forty seven percent of the trees were up to 300 years old, 30.5% were between 300 and 400 years old, and 22.2% were over 400 years old. Both pedunculate oak and sessile oak were of Apennine origin.

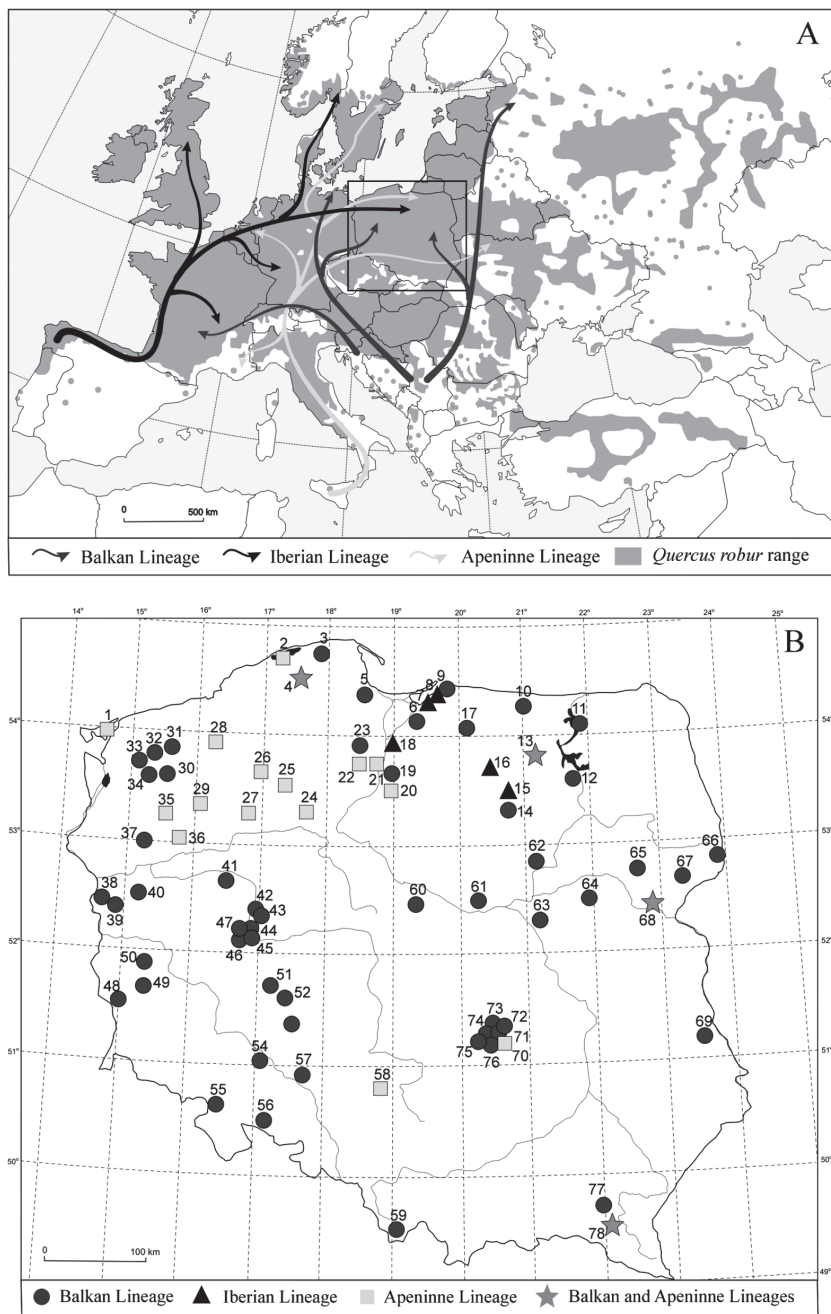


Fig. 1. A) Postglacial recolonization routes of *Quercus robur* and *Q. petraea* (based on Petit et al. 2002); B) Geographic distribution of maternal lineages in Poland.

4 Discussion

Our results indicate that haplotypes of Balkan lineage (A) are the most common in Poland, which is in agreement with findings by Csaikl et al. (2002). This lineage probably appeared in Poland earlier than the other two lineages. Isopollen maps show that 10 000 BP in southern Poland, oak pollen values were slightly greater than 0.5%. This is suggestive of a forthcoming oak migration front from southern Europe. By 9 000 BP, three distinct routes of oak colonization can be recognized including one along the Baltic Sea, southern one through the Moravian Gate and from the southeast. The maximum distribution of oak took place at 4500–4000 BP. From 3500 BP until the present, oak has consistently declined in pollen profiles (Milecka et al. 2004).

The Apennine lineage, the second most frequently found group (23.2% of individuals), is represented by two haplotypes. Haplotype 1 occurred frequently in the north of Poland. This haplotype is commonly found in Central and Eastern Europe and it even reaches Scandinavia, where it is the most frequent haplotype (König et al. 2002, Jensen et al. 2002). Dendrochronological age assessments showed that individuals with haplotype 1 are 200 to 613 years old, which strongly suggests that this lineage is native to Poland. Haplotype 2 was observed only in southern Poland and was detected in four populations. The movement of haplotype 2 in Europe proceeded from Italy northward through eastern Austria, Hungary, and Slovakia to Poland, and then further east. Isolated populations in Lithuania also carry this haplotype, as well as a group of five populations in Slovakia (Tutkova-van Loo and Burg 2004). Based on the results presented here, migration of haplotype 2 in Poland seems to be marginal.

Unlike the Balkan and the Apennine lineages, the Iberian lineage has not been previously considered autochthonous in Poland (Csaikl et al. 2002). Iberian haplotypes were found in a restricted area, mainly along the coast of the Baltic Sea in Poland. Furthermore, only 2% of Iberian individuals were noted in monotypic stands, lending further support to assertion that the Iberian lineage is not autochthonous. In the

data presented here, members of Iberian lineage were found only in monotypic stands. In regions where different recolonization paths intersect, fixation for one haplotype or a few haplotypes from one lineage in one population suggests its autochthonous origin (Petit et al. 2002). Thus, stands fixed for Iberian haplotype in Poland could be regarded as native. Moreover, the 200-year age criterion used for sampling renders these results especially reliable. The oldest individuals of Iberian origin were estimated to be approximately 700 years old. Hence, natural migration of oak from the Iberian Peninsula to Poland is likely. The Iberian origin has also been documented for Scots pine (Soranzo et al. 2000). This supports our findings that Iberian refugium indeed, could contribute to postglacial colonization process in that part of Europe.

Although Iberian populations are distributed only in the northwestern part of Poland, along the coast of the Baltic Sea, they appear to connect with the general recolonization pattern. The colonization of Europe by oaks from Iberian refugium took place along the coastal areas of Europe (Petit et al. 2002). None of the Iberian haplotypes was found in the eastern regions of Poland or in the other Baltic Countries (Csaikl et al. 2002). The data indicate that the Iberian lineage migration route reached Poland as far east as beyond the Vistula River. This lineage does not reach as far east as Germany, as it was previously stated. The question is, why more Iberian populations have not been detected in the northwestern territories of Poland. There are two reasonable explanations. The Iberian populations may have either become extinct during huge deforestation events noted especially in northern and central Poland since 17th, or they were missed during the sampling procedure due to their low frequency.

In previous study, three Iberian haplotypes were detected in Poland: 10, 11 and the most frequent haplotype 12 (Csaikl et al. 2002). We noted only haplotype 12. Discrepancies can be related to sampling procedure. In this study, old oak trees and stands were selected, as they would resemble past autochthonous populations. Csaikl et al. (2002) studied the most valuable stands, which may not be necessarily the oldest and native ones. Iberian haplotypes were found together with haplotypes from other lineages, which led

the authors to the conclusion that Iberian lineage in Poland is the result of seed transport. At 19th century forests in northern Poland were managed by Germans or German-educated foresters, and intensive seed transport might have occurred. Haplotype 10 found by Csaikl et al. (2002) and undetected in this study, was indeed reported in western Poland but in artificial oak stands younger than 200 years (Kedzierska 2004). Haplotype 10 was described by König et al. (2002) as the most abundant haplotype among those found in Germany (10, 11, and 12). Hence, it seems likely that oak seeds transport from Germany to Poland occurred in the past.

Due to its geographic location, Poland is a point of intersection for the three maternal lineages. The geographic distribution of maternal lineages is clearly structured which may result from succeeding colonization and further competition among populations originated in different refugial areas. The most significant result of this study states the natural presence of the Iberian lineage in Poland. However, due to its low frequency, influence of this lineage is difficult to evaluate. The practical aspect of this work is that the results gained could be used to identify autochthonous oak stands what is crucial in delimitating zones for conservation of genetic resources.

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Total of 31 references