

Czech University of Life Sciences

Faculty of Environmental Sciences.



**Phylogeography, postglacial gene flow, and population
history of boreal tree species *Alnus incana* in Central
Europe.**

Diploma Thesis

Author: Valérie Poupon

Supervisor: doc. Mgr. Bohumil Mandák, Ph.D.

Prague 2017

CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Environmental Sciences

DIPLOMA THESIS ASSIGNMENT

Valérie Poupon

Nature Conservation

Thesis title

Phylogeography, postglacial gene flow, and population history of boreal tree species *Alnus incana* in Central Europe

Objectives of thesis

The study will be based on previous data of the historical pattern of postglacial distribution range expansion of the boreal tree species *Alnus incana*. They indicate the presence of an effective refugium in central Europe located outside classical southern refugia confirming the existence of northern refugia for boreal trees in Europe. The postglacial range expansion of *A. incana* showed that Fennoscandian populations are not derived from eastern European ones, but from central-European ones that originated from populations in the Alps. Hence, populations responsible for the colonization of northern Europe at the end of the last Ice Age currently occurring in the Sudeten Mountains, the westernmost Carpathians and in the eastern Alps represent cryptic refugia situated in lowland regions of central Europe during the Last Glacial Maximum. It is however unclear from which populations or area the migration wave was exactly derived.

Main aim of the work is to collect sufficient amount of data to more precisely locate the founding population of northern Europe by analysis of highly variable nuclear genetic marker from significant number of individuals.

Methodology

We will analyse genetic variation at 18 nuclear microsatellite loci in 400 samples of *A. incana* from 20 populations. The populations will be collected in Sudeten, Šumava, Beskydy and Krušné hory Mountains. On the basis of this data we will reconstruct phylogeography, postglacial gene flow and population history of *Alnus incana* in Central Europe.

The proposed extent of the thesis

60 pages

Keywords

Alnus incana, postglacial migration, gene flow, microsatellites

Recommended information sources

- Bennett KD, Tzedakis PC, Willis KJ. 1991. Quaternary refugia of north European trees. *Journal of Biogeography* 18: 103–115.
- Lascoux M, Palmé AE, Cheddadi R, Latta RG. 2004. Impact of Ice Age on the genetic structure of trees and shrubs. *Philosophical Transactions of the Royal Society B* 359: 197–207.
- Magri D, Vendramin GG, Comps B, Dupanloup I, Geburek T, Gömöry D, Latałowa M, Litt T, Paule L, Roure JM, Tantau I, van der Knaap WO, Petit RJ, de Beaulieu J-L. 2006. A new scenario for the quaternary history of European beech populations: palaeobotanical evidence and genetic consequences. *New Phytologist* 171: 199–221.
- Tollefsrud MM, Kissling R, Gugerli F, Johnsen Ø, Skrøppa T, Cheddadi R, van der Knaap WO, Latałowa M, Terhürne-Berson R, Litt T, Geburek T, Brochmann C, Sperisen C. 2008. Genetic consequences of glacial survival and postglacial colonization in Norway spruce: combined analysis of mitochondrial DNA and fossil pollen. *Molecular Ecology* 17: 4134–4150.
- Tzedakis PC, Emerson BC, Hewitt GM. 2013. Cryptic or mystic? Glacial tree refugia in northern Europe. *Trends in Ecology and Evolution* 28: 696–704.
-

Expected date of thesis defence

2016/17 SS – FES

The Diploma Thesis Supervisor

doc. Mgr. Bohumil Mandák, Ph.D.

Supervising department

Department of Ecology

Electronic approval: 1. 12. 2015

prof. RNDr. Vladimír Bejček, CSc.

Head of department

Electronic approval: 7. 12. 2015

prof. Ing. Petr Sklenička, CSc.

Dean

Prague on 13. 04. 2017

Declaration

I hereby declare that I composed this diploma thesis, entitled “Phylogeography, postglacial gene flow, and population history of boreal tree species *Alnus incana* in Central Europe” independently, under the direction of doc. Mgr. Bohumil Mandák, Ph.D.

I also declare that all used literature and publications have been referenced and have been personally consulted.

I certify that it has not been previously submitted for examination which has led to the award of a degree.

Prague, 2017

Valérie Poupon

Acknowledgment

I would like to express my gratitude toward my supervisor, doc. Mgr. Bohumil Mandák PhD , for offering me the chance to work on a very interesting research topic and for his support every step of the way.

I would also like to personally thank Ing. Jan Douda PhD and Ing. Petr Vít PhD for helping during the sampling procedure on the field as well as Mgr. Martina Hadincová and Mgr. Jan Bílý for being so helpful during the time I spent in the laboratory.

Further, I am grateful to Ing. Lukáš Pospíšil and doc. Peter Kumble PhD for their availability concerning every administrative matter, but also for their kindness all along my master program.

Finally, I would like to thank my mother Claudine for her unconditional support and help as well as my three sisters, Isabelle, Claire and Cécile for encouraging me in my academic work and dreams even if it means that I am not home.

Abstract

This study explored colonization routes of the grey alder, *Alnus incana*, in Central Europe. It relies on data reported by Mandák et al. (2016) using chloroplast DNA and microsatellites analyses on 65 populations all over the tree European range. The authors suggested that Fennoscandia was not colonized by Eastern Europe and that from cryptic refugia in Central Europe, populations spread north to Fennoscandia. Due to the broad scale of the study, only few populations were sampled in central Europe thereby providing an imprecise picture of this area. In order to better understand the migration pattern in this area and maybe discover from which part of Central Europe exactly the Fennoscandian populations take their origin, we sampled and analyzed in this study 13 populations for 18 nuclear microsatellites loci from the mountains of Central Europe (i.e. Krušné hory, Český les, Šumava, Krkonoše and Beskydy Mountains).

Microsatellites dataset of 51 populations from Mandák et al. (2016) was also added in our analyses. In this diploma thesis, the field of study that we explored is phylogeography. More specifically, we analyzed here nuclear markers diversity of present populations to retrace the species' history. Our analyses showed the presence of relatively clearly defined genetic clusters in the Western Alps, Eastern Alps, South Eastern Europe (i.e. Carpathians and Balkans) and Scandinavia as well as a mixture of clusters in all the populations that we sampled in Central Europe. Moreover the common cluster of Scandinavia is fairly present in the North of the Czech Republic indicating that the colonization of Scandinavia probably took place from the cryptic refugia situated in the low land of Central Europe. The results also highlight the possibility that a connection has occurred between the populations of the Carpathians and the Baltic countries.

Keywords: *Alnus incana*, post glacial migration, gene flow, microsatellites

Abstrakt

Tato studie zkoumá kolonizační cesty olše šedé, *Alnus incana*, ve střední Evropě. Spoléhá na data uvedená ve studii autory Mandák et al. (2016), kteří užili DNA v chloroplastech a analýzu mikrosatelitů vyskytujících se v 65 populacích tohoto stromu v celé jeho evropské oblasti výskytu. Autoři předpokládají, že oblast Fenoskandinávie nebyla kolonizována z oblasti východní Evropy, ale že tomu tak bylo díky cryptické refugii ve středí Evropě a tyto populace se tak rozšířili severně do Fenoskandinávie. Vzhledem k širokému rozsahu studie bylo prozkoumáno pouze několik populací ve střední Evropě, které tedy poskytly ne úplně přesný přehled o této oblasti. Právě proto, abychom lépe pochopili migrační vzorec v této oblasti a možná i objevili přesně, ze které části ve střední Evropě pochází současná Fenoskandinávská populace, jsme v této studii shromáždili a analyzovali 13 populací a 18 jejich jaderných mikrosatelitních lokusů z několika pohoří v centrální Evropě (např. Krušné hory, Český les, Šumava, Krkonoše a Beskydy). Dataset mikrosatelitů 51 populací ze studie Mandák et. al (2016) byl také přidán do naší analýzy. V této diplomové práci nás zajímala zejména fylogeografie. Konkrétněji, analyzovali jsme markery jaderné diversity současných populací, abychom zpětně zdokumentovali druhovou historii. Naše analýza ukázala přítomnost relativně čistě definovaného genetického clustru v Západních Alpách, Východních Alpách, Jihovýchodní Evropě (např. Karpaty a Balkán) a ve Skandinávii, stejně jako směs různých clustrů ve všech populacích, které jsme shromáždili ve Střední Evropě. Víceméně běžný Skandinávský cluster je rovnoměrně přítomný na severu České republiky, což ukazuje, že se kolonizace Skandinávie pravděpodobně odehrála jako kryptická refugie pocházející z nižších poloh Centrální Evropy. Výsledky také zdůraznili možnost propojení mezi populacemi Karpat a Balkánských zemí.

Klíčová slova: *Alnus incana*, post-glacial migrace, tok genů, mikrosatelity

Table of Contents

1.	INTRODUCTION.....	10
2.	THE AIM OF THE DIPLOMA THESIS.....	11
3.	LITERATURE REVIEW.....	12
3.1.	Glacial ages causes, evidences and characterization.....	12
3.2.	Tree migration process.....	16
3.3.	Glacial refugia and migration patterns during the Holocene period.....	16
3.3.1.	South refugia hypothesis.....	17
•	Pollen records.....	18
•	Molecular studies.....	18
3.3.2.	Cryptic refugia hypothesis.....	21
•	Pollen limits.....	22
•	High migration rates during the re-colonization process.....	22
•	Large herbivorous species.....	23
•	Macrofossils charcoal and pollen evidence.....	23
•	Boreal versus temperate species.....	24
•	Species distribution modeling in relation to climate.....	25
•	Molecular markers.....	26
3.4.	Molecular markers: chloroplast DNA, mitochondrial DNA and microsatellites.....	26
3.4.1.	Chloroplast DNA and mitochondrial DNA.....	26
•	<i>Pinus sylvestris</i>	27
•	<i>Betula sp.</i>	28
•	<i>Salix sp.</i>	28
•	<i>Alnus incana</i>	29
3.4.2.	Microsatellites.....	30
3.5.	<i>Alnus incana</i>, general information.....	32
4.	METHDOLOGY.....	34
4.1.	Samples collection.....	34
4.2.	Sample processing.....	34
4.2.1.	DNA extraction.....	34
4.2.2.	Measurement of the absorbance and dilution.....	35
4.2.3.	DNA amplification and sequencing.....	35
4.3.	Data processing.....	36
4.3.1.	Microsatellites analyze.....	36
4.3.2.	Basic statistics.....	37
4.3.3.	Statistical analyses.....	37
5.	RESULTS.....	38

5.1. Basic population genetic parameters characterizing Czech and Slovakian populations.....	38
5.2. Population genetic structure characterizing Czech and Slovakian populations.....	40
5.3. Comparison of Czech and Slovakian populations with the rest of Europe.....	41
6. DISCUSSION.....	45
7. CONCLUSION.....	49
8. GLOSSARY.....	51
9. REFERENCES.....	53

1. Introduction

The end of the quaternary period was marked by 100 Kyr cycles of climatic variations composed of glacial and interglacial stages (Coluccia & Louse 2004). These variations are believed to have been greatly determining in today's species range and population genetic structure in both animals and plants (Hofreiter & Stewart 2009). As a matter of fact, environmental stressors can force population through three different ways: adaptation, migration or extinction (Aitken et al. 2008)

Primarily, early studies using pollen records (Huntley & Birks 1983; Bennett et al. 1991) demonstrated that, in Europe, animal and tree populations survived the glacial times in three South refugia i.e. Balkan, Italian and Iberian peninsulas. This can be referred as the classical "refugium theory" or "South refugia hypothesis".

However, more recent studies including new evidences such as macrofossils (Willis & Van Andel 2004), indication concerning large herbivorous species (Guthrie 1990), new climate assessment (Svenning et al. 2008) and the use of new molecular markers (Willis & Van Andel 2004; Pyhäjärvi et al. 2008; Tzedakis et al. 2013) tend to prove that some tree species, and more precisely boreal tree species, have survived the cold period further north than previously thought as small scarce populations in area called cryptic refugia. With the genetic studies on molecular markers, one fundamental idea is that the present population structure of one species encompasses the signature of its evolutionary events. It means that the study of one species current distribution of genetic variation can give insight into its history such as locations of refugia during the cold period and the colonization routes used afterwards (Palme & Vendramin 2002; Pyhäjärvi et al. 2008). The present study will analyze the molecular variation of the grey alder, *Alnus incana*, in Central Europe to explore the possibility that populations spread north from cryptic refugia in Central Europe, as proposed by Mandák et al. (2016).

2. The aim of the diploma thesis

The study is based on previous data of the historical pattern of postglacial distribution range expansion of the boreal tree species *Alnus incana*. They argue the following facts. *Alnus incana* was present within an effective refugium outside the classical Southern refugia during the last glacial period. The postglacial range expansion of *Alnus incana* showed that Fennoscandian populations are not derived from eastern European ones, but from central-European ones. The populations responsible for the colonization of northern Europe at the end of the last Ice Age are currently occurring in the Sudeten Mountains, the westernmost Carpathians and in the eastern Alps represent cryptic refugia situated in lowland regions of central Europe during the Last Glacial Maximum.

The main aim of the thesis was to collect sufficient amount of data to more precisely locate the founding population of northern Europe by analysis of highly variable nuclear genetic marker from significant number of individuals.

The research questions are:

- Can we confirm the presence of cryptic refugia in Central Europe?
- Can we discover from which part of Central Europe exactly the Fennoscandian populations take their origin?

3. Literature review

In the following literature review, will be discussed the causes and characteristics of the climatic variations of the end of the quaternary period, the migration process for tree species, the two current hypothesis concerning the location of tree refugia during the last glacial period and finally the research previously done concerning *Alnus incana*.

3.1. Glacial ages causes, evidences and characterization:

The climatic conditions on Earth have not always been the same; we can observe tremendous changes over the geological time scale, often correlated with the carbon cycle. A low value of the concentration of atmospheric CO₂, for example due to the burying of organic matter during the Carboniferous period, would be responsible for a limited green house effect and so a colder period. A high Value of the concentration of atmospheric CO₂, for example due to an intense volcanic activity during the Cretaceous period, would be responsible for a strong green house effect that would induce warmer temperatures.

During the Tertiary era (65 Myr), the climate became cooler and temperature oscillations have increased. These phenomena led to cycles of glacial and interglacial ages during the Quaternary period. While at first cycles were lasting 41Kry (2.4-0.9 Myr) they are now ongoing at a rate of 100 Kry (Hewitt 2000). In the 1930s Milutin Milankovitch, using the hypothesis on precession of the mathematician Joseph Adhemar (from 1842) as well as James Croll's work about precession¹, obliquity² and eccentricity³, built a mathematical theory known as the "Croll-Milankovich theory" (Raymo & Huybers 2008). This theory states that the three phenomena (i.e. eccentricity, obliquity and precession) are modifying the Earth's insulation and the energy received and explains climate change as a function of the Summer insulation received in the high latitudes of the northern hemisphere (Feng 2015). However, this theory is not able to explain completely this 100 Kry ice age cycle, or why it switched from 41Kry to 100Kry cycles, because from the three previously mentioned phenomena influencing the Earth's

¹ Precession: glossary p52

² Obliquity: glossary p52

³ Eccentricity: glossary p51

climate, the eccentricity (100 Kry period) is the one that should have the lowest impact. It has been proposed that various feedbacks may increase the eccentricity impact (Loutre 2003; Tuenter et al. 2005). However there are still doubts about the exactitude of the theory and additional data about the previous climate may be necessary to statistically prove or disprove the eccentricity theory.

Even though all the processes involved are not completely understood, there are many evidences of the climatic variations of the last 400Kry. We have cores⁴ of ice sheets, sea bed and lake bottoms that were analyzed for several clues.

One ice-drilling project, based on a collaboration between Russia, France and the United States at the Russia Vostok station in the East Antarctica, enabled the drilling of a 3,623m ice core in January 1998 (Coluccia & Louse 2004), which is the deepest ever recovered. This core represents a time period longer than 400 Kry and represents about the last four glacial-interglacial cycles. The researchers especially recorded the content in deuterium (proxy for local temperature changes), in dust (desert aerosols), the sodium concentration (marine aerosols), entrapped air bubbles (greenhouse gases, CO₂ and CH₄) and the Delta O¹⁸ of the O₂ (That was transposed afterwards to the Delta O¹⁸ of the atmosphere) informing about the global ice volume as well as the hydrological cycle. In addition to confirm the four cycles of climatic changes during the last 400 Kry, and to describe them, they characterized more in detail the last cycle (Figure 1). This cycle can be divided in several portions corresponding to different phases of the global temperature (Tzedakis et al. 2013). Notably we can see that the glacial period was characterized by cold but also warmer phases; the last glacial maximum was about 21Kry ago during the Last Pleniglacial⁵ (LPG); the current interglacial period (i.e. Holocene) started about 10-12 Kry.

⁴ Cores: glossary p51

⁵ Last Pleniglacial: glossary p51

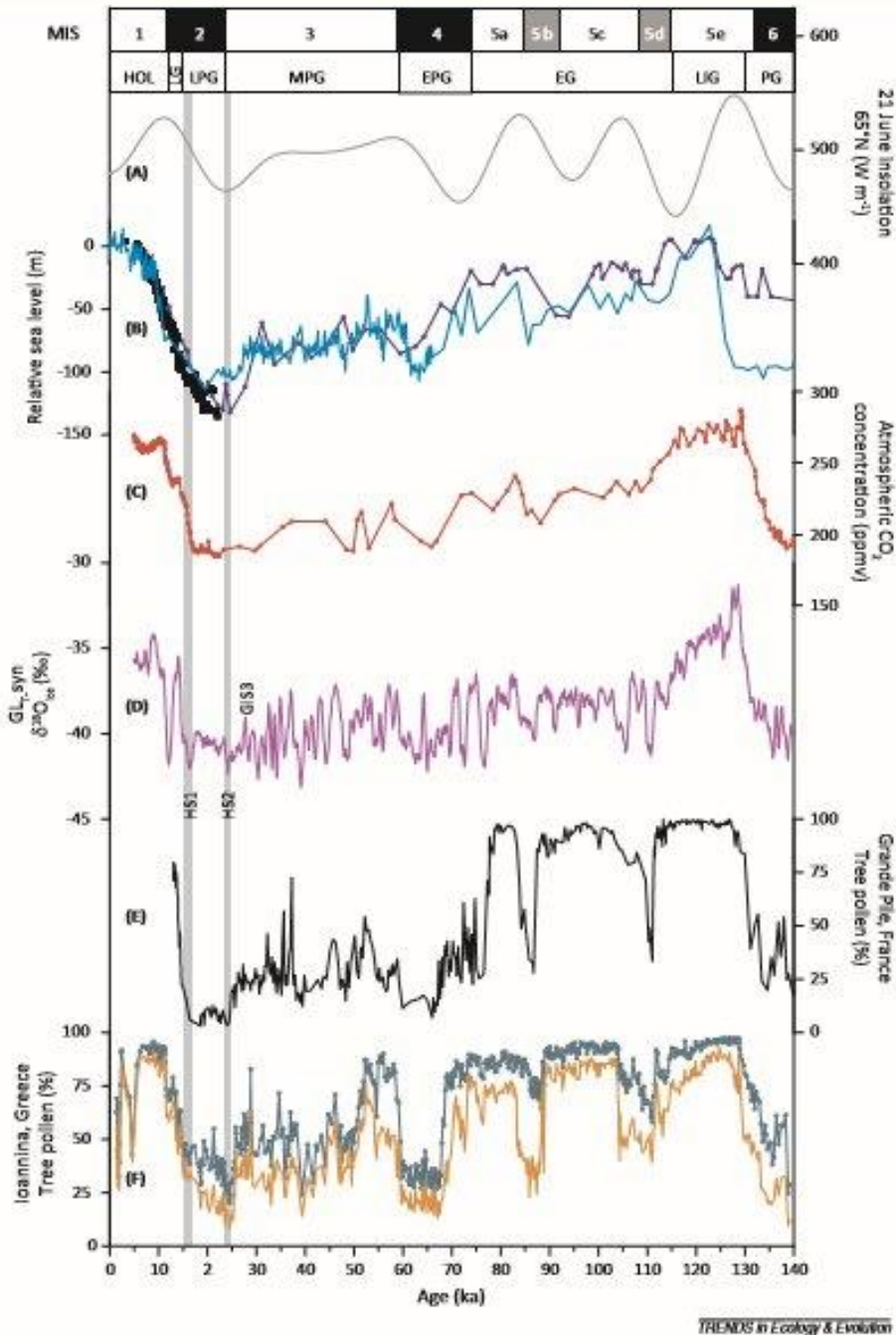


Figure 1: Graph representing the climate changes and vegetation responses during the past 140 Kyr. (A) Insolation on the 21st of June 65°N (W/m^2), (B) Sea-level reconstructions, (C) atmospheric CO_2 concentration in Antarctic ice cores; (E) changes in arboreal pollen percentages in the Grande Pile record, Vosges Mountains, France; (F) changes in temperate and pioneer (Pinus, Betula, and Juniperus) pollen percentages in northwest Greece. LIG: Last Interglacial, EG: Early Glacial 114-74kyr, EPG: Early Pleniglacial 74-59kyr, MPG: Middle Pleniglacial 59-24kyr, LPG: Late Pleniglacial 24-14.6kyr (which include the Last glacial maximum 21 +/- 2kyr), LG: Last Glacial 14.6-11.7kyr (that include the “late glacial maximum”), HOL: Holocene 11.7Kyr-today (Tzedakis et al. 2013).

Figure 2 illustrates the area covered by the Ice sheet during the last glacial maximum (Tzedakis, Emerson, and Hewitt 2013). The Ice sheet has receded for the last glacial period and has melted afterwards to its current extends. The extent of the ice sheet during the last glaciations is not the only difference that could be observed in the landscape during the LPG. In addition to an average 8°C colder temperatures (Willis and Van Andel 2004; Lomosino et al. 2006), the climate in Western Europe was more continental, which means colder winter and hotter summer, than the Eastern one which is in opposition with the current climate. As seen in the Figure 1, the concentration of atmospheric CO₂ was lower, which may have impacted the plant productivity and water use efficiency (Cowling & Sykes 1999). There is also evidence of a strong wind activity that might have forced the tree species to grow in more protected area (Leroy & Arpe 2007). It is important to know about this natural process, because the alternation of glacial and interglacial periods and their associated changes has had consequent impact on the Earth's living organism causing variations in their ranges over time (Hofreiter & Stewart 2009).



Figure 2: European paleoenvironments during the Late Pleniglacial. Maximum extent of ice (white) and continuous and discontinuous permafrost (large and small crosses) are shown(Tzedakis et al. 2013).

3.2. Tree migration process

To survive in a changing environment plants are following two main strategies, the adaptation and the migration (Aitken et al. 2008). While the first one relies on the genetic diversity of the population to promote the most adapted traits for the new environment, the second one is based on the movement of the populations, through seeds dispersal or vegetative growth, to more suitable locations.

Knowing that mature plants are sessile, the migration of population is possible due to the dispersal events that take place at the seed stage of the life cycle of the individuals (Petit et al. 2004). Depending on the species, the dispersal of the seeds can rely on different transportation modes such as wind, water, animals, gravity and human activity. While the migration itself is seed-dependent, the gene flow between populations can also be achieved through pollination.

It is important to remember that even if we talk about migration from one location to another, there is no deliberate intention. In nature the seed dispersal will occur in all directions, but only the one situated in favorable conditions will grow to a new tree that will produce new seeds. In a changing environment, such as a transition between glacial and interglacial phases, we will observe a latitudinal (and altitudinal) movement with migration at the leading edge, associated with long-distance seed dispersal, and extinction at the rear due to new unsuitable conditions (Petit et al. 2004). In Europe such migration process from South to North has started around 15-10 Kya ago (beginning of the Holocene period) (Heuertz et al. 2004). This climatically driven migration process has originated from the refugia locations in which tree species had survived the glacial times.

3.3. Glacial refugia and migration patterns during the Holocene period

As mentioned above, the northern part of Europe was covered with an ice sheet and central Europe was under permafrost during the LGM. Because of these climatic conditions, tree populations survived the cold period in more hospitable conditions further South than their today's location. This tree distribution preceding the Holocene

period as well as their colonization routes during the Holocene have been studied since a few decades and two main hypothesis have raised and will be discussed below.

3.3.1. South refugia hypothesis

This hypothesis was the first to emerge and describes North and Central Europe as tree-less. The North was mainly under an ice sheet while Central Europe was covered by cold steppe, tundra and permafrost plains. It explains that populations survived in Southern European regions. The three main refugia⁶ locations were the Iberian peninsula, Italy and the Balkans (Bennett et al. 1991; Taberlet et al. 1998; Hewitt 2000) as we can see on the figure 3. This hypothesis is supported by pollen records (Huntley & Birks 1983; Bennett et al. 1991) as well as molecular studies (Konnert & Bergmann 1995; Demesure et al. 1996 Dumolin-Lapègue et al. 1997; Lagercrantz et al. 1990; Taberlet et al. 1998). Concerning the post glacial colonization routes, they seemed to differ depending on the species (Taberlet et al. 1998).

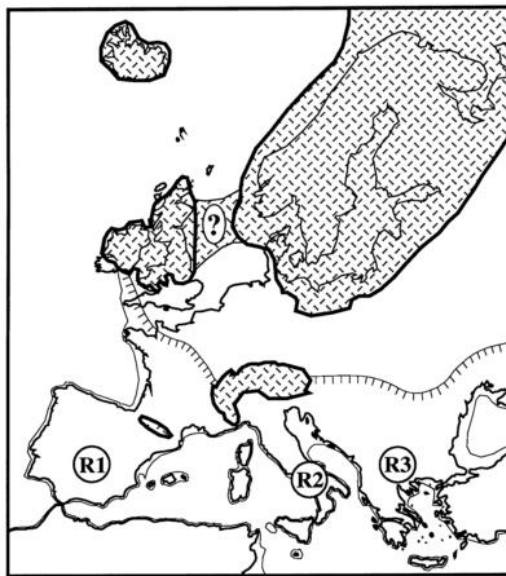


Figure 3: Maximum extension of ice sheets in Europe during the last cold period 20000 – 18000 years ago. R1, R2 and R3 indicate the three main potential refugia in Portugal-Spain, in Italy, and in the Balcans, respectively. The southern limit of the permafrost is indicated by the scale line (Taberlet et al. 1998).

- Pollen records

⁶ Refugia : glossary p52

First studies were based on pollen records, such as the work of Huntley and Birks (1983) gathering pollen data of the major tree and shrub species of Europe. They created maps at 500- or 1000- year intervals of the pollen frequency for the last 13,000 years. They suggested that for each species, the location of their first appearance would broadly match their glacial refugia areas. From there was inferred that during the last glacial period, European trees were located in East, South and West refugia such as the Balkans, Italy and the Iberian Peninsula (Huntley & Birks 1983). Another study (Bennett et al. 1991) used past and present pollen records as well as the modern tree distributions, a model of past climate and the physiography of southern Europe. These authors were arguing the existence of South refugia for current North European tree during the cold stage. According to the study, the trees mainly occupied mid-altitude sites in the Western Balkan, Alps and Italian mountains where the climate would have been suitable. They discussed that the reason why the Iberian Peninsula was not a significant refugia area might be that it was a too arid region and that mountainous massif were too small to support viable populations through all the cold stage.

- Molecular studies

Early genetic studies were also supporting the South refugia hypothesis. *Abies alba* DNA, extracted from seeds or buds, was studied (Konnert & Bergmann 1995) to describe the biogeographic history of the Silver fir populations in Europe. Polymorphic enzyme loci were investigated in order to analyze statistically the allele frequency distribution. Each of the low-frequency alleles that was found matched small specific areas that were already believed as being refugia for the fir populations. That way they highlighted five refugia that were used during the last glaciation (i.e. Balkans, two in Italy and two in France). However, silver fir populations in the Pyrenees and in the Southern Italy did not participate in the re-colonization process which was demonstrated by distinct gene pools.

Concerning *Fagus sylvatica* (Demesure et al. 1996) the analysis of chloroplast DNA⁷ in 85 European populations revealed two patterns. First, a clear homogeneity among populations in most of the tree range which can be explained by the re-colonization processes from one area only (Carpathians) that gave only a part of the genetic diversity to most of the nowadays populations. Second, the southern part of the range (i.e. Italy, Carpathian and eventually Pyrenees) has a higher variability explained by the fact that these regions represent the other glacial refugia that did not take place in post glacial re-colonization. Silver fir was also investigated later on by Magri et al. (2006). The study was based on an extensive amount of data (i.e. pollen, macrofossils, chloroplast DNA and nuclear markers) published or unpublished that they reviewed. The results agree with the presence of refugia in the Southern regions with confirmation of French populations and the addition of Balkan, Slovenian, South Moravian and South Bohemian populations. Moreover, the authors argued that most of these regions have expanded with a late start and did not spread much north. The colonization of Europe would be in fact from population that survived in Slovenia and France.

Another study (Dumolin-Lapègue et al. 1997) concerned *Quercus sp.* where 345 oak populations were sampled throughout Europe. Eight species of white oaks were included since through hybridization they share the same set of haplotypes. 23 haplotypes were identified and phylogenetically ordered. Afterwards they mapped potential postglacial routes. These routes seem to originate from three potential refugia (e.g. Italy, Balkans, and Spain) and are displayed Figure 4. They also noticed that most of the cpDNA polymorphism seemed to be anterior to the Holocene and that the observed structure was due to preexisting haplotypes divided between “expansion to the North” and “left behind in the South”. This also indicates a slow mutation rate within cpDNA marker.

⁷ Chloroplast DNA : Glossary p51

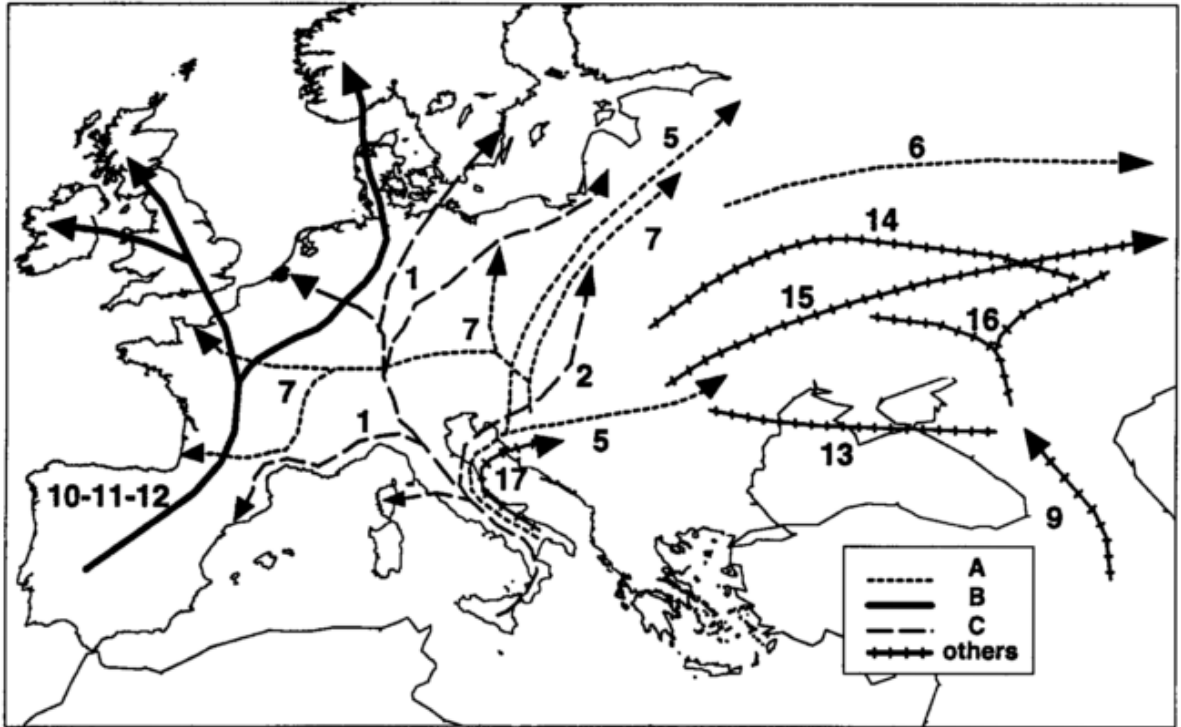


Figure 4: Probable colonization routes of *Quercus* sp. during the Holocene. The different types of arrows represent different lineages (Dumolin-Lapègue et al. 1997).

Even though the South refugia hypothesis mainly involved the survival of the glacial ages in the Balkan, Iberian and Italian peninsulas, studies of Norway spruce in the 80s-90s were showing a different pattern. *Picea abies* population structure was assessed using electrophoretically detectable genetic variability of 22 isozymes protein loci (Lagercrantz & Ryman 1990). The results were consonant with the pollen record of the species (Huntley & Birks 1983) showing three refugia areas in more Eastern refugia (e.g. Carpathians, Dinaric Alps and the area of Moscow) than the typical South refugia. The four species mentioned above (e.g. *Picea abies*, *Abies alba*, *Fagus sylvatica* and *Quercus* spp.) were reanalyzed (Taberlet et al. 1998) using a Brooks parsimony analysis that produced unrooted area phylogram. From this phylograms, refugia locations were mapped as well as the general pattern of re-colonization of each species (Figure 5).

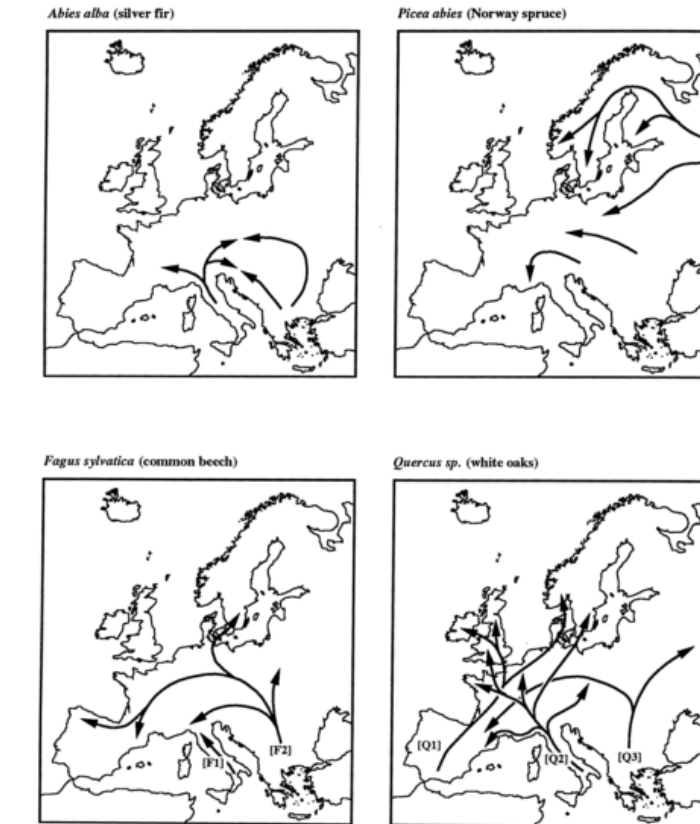


Figure 5: Post glacial colonozation routes for four tree species: *Picea Abies*, *Abies Alba*, *Fagus Sylvatica*, *Quercuss spp.* (Taberlet et al. 1998).

3.3.2. Cryptic refugia hypothesis

The fact that tree species have survived in southern refugia is now largely accepted. However, recent studies show more complicated patterns of the glacial and postglacial history. Several findings favor the idea that refugia, called cryptic refugia, were located further North than previously thought. These populations would have been small and/or presenting a low density. In the following paragraphs will be presented the different clues supporting this hypothesis including the pollen use limits (McLachlan & Clark 2004; Tzedakis et al. 2013), the “too” high migration rates inferred for the post-glacial times (Clark et al. 1998; Gugger & Sugita 2010; Tzedakis et al. 2013), the presence of large herbivorous species (Guthrie 1990; Guthrie 1991; Willis & Van Andel 2004; Sommer & Nadachowski 2006; Svenning et al. 2008), macrofossils evidences (Willis & Van Andel 2004), the difference between temperate and boreal tree patterns (Tzedakis et

al. 2013), the use of species distribution modeling (Svenning et al. 2008) and finally the recent studies on molecular markers (Willis & Van Andel 2004; Tzedakis et al. 2013).

- Pollen limits

As mentioned earlier, pollen records were largely used to assess the location of tree species during geological time. However, new studies are now supporting the fact that low density populations would not be accurately mapped using only fossil records (McLachlan & Clark 2004). In this article, the current location of beech tree (*Fagus grandifolia*), in North America, was extrapolated using pollen records in the recent sediments. They compared afterwards the modern range of the American beech with their results. They showed that for areas with a high density of individuals, the results were accurate while when individuals were broadly distributed at a low density, the distribution was not accurately mapped. This is due to the fact that populations are mapped when the proportion of pollen is greater than 0.5%. Indeed, for a lower amount we are not able to make the difference between a close low density population and a distant high density population. Within the cryptic refugia hypothesis, this result means that if cryptic small and scattered populations would have existed further North than in the South refugia hypothesis, they would not have been mapped. By consequence, an absence (<0.5%) of pollen data is not indicative of an absence of tree.

There are other issues related to the use of pollen records. First, there can be taphonomical biases due to mixing of geological layers, containing pollen, through erosion. Second, it is hypothesized that due to rough environmental conditions we could observe palynologically “silent tree” growing only by asexual reproduction (Tzedakis et al. 2013). Finally, trees are not all producing the same amount of pollen so the low concentration in one area might only mean a low production of pollen of the tree species nearby.

- High migration rates during the re-colonization process

These migration rates were inferred from the alleged locations of the tree species populations during the last glacial period and their current ranges. The North American tree-shift after the Last glacial period enabling to reach favorable climates was estimated at a rate ranging from 100 to 1000 m/y (Gugger & Sugita 2010). In Europe, this migration rate was estimated up to 2000m/y. But these rates are far to match with the empirical migration rate currently observed (<100m/y) as described by Reid's paradox of rapid plant migration (Clark et al. 1998). One of the possibilities that may explain the rapid migration of trees up North would be events of seed long distance dispersal. Another possibility would be the presence of populations in cryptic refugia that would also explain the bias in the migration rates (Tzedakis et al. 2013). In this case, one explanation does not refute the other one, both could be true.

- Large herbivorous species

The first pollen based studies that were indicating a “tree-less tundra” model in Central and North Europe during the LPG were repeatedly questioned by researchers of the ice-age mammalian fauna (Guthrie 1990); Guthrie 2001) that found evidence of numerous large herbivores species such as mammoth, woolly rhinoceros, reindeer, giant deer, bison or aurochs which need very productive environments (Willis & Van Andel 2004). The occurrence of trees during the LGM is consistent with the occurrence of mammal assemblages with mixtures of forest, tundra and steppe species at many locations (Sommer & Nadachowski 2006; Svenning et al. 2008)

- Macrofossils charcoal and pollen evidence:

Willis and Van Andel (2004) reviewed the presence of macrofossils charcoal in Central Europe that were found within a time spans ranging from 40Kr to 15KR years ago. For our concern, only macrofossils dated between 25Kr and 15Kr years ago are of interest because they represent remains of trees during the LPG. On the Figure 6 we can see that few species (i.e. *Picea sp.*, *Larix sp.*, *Pinus sp.*, *Salix sp.*) were to be found in the Hungarian regions during the LPG which is a bit further North than the classical South

refugia hypothesis. Moreover, like for the pollen data described above, the absence of microfossils does not necessarily indicate that there were no trees. In this case it is because of their rareness. As for the pollen evidence, they reviewed that the rare lake and peat sediments in central Europe (i.e. Czech Republic, Romania and Hungary) which can be considered as stratigraphically accurate, also support the presence of trees. Was found mostly *Pinus sp.* and *Betula sp.* pollen's but gender such as *Larix*, *Salix*, *Juniperus* and *Alnus* were also detected.

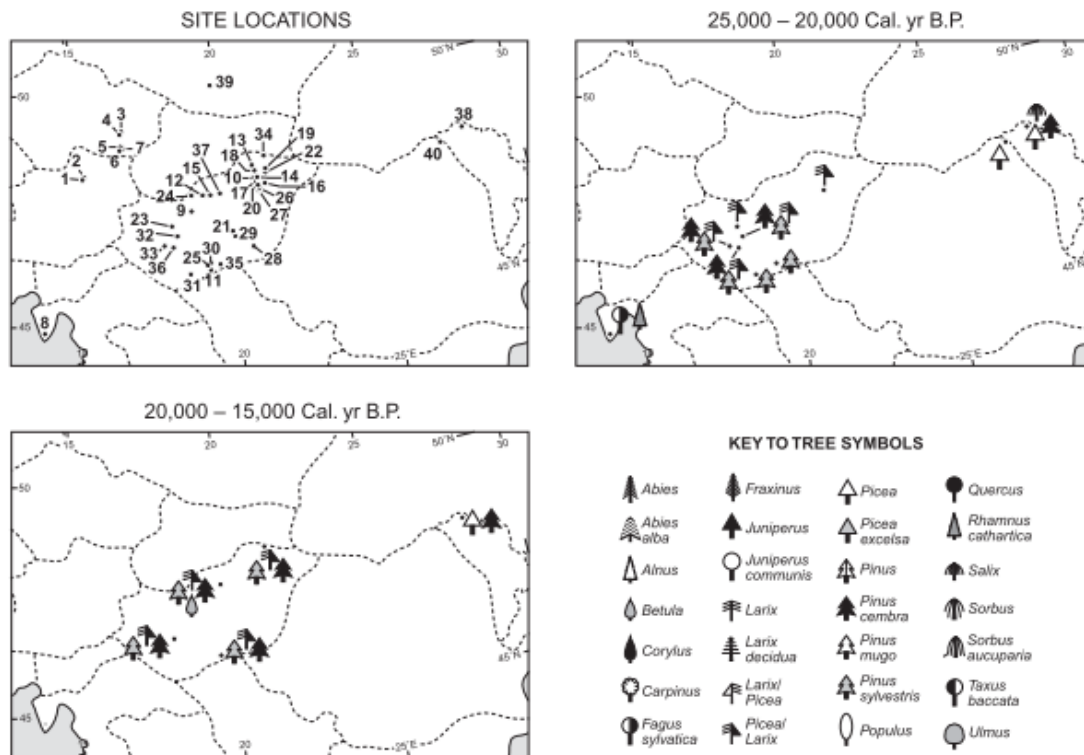


Figure 6: Location of the macrofossil charcoal sites and the tree species identified at each site for the time intervals 25,000–20,000 and 20,000–15,000 ka BP (Willis & Van Andel 2004).

- Boreal versus temperate species

Using evidence of pollen, macrofossils and genetic data as well as results of paleoclimate models was estimated the potential tree distribution in Europe (Tzedakis et al. 2013). Results clearly make the difference between temperate and boreal trees. Temperate trees were mainly absent north of the 45°N (except restricted locations in Eastern Europe) while boreal trees as well as being distributed further North were also

showing a West-East asymmetry. In Western Europe, boreal trees were not to be seen further than 46°N while in the Eastern Europe they could persist up to 49°N and also in higher latitudes on the East of the Fennoscandian ice-sheet.

- species distribution modeling in relation to climate

One of the main reasons for the Central and Eastern Europe tree-less landscape assumption is that the climate during the LPG was too extreme to allow tree growth (Willis & Van Andel 2004). But new climate simulations such as the Stage 3 Project simulation and the Laboratoire de Météorologie Dynamique's LMDZHR simulation (Svenning et al. 2008) are supporting the idea that climate weren't nearly as severe and inhospitable as previously anticipated and would have allowed the growth of boreal and boreal plus temperate trees in Central and Eastern Europe as we can see on the Figure 7. However, the low CO₂ concentration and strong winds would be responsible for low density populations restricted in moist microsite.

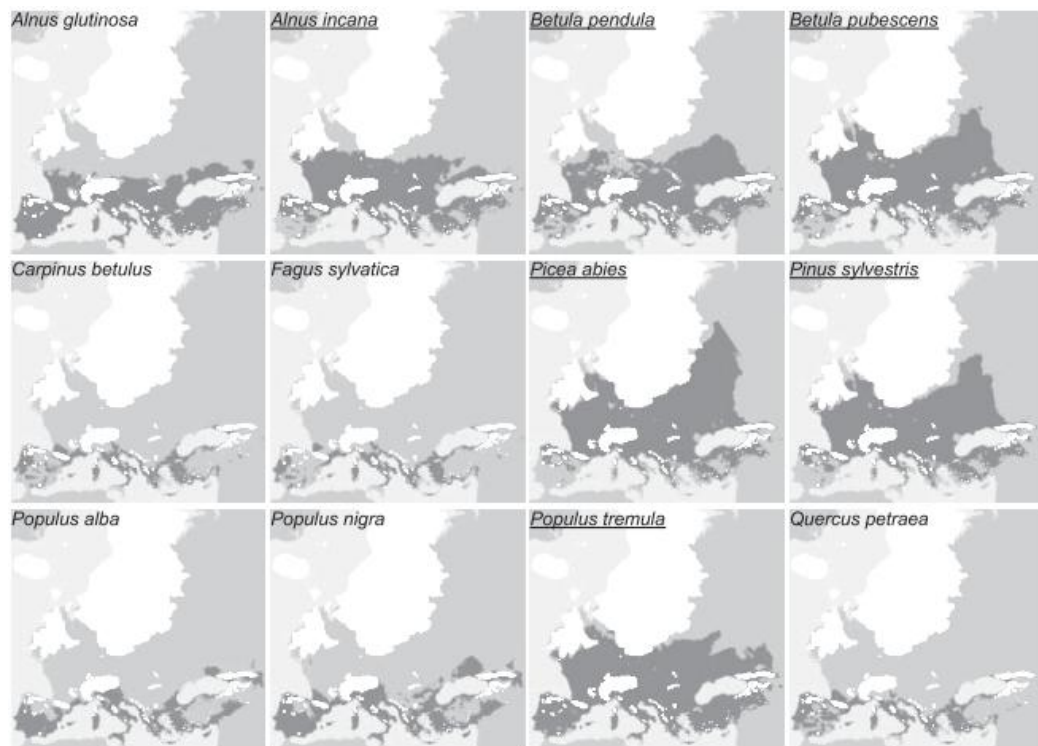


Figure 7: Modelled potential distributions of 12 temperate and boreal (underlined) trees in Europe during the LGM (LMDZHR simulation)(Svenning et al. 2008).

- Molecular markers

Since the past decade, several studies using genetic legacy of several tree species in Europe have supported the importance of the South refugia during LPG. However, some of them, concerning boreal tree species such as *Picea abies*, *Alnus*, *Populus*, *Betula* and *Salix* are showing a more Northern and scattered distribution which support the North refugia hypothesis (Willis & Van Andel 2004).

The different molecular markers used to retrace the history of tree populations, and findings concerning refugia locations and migration routes of several boreal species will be summarized in the next section.

3.4. Molecular markers: chloroplast DNA, mitochondrial DNA⁸ and microsatellites⁹

The study of the past movements of species populations using genetic markers is called phylogeography and has been developed only recently compared to the field of palynology. Genetic studies are using DNA analysis of present day species to help understanding the past movements of populations. They are usually using cytoplasmic DNA (maternally inherited) such as chloroplast DNA and mitochondrial DNA but nuclear markers such as isosymes and microsatellites have also been used recently (Box 4). To reconstruct the tree postglacial history, the studies are based on the assumption that long term isolation of populations during the LPG would increase their genetic differentiation. Moreover the populations issued from post-glacial colonization should have a lower genetic diversity due to consecutive founder events (Willis & Van Andel 2004). Refugia populations are assumed to have a high genetic diversity due to their survival through several glacial cycles with the presence of endemic and ancestral alleles (Tzedakis et al.2013).

3.4.1. chloroplast DNA and mitochondrial DNA

⁸ Mitochondrial DNA : glossary p52

⁹ Microsatellites : gloassary p52

- *Pinus sylvestris*

The history of Scots pine, *Pinus sylvestris*, was inferred from mitochondrial DNA that was sampled for 37 populations in all its European range (Pyhäjärvi et al. 2008). An indel (insertion of base in the DNA) of 32pb was found in the Northern European regions while it was not found in the Mediterranean peninsulas as we can see on Figure 8. This difference is supporting the cryptic refugia hypothesis in accordance with macrofossil data that suggest a refugia near Hungary and Czech Republic (Willis & Van Andel 2004). However, the colonization route used by *Pinus sylvestris* is unclear between an Eastern origin and the colonization from Central Europe refugia. In the study of Vidyakin et al. (2012) the mitochondrial DNA of *Pinus sylvestris* was studied in the Northern part of European Russia. The results suggested that Fennoscandian and Northern Russia Scots pine populations do not originate from the same glacial refugia. In consequence Fennoscandia was probably not colonized by North Eastern Russia refugia. However, the origin of the colonization is still unclear. Both studies insist on the use of more genetic marker and more sampling for a better view of *Pinus sylvestris* history.

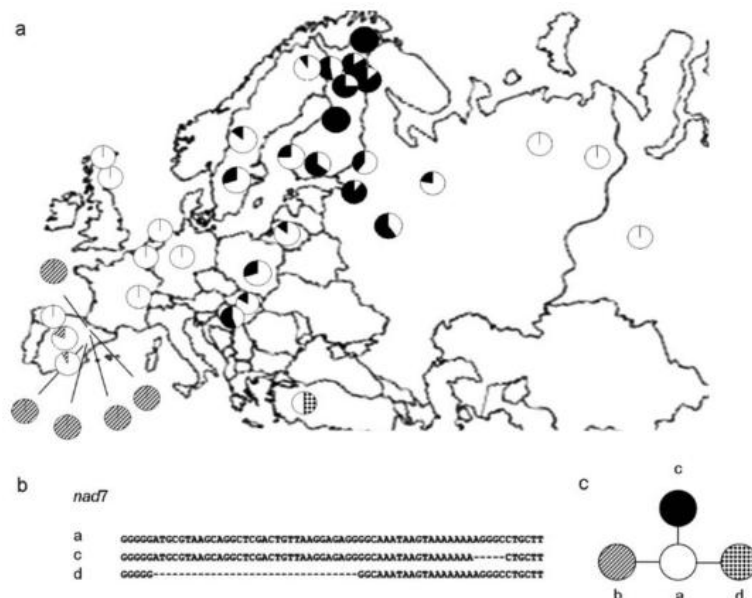


Figure 8: Pie diagrams describing the distribution of mitochondrial haplotypes in *Pinus sylvestris* populations. Patterns in diagrams are equivalent to the patterns in haplotype network (Pyhäjärvi et al. 2008).

- *Betula sp.*

Concerning *Betula sp.* (Maliouchenko et al. 2007), analyses on chloroplast DNA are showing that south-west populations probably did not contribute to the postglacial colonization and that Eastern and Western populations were not sharing the same history and were probably not colonized from the same refugia. In addition, while the Icelandic birch population may have taken origin in the North Scandinavian part (more data needed), colonization routes all over Europe are not clear yet.

- *Salix sp.*

In the case of *Salix sp.* (Palmé et al. 2003), 24 European populations were studied using chloroplast DNA. However the results lack phylogeographic structure as we can see on the Figure 9 and the authors argued that it “might be the consequence of one or more of several factors: (i) lack of well-defined southern refugia, (ii) rapid speed of re-colonization and high dispersal ability, (iii) high mutation rates and (iv) extensive hybridization with other willow species.”¹⁰

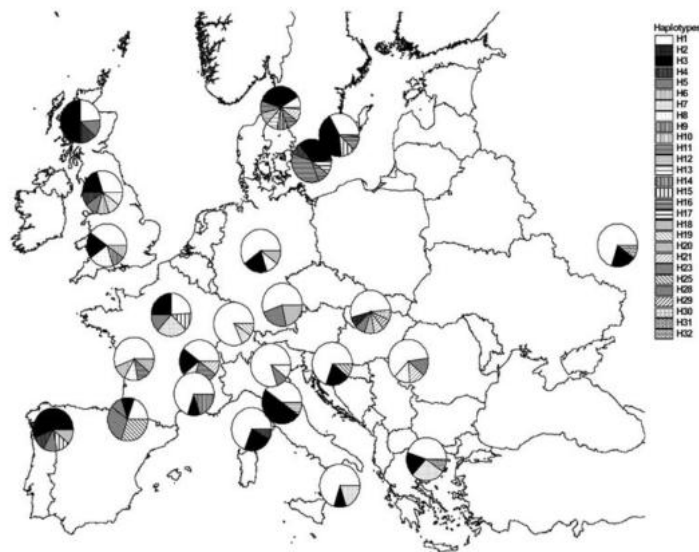


Figure 1 Geographic distribution of chloroplast PCR-RFLP haplotypes in *S. caprea*.

Figure 9: Geographic distribution of chloroplast PCR-RFLP haplotypes in *Salix caprea* (Palmé et al. 2003).

¹⁰ p. 471

- *Alnus incana*

Mandák et al. (2016) sampled 65 populations (1004 individuals) of *Alnus incana* species and performed chloroplast DNA as well as microsatellites analyses. Concerning the chloroplast DNA analyses, they found that Southeastern Europe did not take part in the re-colonization of Europe during the postglacial times. According a BEAST analysis (Bayesian Evolutionary Analysis Sampling Trees), Figure 10 (c), the separation of these lineages was dated roughly around the beginning of the Quaternary period (2.4Mr years ago (Hewitt 2000)). Moreover, the analysis revealed that the haplotype of the lineage 1 and its derivatives spread in Central and Northern Europe about 81Kr years ago, which could be associated with the last Weichselian glacial period that was a warm period within the last ice age. By extension, this happened before the beginning of the Holocene. Moreover, the haplotypes present in Russia are not present in Fennoscandia (Figure 10 (a)) and this indicates that Fennoscandian populations did not originate from Eastern Europe during the post glacial re-colonization. We can also clearly see that the lineages found in the Carpathian and Balkan regions are highly differentiated with the rest of the Europe and these data indicate that they were probably no connections between them and the rest of Europe during the re-colonization period.

The review of these different studies shows unclear patterns as it comes to glacial refugia locations or migration routes. But *Pinus sylvestris* study as well as *Alnus incana*'s shows the presence of cryptic refugia in Central Europe. Moreover, some elements of migration routes are highlighted, such as the presence different refugia origins for *Pinus sylvestris*' current range (Vidyakin et al. 2012), the fact that South-West populations of *Betula sp.* did not take part in the re-colonization process (Maliouchenko et al. 2007), and that Fennoscandia was not colonized from Eastern Europe (Mandák et al. 2016). Disturbingly, as we can see in the study of Mandák et al. (2016), the divergence time between the different lineages can consequently predate the Holocene period and may as a consequence induce biases while interpreting haplotype data.

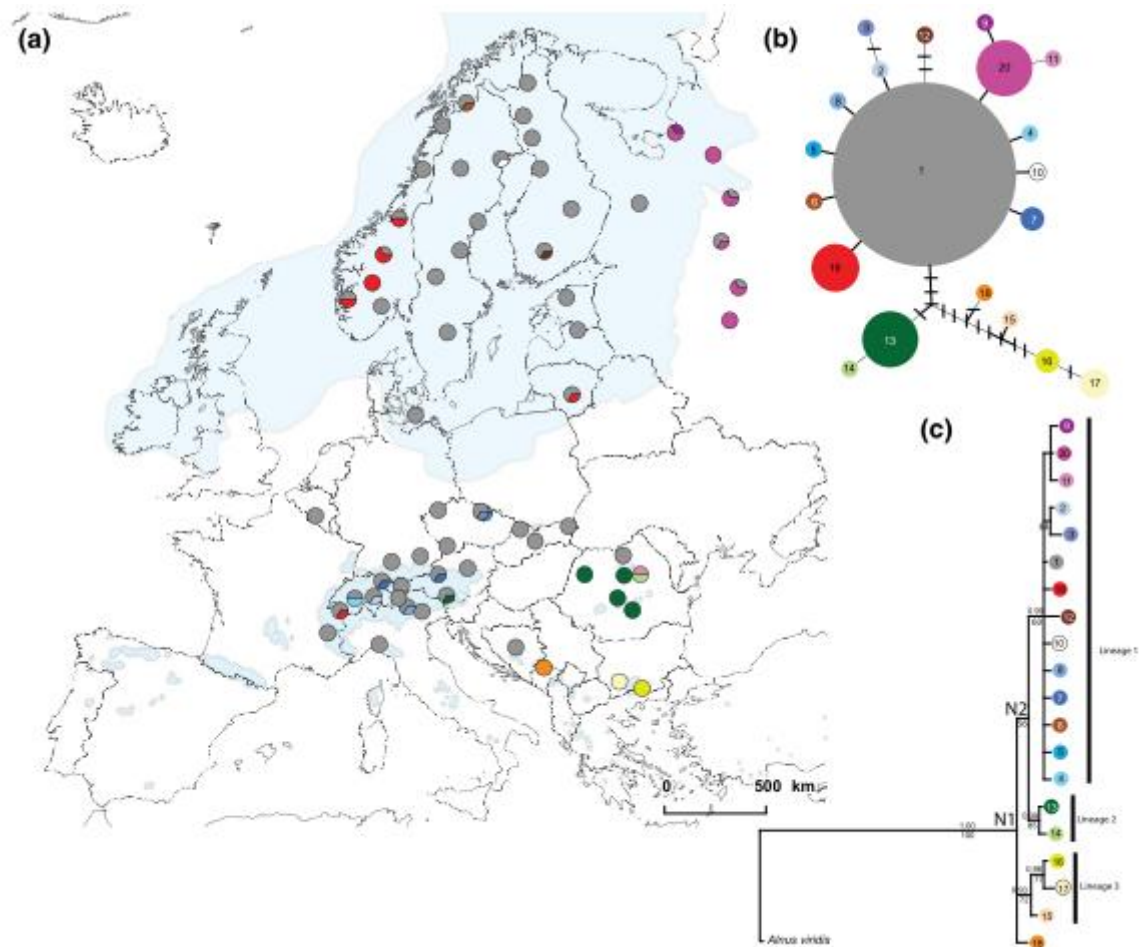


Figure 10: Analyze of the chloroplast DNA haplotypes of *Alnus incana*. (a) Pies charts of haplotypes frequencies across the sampled populations; (b) Chloroplast DNA haplotype network; (c) Bayesian phylogenetic analysis (Mandák et al. 2016).

3.4.2. Microsatellites

Microsatellites, as opposed to chloroplast DNA and mitochondrial DNA, have a higher mutation rate (Anderson et al. 2011; Tzedakis et al. 2013). This may be useful when reconstructing population migration history of the Holocene period.

The study by Anderson et al. (2011) took place in Alaska and pointed out the better resolution in post glacial population history of the white spruce using microsatellites instead of cpDNA. Indeed, a previous study (Petit et al. 2006), using cpDNA was able to show the presence of a glacial refugium somewhere in the North-Central Alaska.

However, using microsatellites, the authors were able to refine the results. In addition to confirm the presence on a North-Central Alaskan refugium, they were able to understand

more clearly which genetic processes were dominant in Alaskan and surrounding regions. In the first case genetic drift played a major role while in the second case gene flow among populations was more important. The results also suggested the presence of other cryptic refugia.

Another study (Tollefsrud et al. 2009) was focusing on the postglacial migration routes of *Picea abies*, the Norway spruce. This study was based on previous results (Tollefsrud et al. 2008), which were obtained by using paleoecological records as well as mitochondrial DNA data, to infer that while the Southern part of Europe was colonized from several refugia (Balkan peninsula non-included), the Northern part was colonized from a unique and large refugium in Russia. Further Tollefsrud et al. (2009) explored 37 populations for microsatellite loci. The results supported the existence of a Russian refugium from where originates the Scandinavian populations, but also showed that two routes were used to colonize Scandinavia from that refugium. One route through Finland and one route through the Baltic Sea area.

The study of *Alnus incana* (Mandák et al. 2016) was not only using cpDNA markers but also microsatellite analyses. While for the cpDNA results different haplotypes were showing lineages separation highly predating the Holocene period, the study of the microsatellites indicated the colonization of the Northern parts of Europe from the Central-European populations (Figure 11) starting from the beginning of the Holocene. This emphasizes the presence of cryptic refugia in the low land of Central Europe. Moreover, populations confined to the south during the cold period also spread north to the Alps and the Carpathians (Figure 11) around the same time period. However, their migration pattern stopped there and they do not influence the genetic diversity that could be observed in further North regions. By consequence, as mentioned above, it seems that chloroplast DNA do not show the Holocene migration, however it might be the case for the variations in microsatellites (Mandák et al. 2016)

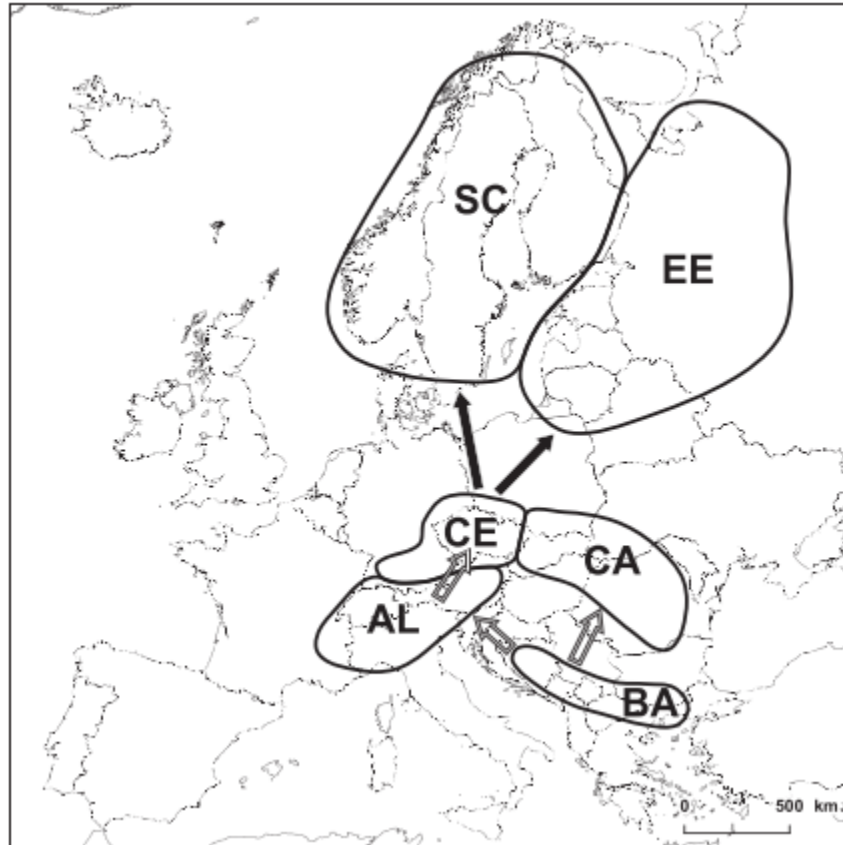


Figure 11: Bayesian computation analysis have been used to highlight the colonization pattern of European *Alnus incana* that was the most probable. CA: Carpathians; AL: Alps; CE: Central Europe; SC: Fennoscandia; EE: Northeastern Europe and Baltic. The open arrows represent the Southern refugia population most probable colonization pattern and the black ones represent the most probable colonization pattern of the LPG ice sheet covered areas (Mandák et al. 2016).

3.5. *Alnus incana*, general information

The grey alder from the Latin name *Alnus incana* (L.) Moench is part of the Betulaceae family. Its height can be up to 20m. The bark is dark-grey with greening shading, mainly smooth but can be cracked at the base. Its leaves are elliptic to roundish, with a tapering tip, at least slightly pubescent. Their margins are doubly dentate and rarely incised. The upper side is matt green while the underside is grayish with hairs. The buds are dark purple-red, oblong, with two external scales covered by warty little scales. Flowers bloom early spring with the male flowers forming a pendent catkin while female catkins erect, they are monoecious (individual flowers are either male or female, but

both sexes can be found on the same plant). The fruit consists of an oval-shaped dark-brown cone (Rushforth 2000) It is a diploid tree ($2n = 2x = 28$) wind-pollinated, self-incompatible, relatively short-lived woody species (ca. 20–50 years) of riparian and water-logged habitats (Tallantire 1974; Douđa et al. 2009; Douđa 2010). It can reproduce after 6 to 15 years of age with a maximum lifespan being 60 to 100 years (Tallantire, 1974). In its Northern range, the species covers the entire Fennoscandia and extends in the East through the European Russia and the Western part of Siberia. However, *Alnus incana* is found only in patches within the mountains of its Southern range. We can find populations in high altitude shallow valleys of the Alps, Hercynian Mountains, Carpathians and Dinaric Alps (Figure 12)(Douđa et al. 2014). It is considered a boreal tree.

The study of the grey alder history via pollen is not easy due to our inability to precisely make the difference between *Alnus glutinosa* and *Alnus incana*. However, we can confirm the presence of the genus *Alnus* in the classical three southern refugia (Balkan, Italian and Iberian peninsulas) (Douđa et al. 2014). Only few macrofossils were found in the Northern part of Europe dated from the beginning of the Holocene, but because of the difficulty to find such evidence, it does not prove the absence of cryptic refugia in Eastern Europe (Douđa et al. 2014).

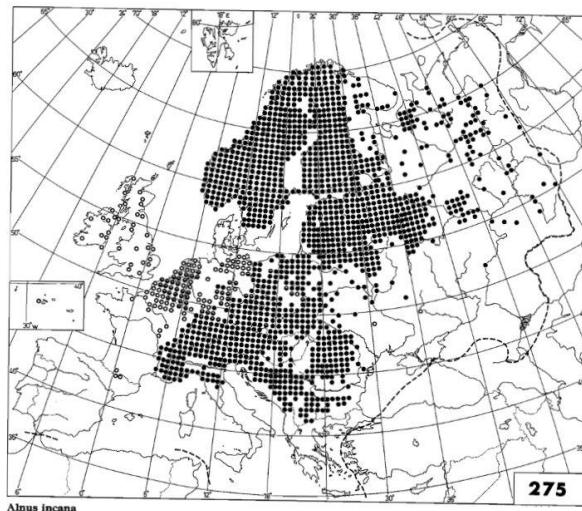


Figure 12: This is the distribution range of *Alnus incana* (L.) Moench – Map 275

4. Methodology

4.1. Samples collection

While the previous study (Mandák et al. 2016) covered most of *Alnus incana* range, this study focused more on Central Europe populations. Because this species is a boreal tree, it can be found in Central Europe and Southern regions only in higher altitudes where the climate is more appropriate for its growth. Moreover due to its light and moisture requirement, the sample collection could be limited to shallow valley where we can have river stream as well as good sun exposition. Using a map of central Europe, were determined potential sites of *Alnus incana* population in the Krušné hory, Český les, Šumava, Krkonoše and Beskydy Mountains and then each of these locations were visited during summer 2015, when the trees had leaves. Populations were not found in each of these locations and sometimes the populations were too scarce to enable the collection of enough samples. For each locality (13 in total) was collected 20 individuals per population if possible (15 samples at least). Within population, individuals were collected along linear transects at least 50 m apart which means that each population sample represents a one-kilometer-long or longer transect. The spacing between the individuals' samples is important in order to prevent collecting two samples of the same DNA due to vegetative growth. For each tree sampled, were collected 2-3 leaves that were stored in perforated bags. Then, the perforated bags were put in silica gel to allow the samples to dry and be stored before further processing. Five populations previously sampled within the previous study (Mandák et al. 2016) were added to this study to complete the sampling design of Central Europe.

4.2. Sample processing

4.2.1. DNA extraction

Once all the samples were collected and dried, the next step was the DNA extraction. Was used the DNeasy 96 plant kit along with the protocol: “Purification of Total DNA from frozen or Lyophilized Plant Tissue” to extract the DNA from all the samples. Once extracted, the samples were stored in the freezer. The few adjustments that were made compared to the protocol are listed below:

- Registered cards were filled with each samples number to know at any moment which sample is on which rack, strips or tubes. This means that each rows and racks had to be marked all along the process.
- For the first step, was used 20 mg of dried tissue instead of 10 mg because the tissues were not as desiccated as lyophilized tissues.
- For the step 24, the buffer AW is now called AW2.
- For the step 26, product was eluded 2 times 50 ul instead of 2 time 100 ul to favor a higher concentration of DNA rather than a higher overall DNA.
- The DNA extraction using DNeasy kit enables to handle two times 96 samples. For a total of 250 samples, two runs were necessary to complete the extractions.

4.2.2. Measurement of the absorbance and dilution

Using the UVS99 Nanodrop device, the absorbance of the samples was measured. Each sample was measured twice to have a more accurate result. In order to perform the PCR, each sample has to have a concentration as close as possible of 20ng/uL. Samples with a concentration greater than that were diluted with purified water as many times as necessary to achieve 20ng/uL. Samples with a lower concentration were used as such.

4.2.3. DNA amplification and sequencing

Once the samples were diluted, they were amplified through Polymerase Chain Reaction (PCR) in a Mastercycler (Eppendorf, Germany) with QIAGEN Multiplex PCR kit (QIAGEN, Hilden, Germany). Two multiplexes of nine set of primers each were used (A2, A6, A10, A18, A22, A26, A35, A37, A38)(Drašnarová et al. 2014) and (Ag1, Ag5, Ag9, Ag10, Ag13, Ag14, Ag20, Ag27, Ag30)(Lepais & Bacles 2011). The amplification

conditions are as described in the supporting information (Methods 1) of Mandák et al. (2016) “PCR1: 15 min of denaturation at 95 °C, followed by 40 cycles at 94 °C for 30 s, 30 s at 58 °C, 60 s at 72 °C and a final extension of 10 min at 72 °C, and for multiplex PCR2: 5 min denaturation at 95°C, 30 cycles at 95°C for 30 s, 58°C for 3 min, 72 °C for 30 s and extension of 30 min at 60 °C”.

4.3. Data processing

4.3.1. Microsatellites analyze

The length of the PCR products were then determined using GeneMarker V2.4.0 (SoftGenetics, State College, USA). In the figure 13 is illustrates the data that could be visualized by the program GeneMarker V2.4.0. The calibration was manually done to align with the results of the previous *Alnus incana* study (Mandák et al. 2016). This allowed to use the results obtained on both studies for the present research.

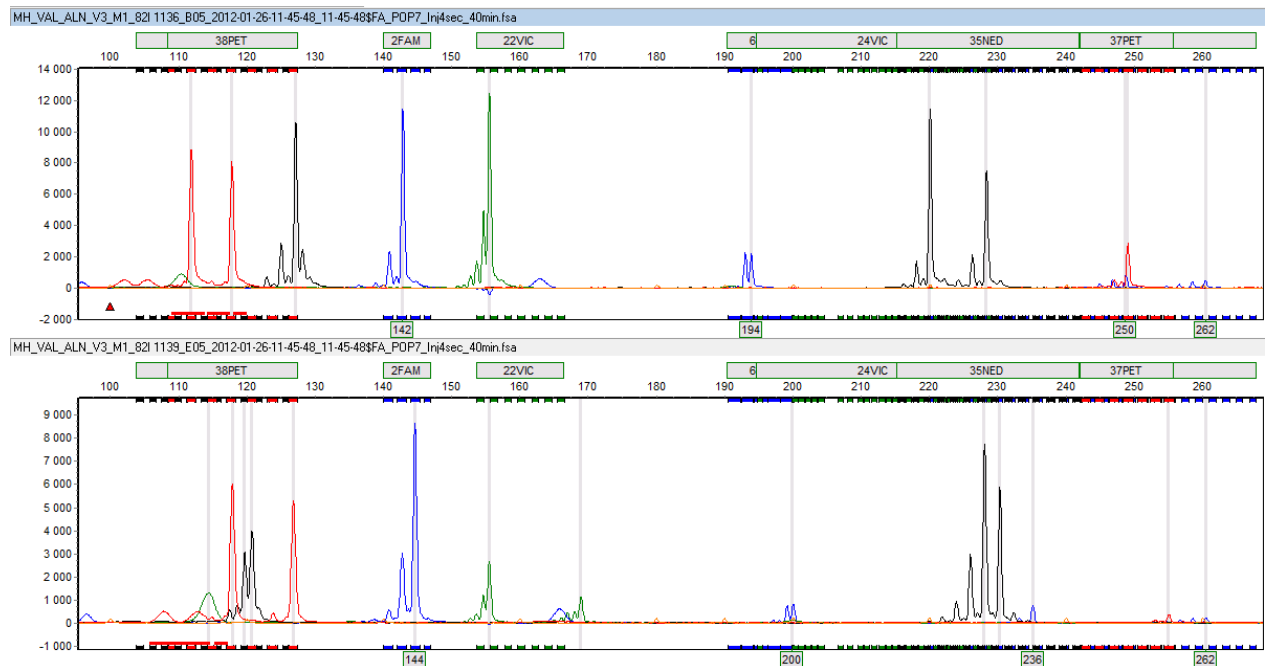


Figure 13: Visualization of two samples from the Multiplex 1 using GeneMarker V2.4.0.

4.3.2. Basic statistics

Using data of our 18 populations established using GeneMarker V2.4.0., my supervisor run the program Fstat 2.9.3 (Goudet 1995) and ARLEQUIN (Excoffier et al. 2005) to obtain population genetic statistical data. As described by Mandák et al. (2016), from Fstat 2.9.3 was extracted the: “average number of alleles per locus (A), mean gene diversity over all loci (HS), mean allelic richness (RS) and Weir & Cockerham’s parameter f(FIS)” and from ARLEQUIN we extracted “observed (HO) and expected (HE) heterozygosities” . We also used Fstat 2.9.3 to do comparison among three groups of population geographically selected (CA= Carpathian mountains; SU= Sudeten mountains; HE= Hercynian mountains). There is “comparisons of allelic richness (RS), observed heterozygosity (HO), gene diversity (HS), inbreeding coefficient f(FIS) and levels of differentiation among populations h(FST) for *Alnus incana* populations” (Mandák et al. 2016) .

4.3.3. Statistical analyses

The data obtained with GeneMarker V2.4.0 were submitted to STRUCTURE V2.3.2.1 (Pritchard, Stephens, and Donnelly 2000) by my supervisor. The first analysis was performed with our 18 populations of Central Europe and the second analysis with the entire set of 64 populations collected all over Europe. This program creates clusters among the populations and it fractionally attributes each individual sample to the created clusters. Ten repetitions were run for each number of clusters to verify the outcome repeatability. The results were then analyzed to provide insight regarding the relevance of the results for each cluster number: an ad hoc statistic ΔK was used as described by Evanno et al. (2005) and the symmetric similarity coefficient as described by (Nordborg et al. 2005) was used. Then using CLUMPP V.1.1.2 (Jakobsson & Rosenberg 2007), averages of the 10 runs for each of the relevant number of cluster were made. The results were visualized using QGIS 2.18 and DISTRUCT 1.1 (Rosenberg 2004).

5. Results

5.1. Basic population genetic parameters characterizing Czech and Slovakian populations

Within the eighteen microsatellites of our eighteen populations, 182 alleles were found. This gives us an average of 10.1 alleles per locus. In the Table 1 is displayed the genetic diversity summary of our populations. The average number of alleles was $A = 4,4$ (s.d. = 0.7); the mean gene diversity was $H_s = H_e = 0,49$ (s.d. = 0.04); the mean observed heterozygosity $H_o = 0.49$ (s.d. = 0.04); the mean allelic richness was $R_s = 4.05$ (s.d. = 0.53). The inbreeding coefficient was low $f(F_{is}) = 0.01$ (s.d. = 0.04) and non-significant indicating that populations were in Hardy-Weinberg equilibrium. The only population that were not in Hardy-Weinberg equilibrium (slightly inbred) was marked by in asterisk in the Table 1.

Pop.	Grp.	Locality	Latitude	Longitude	N	A	Hs	Rs	He	Ho	Fis	Fis pvalue
80	CA	Rožnov pod Radhoštěm	49,4201	18,3574	15	4,1	0,495	3,965	0,496	0,514	-0,035	0,797
81	HE	Rejštejn	49,1403	13,5107	20	4,2	0,455	3,757	0,455	0,441	0,03	0,2424
82	SU	Harachov	50,7688	15,4386	20	6,5	0,605	5,638	0,605	0,574	0,05	0,0612
83	SU	Svoboda nad Úpou	50,6363	15,7881	17	4,9	0,542	4,546	0,542	0,539	0,005	0,472
84	SU	Vrbno pod Pradědem	50,0763	17,2986	20	5,3	0,516	4,643	0,517	0,532	-0,029	0,8051
85	SU	Kouty nad Desnou	50,0954	17,1570	20	4,5	0,496	4,065	0,496	0,509	-0,025	0,7459
86	HE	Novohradské hory	48,6899	14,2371	20	3,8	0,446	3,406	0,446	0,45	-0,01	0,6221
87	HE	Klínovec	50,4130	13,0659	20	4,6	0,531	4,149	0,530	0,502	0,053	0,081
88	HE	Rybník	49,5332	12,6730	20	4,1	0,499	3,785	0,499	0,457	0,083	0,0186*
89	HE	Špičák	49,1594	13,2245	20	3,8	0,453	3,489	0,452	0,441	0,025	0,2971
90	CA	Osturňa	49,3423	20,2856	18	4,2	0,481	3,974	0,481	0,467	0,029	0,2602
91	CA	Nižná Boca	48,9613	19,7744	20	4,4	0,493	3,981	0,493	0,495	-0,004	0,5654
92	CA	Podspády	49,2793	20,1820	20	4,3	0,474	3,910	0,473	0,455	0,039	0,1708
14	HE	Lenora	48,9127	13,8231	20	4,5	0,456	3,983	0,455	0,424	0,068	0,0459
15	HE	Chomutov	50,5006	13,3298	20	3,4	0,454	3,389	0,454	0,466	-0,026	0,7244
57	CA	Pohronská Podhora	48,7489	19,8123	20	4,3	0,524	3,985	0,524	0,519	0,01	0,3916
58	CA	Klubina	49,3597	18,9084	20	4,8	0,499	4,243	0,499	0,503	-0,008	0,5776

Table 1: Genetic diversity summary of the 18 microsatellites of the 18 populations of *Alnus incana* studied. Pop. = Population with CZE = Czech Republic, SVK = Slovakia. Grp. = geographically defined areas with CA= Carpathian mountains, SU= Sudeten mountains and HE= Hercynian mountains. Locality= the sampling location. Latitude and Longitude represents the GPS coordinates, N = number of individuals per population, A = average number of alleles per locus, HS = mean gene diversity over all loci, RS = mean allelic richness, HO = observed heterozygosity, HE = expected heterozygosity and f(FIS) = inbreeding coefficient (Weir & Cockerham 1984). Populations deviating from the Hardy-Weinberg equilibrium with $p < 0.05$ are signaled by asterisk.

The eighteen populations were then subdivided in 3 geographical groups (i.e. HE= Hercynian mountains, CA= Carpathian mountains, SU= Sudeten mountains) to compare the population's genetic characteristics, results can be seen in Table 2. The comparison of allelic richness (Rs) and observed heterozygosity (Ho) were significantly different among the three regions with the highest values for the Sudeten region followed by the Carpathian and finally the Hercynian regions. The expected heterozygosity (He), the inbreeding coefficient f(Fis) and the levels of differentiation among populations (Fst) were not significantly different.

Grp.	Rs	Ho	Hs=He	Fis	Fst
HE	3,708	0,454	0,471	0,036	0,049
SU	4,498	0,525	0,523	-0,004	0,035
CA	4,01	0,492	0,495	0,006	0,027
Two-sided P-values	0,0112	0,0055	0,0911	0,1617	0,4518

Table 2: Statistical comparison between the three defined geographical locations (Grp.) with CA= Carpathian Mountains, SU= Sudeten mountains and HE= Hercynian mountains. Rs = allelic richness, Ho = observed heterozygosity, Hs = gene diversity, f(Fis) = inbreeding coefficient and Fst = levels of differentiation among populations. The probability values for differences among individual areas are for two-sided t-tests after 10 000 permutations.

5.2. Population genetic structure characterizing Czech and Slovakian populations

Using the similarity coefficient (Nordborg et al. 2005; Ehrich et al. 2007) (Figure 14) and ΔK (Evanno et al. 2005) (Figure 15) analyses, we were able to determine which cluster numbers will be the most informative. Both analyzed pointed at $K=3$ so was decided to further analyze results with $K=3$.

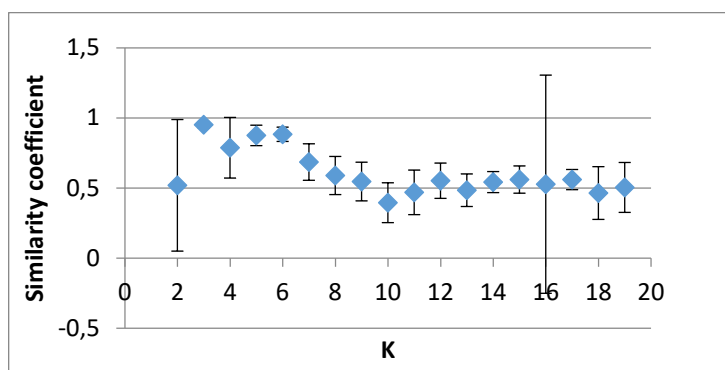


Figure 14: Similarity coefficient according Nordborg et al.(2005) and Ehrich et al. (2007) for the 18 populations.

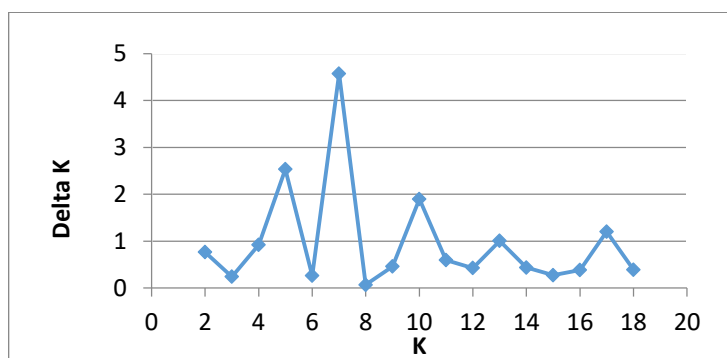


Figure 15: ΔK calculated according Evanno, *et al.* (2005) for the 18 populations.

On the Figure 16 (a) and (b), we can see that individuals of each population were assigned to the three different clusters. The mixture of cluster did not allow the visualization of a clear pattern.

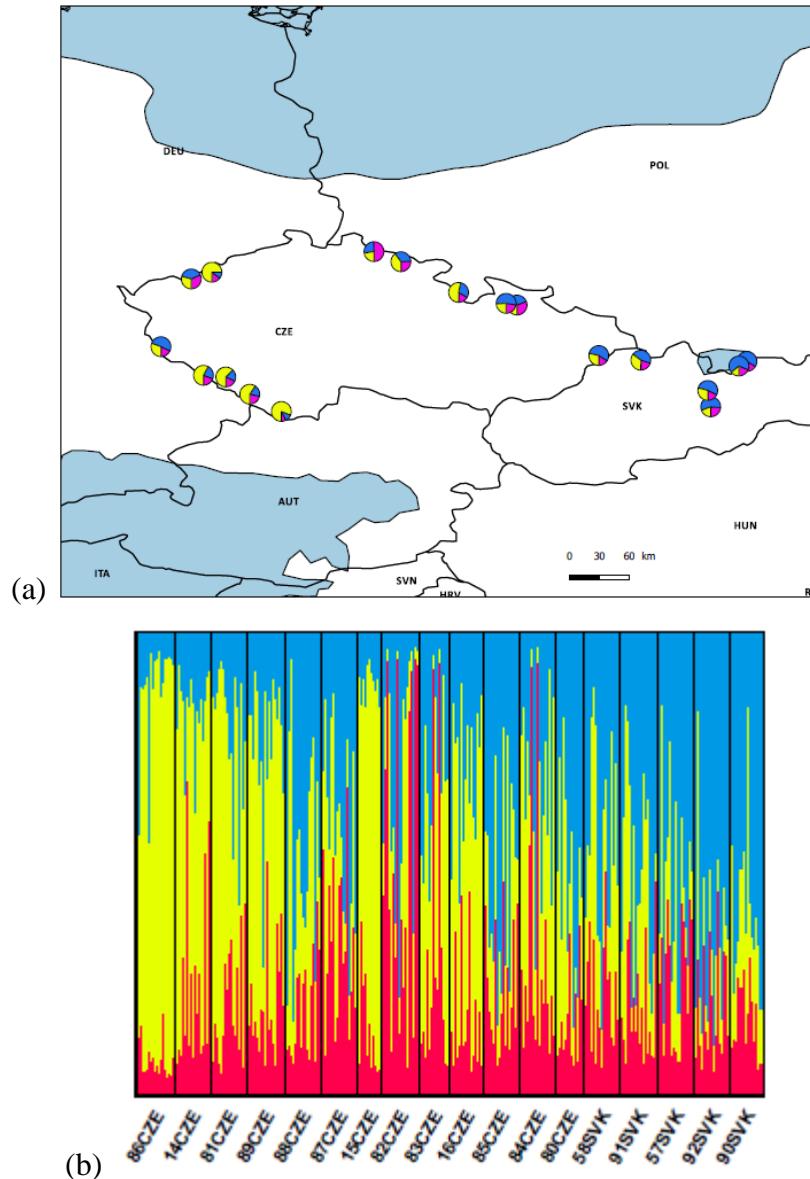


Figure 16: Mean membership proportions of each of the 18 populations of *Alnus incana* for $K=3$. (a) QGIS maps; (b) STRUCTURE 1.1 display.

5.3. Comparison of Czech and Slovakian populations with the rest of Europe

This time for the 64 populations across all Europe, the similarity coefficient (Nordborg et al. 2005; Ehrich et al. 2007) (Figure 17) and ΔK (Evanno et al. 2005) (Figure 18) analyses were used. The first analyze pointed at $K=2, 3$ & 5 to 8 and the second at $K=3$ as the most informative. We then decided to further analyze results with $K=2, 3$ and 5 .

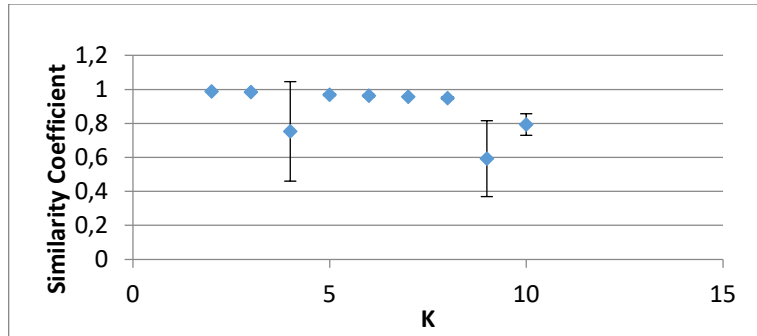


Figure 17: Similarity coefficient according Nordborg et al.(2005) and Ehrich et al. (2007) for the 64 populations.

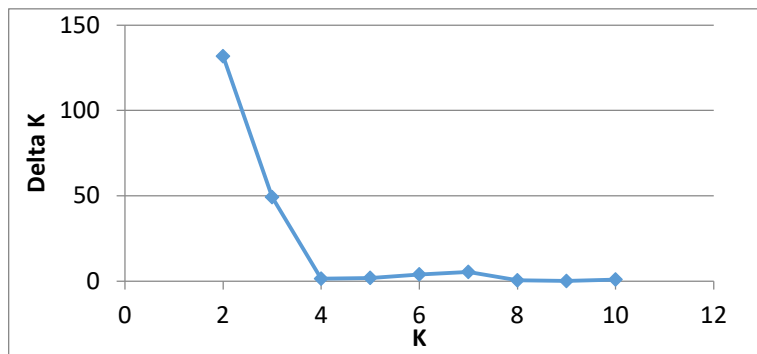


Figure 18: ΔK calculated according Evanno, *et al.* (2005) for the 64 populations.

On the Figure 19, populations are assigned to two clusters and we can clearly visualize two lineages. On the Western side, in the Hercynian regions, we can see one group of populations in pink and on the Eastern side, on the Carpathian and Balkan regions, we can see another group in yellow. Central Europe, North-Eastern Europe and Fennoscandia are represented with a mixture of these two clusters. When the populations are assigned to three clusters (Figure 20), we can see that the blue cluster is very common in the North of the Czech Republic, the Western Carpathian in Slovakia and all over the Northern Europe. We can also see that the yellow cluster of the Carpathian regions in the Eastern Europe present some introgression in the Baltic region. The Hercynian region is still represented by the pink cluster. With five clusters, we can see three South lineages, one on the Carpathian and Balkan region in blue as with two clusters but this time, the Hercynian region is separated in two with the Western Alps in pink, and the Eastern Alps in red. Northern Europe is represented by another lineage in green. The Central Europe and the Baltic regions are represented by a mixture of all 5

clusters. All these results are also visible on Figure 22 with the DISTRUCT 1.1 graphical display.

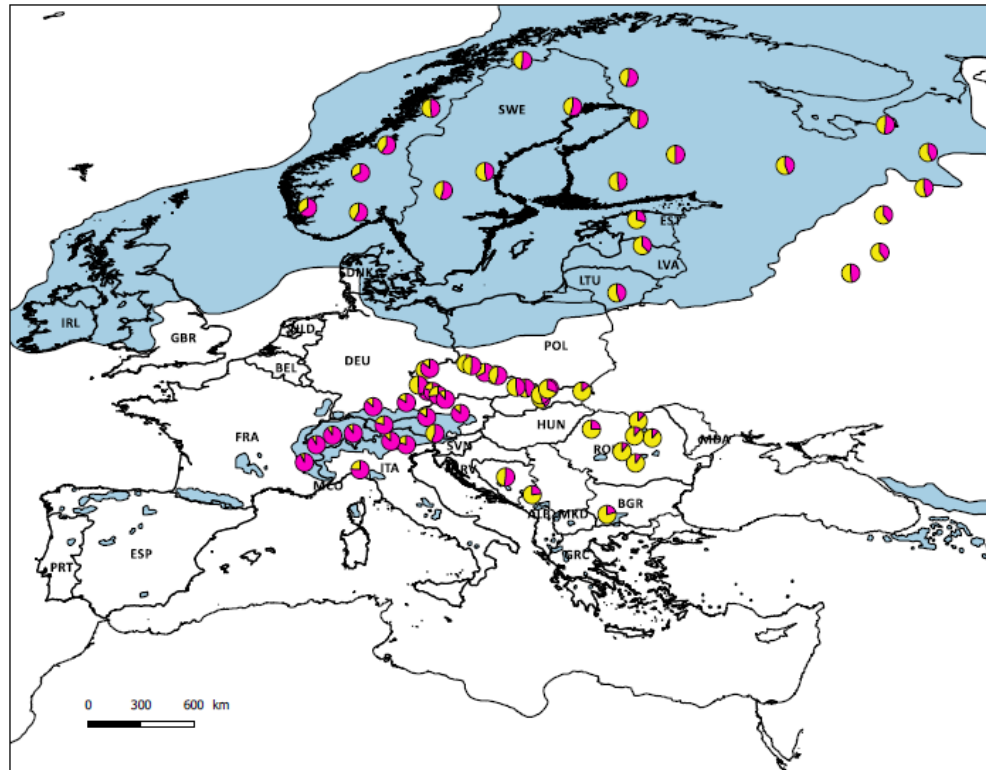


Figure 19: Mean membership proportions of each of the 64 populations of *Alnus incana* for K=2 displayed using QGIS maps.

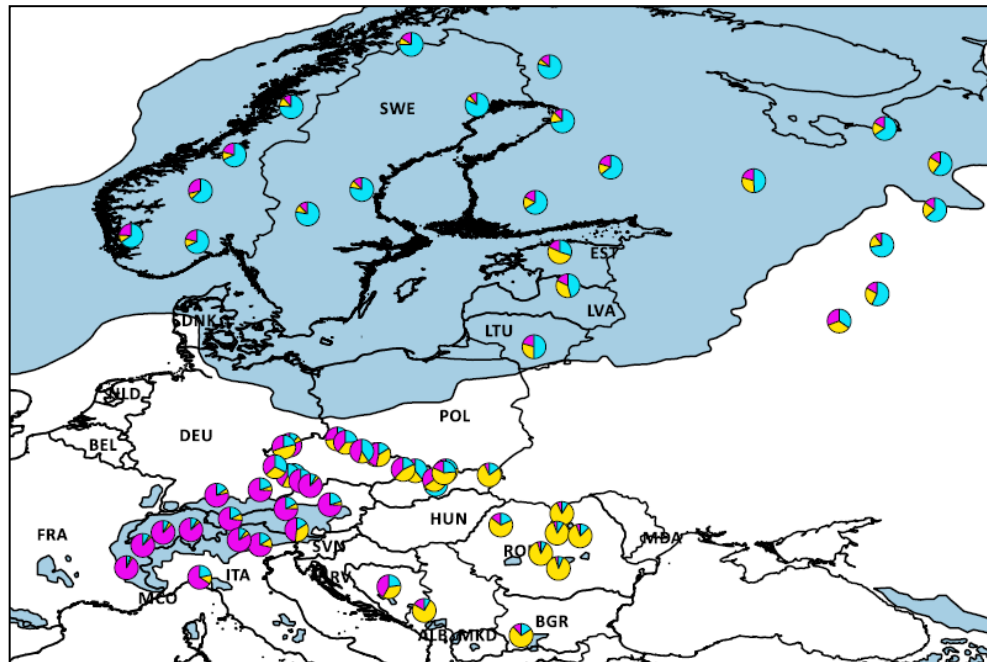


Figure 20: Mean membership proportions of each of the 64 populations of *Alnus incana* for K=3 displayed using QGIS maps.

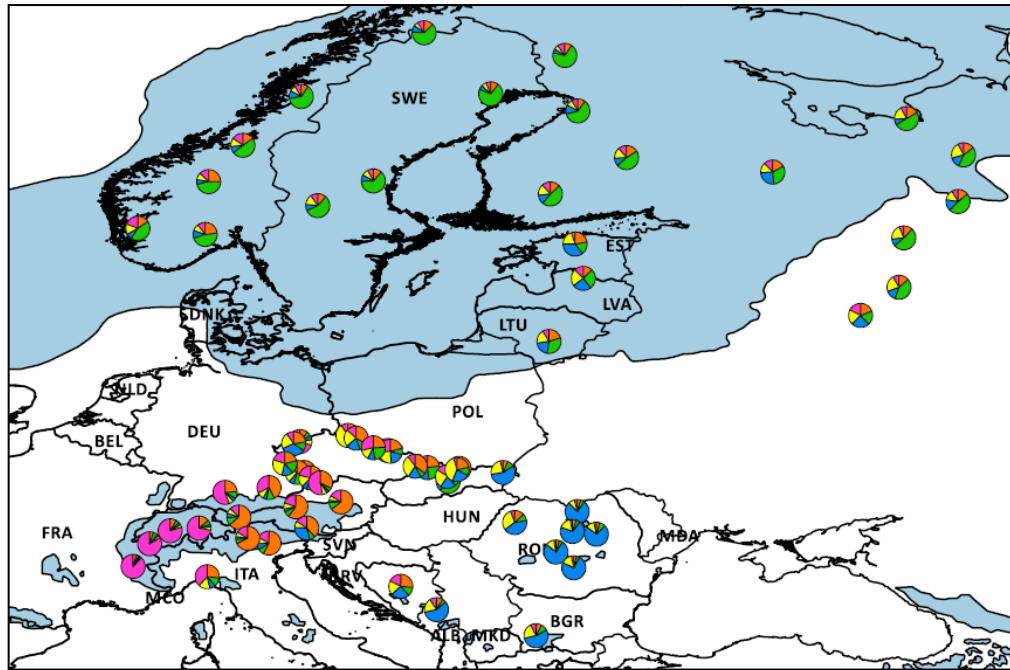


Figure 21: Mean membership proportions of each of the 64 populations of *Alnus incana* for K=5 displayed using QGIS maps.

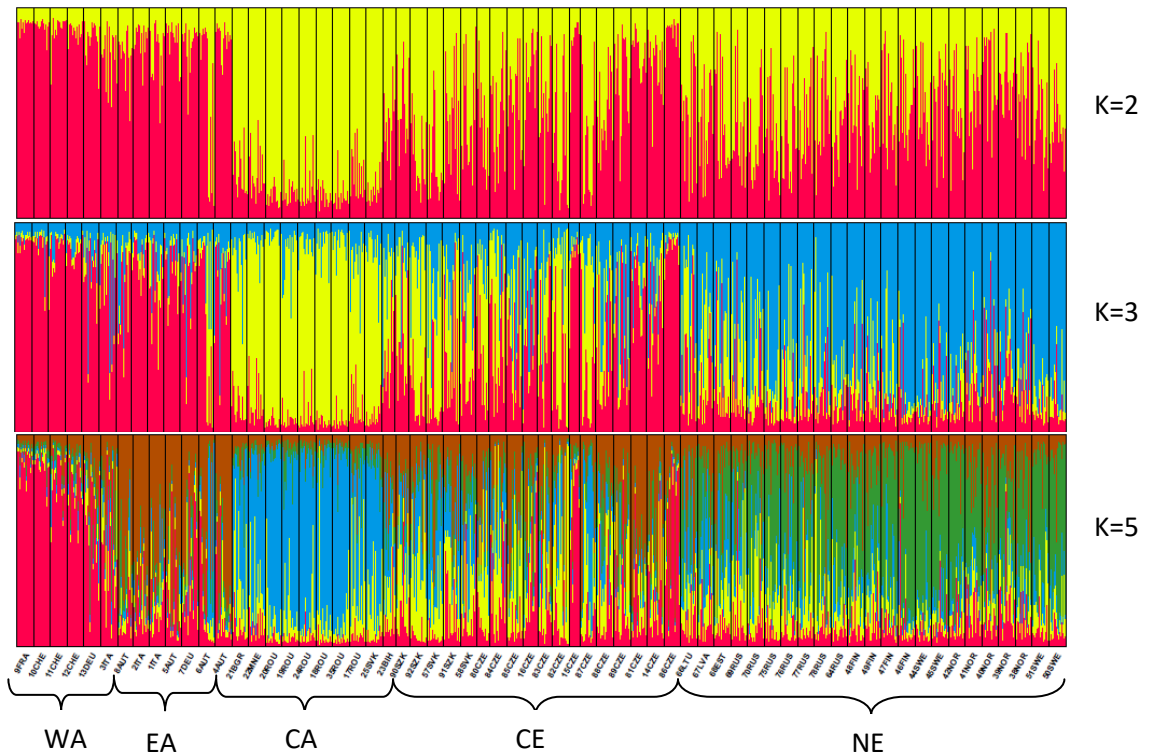


Figure 22: Graphical display of the 64 populations of *Alnus incana* for K=2, 3 and using DISTRUCT 1.1. WA= Western Alps; EA= Eastern Alps; CA= Carpathian and Balkan regions; CE= Central Europe; NE= Northern Europe.

6. Discussion

Studies concerning the biogeographical history of the genus *Alnus* have been carried out using pollen and macrofossils records. They indicate its presence during the LPG in the classical South refugia but also further North in the foothill of the Alps and in the Carpathians (Huntley & Birks 1993; Douda et al. 2014). Douda et al. (2014) also found macrofossils of *Alnus incana* dated from the LG period in the Baltic region, where populations still occur and in the Netherlands which do not belong to the current range anymore. These records are not from the LPG (24-14.6 Kry) but from the LG (14.6-11.7 Kry) consequently, they can be interpreted by a colonization of these regions at the very beginning of the Holocene period (Douda et al. 2014). Moreover this colonization probably took place quickly (around 3Kry) as we can see on the pollen profiles of the Baltic and Scandinavian regions in Douda et al. (2014) study. So far the macrofossils evidences are scarce and they do not provide information concerning potential refugia in Europe or Western Russia. However, more can still be found and radiocarbon-dated in the future and it would help the reconstruction of *Alnus incana* history.

The study conducted by Mandák et al. (2016) is showing very interesting results. Using chloroplast DNA they were able to clearly visualize the distribution of haplotypes diversity all over Europe. From these results, they were able to draw two main conclusions and express several leads concerning the history of *Alnus incana*. They were first able to rule out that this colonization took place through Western Russia because of a haplotype lineage, older than LPG (i.e. 81Kry), that exists there but not in Scandinavia. By its own, this output is very interesting since it shows that *Alnus incana* postglacial history strongly differs from *Picea abies* one. Indeed, in the case of *Picea abies*, microsatellites and mitochondrial DNA supported the idea that Scandinavia was probably colonized by Western Russia (Tollefsrud et al. 2009). Secondly, they concluded that South Eastern Europe was not linked with the re-colonization process. This assertion is justified by the fact that the haplotypes situated in the Carpathian and the Balkan regions differed from the common lineage that can be visualized in most of the Europe. However, our microsatellites results combined with the ones by Mandák et al. (2016) seem to show that a connection does exist between the cluster mainly

represented in the Carpathians and Slovakia, the Baltic countries as well as Western Russia (Figure 20). Our ability to see this link using microsatellites may be because microsatellites have higher mutation rates than chloroplast DNA (Anderson et al. 2011) and enable us more resolution. That said, with the information in our possession it is difficult to infer the datation of this connection. We can propose several hypotheses such as the existence of some connection between the Carpathians and the Central Europe that precedes the Holocene colonization. We can also advance the possibility that an introgression from the Carpathian occurred after the colonization took place. Concerning the leads mentioned above, the chloroplast DNA showed some connections between regions as follows: Carpathian and Eastern Alps, Western Alps, Baltic and Norway as well as Carpathian with Eastern Russia. These three connections that have been found by Mandák et al. (2016) and the one that we expressed above may be consistent with the idea that *Alnus incana* range during the LPG may have been fairly continuous in the European periglacial zone. We can add that the climatic simulations conducted by Svenning et al. (2008) indicate that *Alnus incana* species as well as other boreal trees could have indeed had the climatic possibility to survive in small scarce populations in Central Europe.

The other outputs we got from our microsatellites data combined with the ones from Mandák et al. (2016), that enabled us to visualize genetic clusters (Figure 19-22), also agree with the presence of cryptic refugia in Central Europe. As was already visible by Mandák et al. (2016) clear differentiations can be seen between Western Alps, Eastern Alps, Carpathian region and Scandinavia. But with our addition of new data, we can also visualize with more accuracy in Central Europe highly mixed populations. Moreover, as we can see on the Figure 20, the blue cluster on the Northern part of the Czech Republic seems very common, and it is the one that mainly covers Scandinavia. This is in accordance with a colonization that took place from the low land of Central Europe within a periglacial migration corridor where populations would have survived the LPG. Using approximate Bayesian computation analyses (ABC) Mandák et al. (2016) documented that populations situated in the Alps probably colonized Central Europe from the beginning of the Holocene (approximately 7.2Kr years ago). This might be a reason for the presence of the Alpine cluster in Central Europe.

The analyses of our records of the 18 populations alone in Central Europe was unfortunately not as informative as we wished when we formulated the aim of the thesis. Indeed, on the figure 16 representing the results from STRUCTURE, we can see that all the populations are fairly similar with no clear geographical pattern. However; this result might simply be explained by a gene flow that occurred between them. Indeed, on one hand, the sampling area is relatively small and on the other hand *Alnus incana*'s pollen, small and light grains, has high dispersal ability through wind (Douda 2014 ex. Eisenhut 1961).

These populations were further analyzed using statistical comparison of three geographical regions (FSTAT) as Mandák et al. (2016) did with six geographical regions. It is interesting to notice that the highest allelic richness (R_s) was found for the Sudeten region ($R_s = 4.49$) in our analysis and this may be discussed in the light of the highest result of Mandák et al. (2016) that they found for the Carpathian region ($R_s = 4.38$). This high diversity of the Carpathian region was explained by a continuously large population size over time by Mandák et al. (2016). However, in our case, it could be another indication of glacial refugia in the Northern part of the Czech Republic. Indeed, according to Svenning et al. (2008) if populations would have survived the LPG in Central Europe it would have been in small populations and in that case high genetic variability may be the result of long time survival within a limited area (Hewitt 1996; Tzedakis et al. 2013).

So far in Europe, only two boreal trees, *Picea abies* (Tollefsrud et al. 2009) and *Alnus incana* (Mandák et al. 2016) with fairly the same geographical range have been studied using microsatellites as mentioned by Mandák et al. (2016). These two tree species present different histories, *Picea abies* seems to have re-colonized Scandinavia from refugia in Russia while *Alnus incana* migration pattern started from Central Europe as we can support in the present study. The fact that these two tree species have different histories makes it difficult to strictly compare the results. Therefore it is difficult to interpret the present results in light of those obtained for *Picea abies*.

However, the study of *Picea abies* (Tollefsrud et al. 2009) as well as other studies such as the ones investigating *Fraxinus excelsior* in Europe (Heuertz et al. 2004) or *Picea glauca* in Alaska (Anderson et al. 2011) using microsatellites were successfully able to

highlight the location of refugia and post glacial migration routes. Considering *Picea abies*, the study by Tollefsrud et al. (2009) of 7 nuclear microsatellites loci over 37 populations supported the existence of a glacial refugium in Russia as well as two migration routes from that refugium used to colonized Scandinavia. Concerning *Fraxinus excelsior*, Heuertz et al. (2004) have analyzed 5 nuclear microsatellites loci on 36 populations in Europe. Their result is quite interesting since they showed on one hand that a strong differentiation occurs between populations in Eastern and Western Balkan in accordance with two distinct glacial refugia. On the other hand they found that while differentiation among populations in the rest of the Europe was low, their genetic diversity was surprisingly higher than the one found in the cryptic refugia of Southeastern Europe. The authors suggested a colonization of Europe from several populations with a higher than average admixture of gene pools.

Finally, Anderson et al. (2011), using the analyses of 6 nuclear microsatellites loci over 22 populations in Canada and Alaska were able to highlight the presence of a refugium of *Picea glauca* in the North-Central Alaska during the LPG, as well as a migration route from the Riding mountains (Southern Canada) Northwestwards at the beginning of the Holocene. Taken together, the data obtained for these tree species illustrate the relevance of microsatellite analyses to investigate migration routes

Concerning the limitation of the methodology, I would say that we might have obtained more conclusive results if we would have been able to sample more populations in Central Europe. Unfortunately, the sampling process was a little critical because of the scarcity of populations in Central Europe and because no data exist on where to find them exactly. Their localization on the field was unfortunately quite time consuming and sometimes the populations found were too small to allow an exhaustive sampling.

Moreover the use of additional statistical analyses, and more especially approximate Bayesian computation analyses (ABC) may have given us more information concerning the migration pattern within Central Europe. Indeed, even though this kind of mathematical analysis relies on assumptions and approximations whose effects have to be assessed, it has proved to be useful in population genetics (Beaumont et al. 2002).

7. Conclusion

As was already inferred by Mandák et al. (2016) the hypothesis that refugia existed in the low land of the Central Europe, further north than expected by the classical South refugia hypothesis, seems to be confirmed by the presence of mixed populations sharing the same genetic cluster that can be found in Scandinavia and Western Russia. However, concerning our second hypothesis, our data do not provide information whether the colonization took place from several refugia populations in Central Europe or if the origin was more localized. Indeed, the mixed and fairly similar populations that grow nowadays within the high lands of Central Europe may be a sign that gene flow occurred and in this way obscured a specific population of origin. Moreover, we are not able to specify if this connection occurred during the LPG, during the Holocene or at both times.

As we discussed, a connection seems to exist between Carpathians, Central Europe, Baltic countries and Western Russia. This could be visualized with our microsatellites analyses but also by the fact that an haplotype, derived from Russian lineage, occurs in one of the Carpathian populations (Mandák et al. 2016). This might be the theme of further investigation. Another sampling procedure in these regions could be done with the analysis of new populations using molecular markers. Moreover, more statistical analysis such as Approximate Bayesian Computation modeling (ABC; Beaumont et al. 2002) could be used to obtain probable colonization scenarios.

To conclude, investigations of tree species' history using several types of molecular markers as well as pollen and macrofossil records highlight the fact that it is with a combination of these different results that we obtain the most comprehensive picture (Lascoux et al. 2004; Magri et al. 2006; Tzedakis et al. 2013; Mandák et al. 2016). If we first take *Alnus incana* as an example, microsatellites analyses may unravel the most recent population history during the Holocene. For example we provided this kind of information by showing a clear differentiation of genetic clusters in Europe in our study and from Mandák et al. (2016). However it did not show some differences between populations that would have suggested that Scandinavia was not colonized by Western

Russia. This information was, however, obtained with chloroplast DNA (Mandák et al. 2016). The macrofossils and pollen data studied by Douša et al. (2014) provided other indications such as the presence of *Alnus incana* in the Baltic region at the beginning of the Holocene and the rapid (i.e. 3Kry) colonization time of Scandinavia after the LG. This would support the idea that the different methodologies provide complementary findings and that the investigation of one species' history should be considered by combining different approaches as much as it is possible.

8. Glossary

Chloroplast DNA: It is a circular DNA molecule. Several properties make it useful in phylogeography such as:

- non-recombining molecule, hence only mutations induces variations,
- generally maternally inherited, hence dispersal by seeds so the history reconstructions are not obscured by pollen (Gymnosperms have paternally inherited chloroplast DNA),
- chloroplast among the different plant species are similar enabling the use universal primers.

Cores: They are cylindrical sections of our area of interest. They are drilled perpendicularly of the geological layers. The layers are formed horizontally year by year with the snow (ice sheet) or sediments (underwater) that, under the weight of the subsequent deposition, turn into ice or bedrock. In each layer is trapped material of the corresponding age of the layer. In the ice cores we can find inclusions of dust, ashes, pollen, radioactive elements and bubbles of atmospheric gas. Each of these elements can be analyzed by different processes and give several types of information. The Ice cores provide information about temperature, ocean volume, precipitation, chemistry and gas composition of the lower atmosphere, volcanic eruptions, solar variability, sea-surface productivity, desert extent and forest fires.

Eccentricity: Describes how elliptical the Earth's orbit is around the Sun. The attraction exerted by Jupiter causes the orbit to vary between nearly circular to quite elliptical with an eccentricity of 0.06. There is a period of 100 000 years between two maximal eccentricities.

Last Pleniglacial: Period which is characterized by the most extreme conditions of the last glacial period (24-14.6 Kry).

Microsatellites: Is a repetition of small DNA motifs (1 to 5 base pairs) in the nuclear DNA. They have high mutation rate which can enable us more resolution than chloroplast DNA in phylogeography. However, the development of primers is also more complicated and expensive.

Mitochondrial DNA: It is the DNA founded in the mitochondria and it is usually maternally inherited. In plants, the often slow mutation and the structural rearrangement properties can be limiting for phylogeography studies.

Obliquity: The tilt of the Earth is responsible for the seasons and its cycle lasts 41 000 years. The angle with the perpendicular of the ecliptic varies between 22.1° and 24.6° . When the angle is at its maximum, like 9,500 years ago at the end of the last glacial period, there is a greater differential of solar radiation in the higher latitudes. The winters are longer and colder and the summers are hotter. The obliquity doesn't affect the quantity of solar energy globally received by the Earth but its distribution along latitudes. A small angle may not provide enough solar energy during summer to melt the ice sheet.

Precession: The precession of the equinox is the continuous change of the Earth's axe direction, relatively to the fixed stars, over a cycle of 26,000 years. It influences the season's differences between summer-winter. When the northern hemisphere has greater differences between the seasons, the southern hemisphere has milder ones.

Refugia: In this diploma thesis, a refugium is an area where three populations survived the last glacial period in more favorable conditions.

9. References

- Aitken, S.N. et al., 2008. Adaptation, migration or extirpation: climate change outcomes for tree populations. *Evolutionary Applications*, 1(1), pp.95–111. Available at: <http://doi.wiley.com/10.1111/j.1752-4571.2007.00013.x>.
- Anderson, L.L., Hu, F.S. & Paige, K.N., 2011. Phylogeographic history of white spruce during the last glacial maximum: Uncovering cryptic refugia. *Journal of Heredity*, 102(2), pp.207–216.
- Beaumont, M.A., Zhang, W. & Balding, D.J., 2002. Approximate Bayesian Computation in Population Genetics. *Genetics Society of America*, 2035(December), pp.2025–2035.
- Bennett, K., Tzedakis, P. & Willis, K., 1991. Quaternary Refugia of North European Trees. *Journal of Biogeography*, 18(1), pp.103–115. Available at: <http://discovery.ucl.ac.uk/170139/>.
- Clark, J.S. et al., 1998. Reid ' s paradox of rapid plant migration. *Bioscience*, 48(1), pp.13–24.
- Coluccia, E. & Louse, G., 2004. Climate and atmospheric history of the past 420 , 000 years from the Vostok ice core , Antarctica. *Journal of Environmental Psychology*, 24(3), pp.329–340.
- Cowling, S. a & Sykes, M.T., 1999. Physiological Significance of Low Atmospheric CO₂ for Plant–Climate Interactions. *Quaternary Research*, 52(2), pp.237–242. Available at: <http://www.sciencedirect.com/science/article/pii/S0033589499920653>.
- Dale Guthrie, R., 2001. Origin and causes of the mammoth steppe: A story of cloud cover, woolly mammal tooth pits, buckles, and inside-out Beringia. *Quaternary Science Reviews*, 20(1-3), pp.549–574.
- Douda, J. et al., 2009. Development of alder carr after the abandonment of wet grasslands during the last 70 years. *Ann For Sci*, 66, p.712.
- Douda, J. et al., 2014. Migration patterns of subgenus *Alnus* in Europe since the last glacial maximum: A systematic review. *PLoS ONE*, 9(2), pp.1–14.
- Douda, J., 2010. The role of landscape configuration in plant composition of floodplain forests across different physiographic areas. *Journal of Vegetation Science*, 21(6), pp.1110–1124.
- Drašnarová, A. et al., 2014. Cross-amplification and multiplexing of SSR markers for *Alnus glutinosa* and *A. incana*. *Tree Genetics and Genomes*, 10(4), pp.865–873.
- Dumolin-Lapègue, S. et al., 1997. Phylogeographic structure of white oaks throughout the european continent. *Genetics*, 146(4), pp.1475–1487.
- Ehrich, D. et al., 2007. Genetic consequences of Pleistocene range shifts : contrast between the Arctic , the Alps and the East African mountains. *Molecular Ecology*, 16, pp.2542–2559.
- Evanno, G., Regnaut, S. & Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE : a simulation study. *Molecular Ecology*, 14, pp.2611–2620.
- Feng, F., 2015. Obliquity and precession as pacemakers of Pleistocene deglaciations. *Quaternary Science Reviews*, 122, pp.166–179. Available at:

- <http://dx.doi.org/10.1016/j.quascirev.2015.05.006>.
- Gugger, P.F. & Sugita, S., 2010. Glacial populations and postglacial migration of Douglas-fir based on fossil pollen and macrofossil evidence. *Quaternary Science Reviews*, 29(17-18), pp.2052–2070. Available at: <http://dx.doi.org/10.1016/j.quascirev.2010.04.022>.
- Guthrie, R.D., 1990. Frozen Fauna of the Mammoth Steppe . The Story of Blue Babe. *Science*, 248(4957), pp.900–901.
- Guthrie, R.D., 1991. Ice-age Ecology Evolutionary Novelty. *Tree*, 6(1), pp.33–33.
- Heuertz, M. et al., 2004. Nuclear Microsatellites Reveal Contrasting Patterns of Genetic Structure between Western and Southeastern European Populations of the Common Ash (*Fraxinus excelsior* L .) Published by : Society for the Study of Evolution Stable URL : <http://www.jstor.org/>. *Evolution*, 58(5), pp.976–988.
- Hewitt, G., 2000. The genetic legacy of the Quaternary ice ages. *Nature*, 405(6789), pp.907–913.
- Hewitt, G.M., 1996. Some genetic consequences of ice ages , and their role , in divergence and speciation. *Biological Journal of the Linnean Society*, 58(3), pp.247–276.
- Hofreiter, M. & Stewart, J., 2009. Ecological Change , Range Fluctuations and Population Dynamics during the Pleistocene Review. *Current Biology*, 19(14), pp.R584–R594. Available at: <http://dx.doi.org/10.1016/j.cub.2009.06.030>.
- Lagercrantz, U. & Ryman, N., 1990. Genetic Structure of Norway Spruce (*Picea abies*): Concordance of Morphological and Allozymic Variation Author (s): Ulf Lagercrantz and Nils Ryman Published by : Society for the Study of Evolution Stable URL : <http://www.jstor.org/stable/2409523> Access. *Evolution*, 44(1), pp.38–53.
- Lascoux, M. et al., 2004. Impact of Ice Ages on the genetic structure of trees and shrubs. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 359(1442), pp.197–207.
- Lepais, O. & Bacles, C.F.E., 2011. De Novo discovery and multiplexed amplification of microsatellite markers for black alder (*Alnus glutinosa*) and related species using SSR-enriched shotgun pyrosequencing. *Journal of Heredity*, 102(5), pp.627–632.
- Leroy, S.A.G. & Arpe, K., 2007. Glacial refugia for summer-green trees in Europe and south-west Asia as proposed by ECHAM3 time-slice atmospheric model simulations. *Journal of Biogeography*, 34(12), pp.2115–2128.
- Loutre, M.F., 2003. Ice ages (milankovitch theory). *Elsevier Science*, pp.995–1003.
- Magri, D. et al., 2006. A new scenario for the Quaternary history of European beech populations: palaeobotanical evidence and genetic consequences, A new scenario for the Quaternary history of European beech populations: palaeobotanical evidence and genetic consequences. *New Phytologist*, 171, 171(1, 1), pp.199, 199–221, 221. Available at: <http://onlinelibrary.wiley.com/doi/10.1111/j.1469-8137.2006.01740.x/full>.
- Maliouchenko, O. et al., 2007. Comparative phylogeography and population structure of European *Betula* species, with particular focus on *B. pendula* and *B. pubescens*. *Journal of Biogeography*, 34(9), pp.1601–1610.
- Mandák, B. et al., 2016. Recent similarity in distribution ranges does not mean a similar postglacial history : a phylogeographical study of the boreal tree species *Alnus incana* based on microsatellite and chloroplast DNA variation. *New Phytologist*,

- 210, pp.1395–1407.
- McLachlan, J.S. & Clark, J.S., 2004. Reconstructing historical ranges with fossil data at continental scales. *Forest Ecology and Management*, 197(1-3), pp.139–147.
- Nordborg, M. et al., 2005. The Pattern of Polymorphism in *Arabidopsis thaliana*. *Plos Biology*, 3(7), pp.1289–1299.
- Palmé, a E., Semerikov, V. & Lascoux, M., 2003. Absence of geographical structure of chloroplast DNA variation in willow, *Salix caprea* L. *Heredity*, 91(5), pp.465–474. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/14576739>.
- Palme, A.E. & Vendramin, G.G., 2002. Chloroplast DNA variation, postglacial recolonization and hybridization in hazel, *Corylus avellana*. *Molecular Ecology*, 11(9), pp.1769–1780.
- Petit, J. et al., 2006. Ice-age endurance : DNA evidence of a white spruce refugium in Alaska. *PNAS*, 103(33), pp.12447–12450.
- Petit, R.J. et al., 2004. Ecology and genetics of tree invasions: From recent introductions to Quaternary migrations. *Forest Ecology and Management*, 197(1-3), pp.117–137.
- Pyhäjärvi, T., Salmela, M.J. & Savolainen, O., 2008. Colonization routes of *Pinus sylvestris* inferred from distribution of mitochondrial DNA variation. *Tree Genetics and Genomes*, 4(2), pp.247–254.
- Raymo, M.E. & Huybers, P., 2008. Unlocking the mysteries of the ice ages. *Nature Publishing Group*, 451(January), pp.284–285.
- Sommer, R.S. & Nadachowski, A., 2006. Glacial refugia of mammals in Europe: Evidence from fossil records. *Mammal Review*, 36(4), pp.251–265.
- Svenning, J.C., Normand, S. & Kageyama, M., 2008. Glacial refugia of temperate trees in Europe: Insights from species distribution modelling. *Journal of Ecology*, 96(6), pp.1117–1127.
- Taberlet, P. et al., 1998. Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology*, 7(4), pp.453–464.
- Tollefsrud, M.M. et al., 2009. Combined analysis of nuclear and mitochondrial markers provide new insight into the genetic structure of North European *Picea abies*. *Heredity*, 102, pp.549–62. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/19259114>.
- Tollefsrud, M.M. et al., 2008. Genetic consequences of glacial survival and postglacial colonization in Norway spruce : combined analysis of mitochondrial DNA and fossil pollen. *Molecular Ecology*, 17, pp.4134–4150.
- Tuenter, E. et al., 2005. Sea-ice feedbacks on the climatic response to precession and obliquity forcing. *Geophysical Research Letters*, 32(December), pp.1–4.
- Tzedakis, P.C., Emerson, B.C. & Hewitt, G.M., 2013. Cryptic or mystic? Glacial tree refugia in northern Europe. *Trends in Ecology and Evolution*, 28(12), pp.696–704. Available at: <http://dx.doi.org/10.1016/j.tree.2013.09.001>.
- Vidyakin, a. I. et al., 2012. Spread of mitochondrial DNA haplotypes in population of scots pine (*Pinus sylvestris* L.) in northern European Russia. *Russian Journal of Genetics*, 48(12), pp.1267–1271. Available at: <http://link.springer.com/10.1134/S1022795412120150>.
- Willis, K.J. & Van Andel, T.H., 2004. Trees or no trees? The environments of central and eastern Europe during the Last Glaciation. *Quaternary Science Reviews*, 23(23-24), pp.2369–2387.