

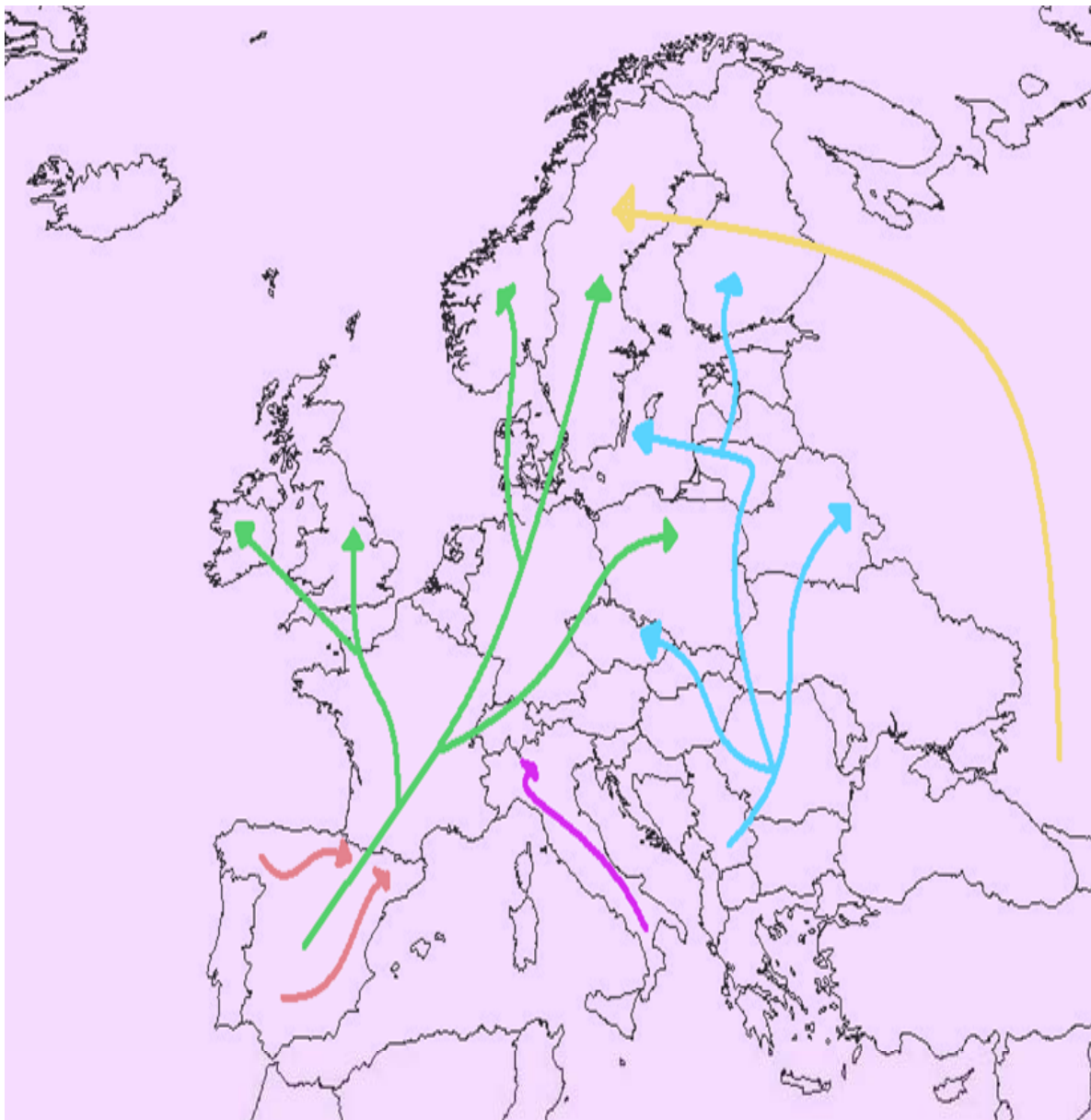
Introductory Literature

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“The phylogeography of plants in Europe”

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**Front page: a map describing the main potential postglacial migration lineages of several animal and plant species found in Europe based on molecular analysis.*

1. Introduction

“Phylogeography is the field of study concerned with the principles and processes governing the geographic distributions of genealogical lineages especially at the intraspecific level” (Avice *et al.* 1987).

Phylogeography is a young and fast-growing field that is primarily concerned with the spatial distribution of molecular lineages within a species but it can also be applied to study the phenotypic traits such as morphological, behavioural and cytological traits. It is a broad integrative field that encapsulates molecular genetics, population dynamics, palaeontology, geology and evolutionary biology (Avice 2000).

Phylogeographic studies generate hypotheses considering the Pleistocene glaciations as a major geographic and climatic factor that could have influenced a species distribution in the past. These studies also take into consideration the effect of recent events such as gene flow, genetic drift, and natural selection on the current geographic distribution of populations and genetic lineages. Because phylogeography deals with genetic variation at the population level at one end and speciation at another end, phylogeography serves as a linking bridge between macroevolution and microevolution (Avice *et al.* 1987).

In this paper I will describe the phylogeography of plants in Europe only, which is my main interest, since my research plants, the two rockrose complexes: *Helianthemum oelandicum* & *H. nummularium* have their main distribution in Europe. I will elucidate the role of the Quaternary cold periods on modifying species distribution and differentiation, and discuss the traditional methods, e.g. fossil records and the new techniques, e.g. molecular markers being used in describing and tracing the potential lineages and refugia of variable animal and plant species. I will also shed light on the role of different evolutionary and ecologically processes such as natural selection on shaping the genetic constitution within species.

2. Approaches

The term “phylogeography” was first introduced in 1987 by Avice and has ever since revolutionized the scientific evolutionary literature and became a popular topic. Numerous studies in the past had been carried out in the same direction as “phylogeography” but under the term “biogeography”, until recently when the new term phylogeography evolved and became firmly established. Many molecular tools have been applied to unravel the historical

scenarios of different plant and animal species to account for their current geographic distribution. It started with protein electrophoresis approaches (Harris 1966) by the late 1960s and progressed through valuable studies on animal mtDNA in early 1974 with the generation of restriction site maps for animal mtDNAs (Brown & Vinograd 1974). Phylogeographic studies blossomed entirely with the advent of PCR (polymerase chain reaction) that opened new doors for numerous molecular studies (Erlich *et al.* 1991) and the development of universal PCR primers for chloroplast (cp) DNAs and mitochondrial (mt) DNAs. Also remarkable advances in statistical programs that are capable of reconstructing the phylogenetic relationships among genes extracted from molecular data, have improved phylogeographic inference of living things on earth.

2.1 Fossil records

The fossil record remains until now the most popular and traditional data source that document changes in the distribution and abundance of plants and animals during the Quaternary. Fossils (palaeontology) combined with rock record (geology) provide valuable data on the relationship between time and space of both extant and extinct species.

Palaeontology plays an important role in describing the present distribution of species with the help of macro and micro fossil records that shed light on the historical distribution of taxa. In plants, pollen records have effectively detected the expansion range of different plant species and their putative refugia during the Last glacial maximum (LGM). Thermophilous forest trees that produce enormous amount of pollen are good examples in which pollen records played a significant role in reconstructing their histories (Hewitt 1996). Palaeo-ecological studies consider the effect of past climatic changes on reorganizing species distribution especially plants. These studies concluded that species response to past climatic changes was through migration / recolonization of new territories and that natural selection / adaptation played a minor role in shaping species' history (Huntley 1991). In earlier studies performed on the forest trees in temperate regions, it was detected that these trees varied in the rate of migration towards north. Huntley & Birks (1983), described in their study based on pollen maps how variable tree species expanded northward at relatively high rate from the southern refugia after the Last Ice Age. The migration rates range from 150-300m / year for most tree species including the common beech *Fagus sylvatica*, 500m / year for the common ash *Fraxinus excelsior* and 1500m / year for hazel *Corylus allevana* and pine *Pinus sp.* to around 2000m / year for alder *Alnus species*. As a consequence of this individualistic response

to climatic changes, the differential in migration rates of plants created mixtures of different species with reduced genetic diversity and increased homozygosity in northern Europe. Consequently, dynamic changes in the composition of communities and ecosystems occur, where the pioneers (earlier migrants) are dominant and well established and the later migrants are adapting to the new environments in the pre-colonized territories.

However, the phylogeography of most herbaceous plants have relatively low pollen production compared to trees and cannot be studied through pollen analysis. So a lot of information regarding the natural range of many plant species during the Last Ice Age and refugial areas cannot be inferred from pollen remnants. Thus, other sources of data are needed to infer the phylogeography and the recolonization pattern of these species.

2.2. Application

Phylogeographical studies cover a wide range of different organisms including: animals, plants, bacteria, viruses and even humans. By applying different molecular techniques that investigate the cytoplasmic (mtDNA & cpDNA) and the nuclear DNA variation, inferences about the species history, distribution and diversification can be made. Consequently, approaches combining the molecular data, climatic changes and geographical distribution of species may shed light on the relative roles of different historical forces on shaping the current genetic variation among species (Avice 1994).

3. The history of life on earth

Life on earth has been continuously subjected to dramatic events, physically (e.g. continental drift) and climatically (e.g. Pleistocene Ice Ages) ever since the origin of life. In this context, I will describe two major historical events that had a significant influence on the distribution and differentiation of all living things on earth.

3.1. Plate tectonics / Continental drift

The Plate tectonic theory describes the surface movement of the Earth. It states that the Earth surface is broken into large plates that continually changes in size and position over time. The edges of these plates that move against each other, represent hotspots of intensive geological activity, such as earthquakes and volcanoes. The Plate tectonic is a combination of two physical events, continental drift and sea-floor spreading (McKenzie & Parker 1967).

The German geologist Alfred Wegener was the first to propose the theory of continental drift, which states that parts of the Earth's crust moved slowly on the top of a liquid core. Wegener used his observations to predict that all the current continents were once part of a single gigantic super continent *Pangaea*. Sea-floor spreading is the formation or extension of a new oceanic floor when two plates move apart. As the plates move apart from each other, the rocks break and form a fracture between the plates (Raven & Williams 1997).

Continental drift theory is supported by several finding (Felton *et al.* 1975):

- i. Similarities in the distribution of macro and microfossils of plants and animals in several continents.
- ii. Similarities in the distribution of rocks along the opposed shores of the continents.
- iii. Radioactivity and magnetic properties of the rocks, along with similar mineral deposits between continents.
- iv. Geographic distribution of extant plant and animal species

Jardine and Mckenzie (1972) described in their paper the history of marsupials, how the continental drift affected the organism's distribution and diversification. Nowadays, native marsupials are found in Australia, north and South America and they exhibit remarkable morphological variation, adaptation and specialization to their current environments. It has been suggested that all marsupial species descend from one ancestor based on cytological studies, which in turn confirms that marsupials were once distributed on a single land mass before they became separated, diverged and isolated into disjunct areas as a consequence of continental drift.

Another study performed on the biogeography of West Arctic plant species Dahl (1958), postulated that these plant species reached Scandinavia via an earlier North Atlantic bridge or by direct migration from northeast America to northeast Europe. Thus, suggesting that theses species were once distributed on a single land mass connecting North America and Europe. However, there is still a lot of debate questioning whether or not North Atlantic Bridge did exist in the past and conclude that the west arctic plant species distribution on both continents can be better explained by long distance dispersal model.

Further evidence on continental drift can be inferred from Colbert's study (1952) that summarized the percentage of similarities in the Triassic reptile families. South America &

Africa had 75 % in common, South America & North America, 63 %, South America & Europe, 75 %, and South America & Australia, 12 %.

The Continental drift / Plate tectonics had obviously a primarily effect on rearranging species' spatial distribution, modification, divergence and differentiation. It is of great interest to use the past and current positions of the continents combined with climatic changes to study distribution and evolution of organisms.

3.2. Ice age cycles and glacial refugia

The Quaternary ice ages that occurred approximately 2.4 Myr ago, had a dramatic influence on the distribution and differentiation of many plant and animal species. The Last glacial cycle ended around 13 000 years ago (more recently compared to continental drift), and it had a profound impact on reorganizing the genetic structure of different species that are living today.

During the Last Ice Age, ice sheets covered large parts of northern Europe, including the whole of Scandinavia and northern Britain (Fig.1). The major mountain ranges in the south i.e. the Carpathians, Pyrenees and Alps were covered with ice caps. Between the main ice sheet and the southern mountain blocks was a plain of permafrost, tundra and cold steppe (Hewitt 1999). The extreme climatic and geological changes associated with glaciations led to extinction most of the northern Europe plants. However, the species that were inhabiting southern refugia such as the Iberian Peninsula, Italy, Turkey and the Balkans, survived where the climate was more favourable. The warm interglacial periods enhanced the expansion of southern species from these refugia towards north. As a consequence, plant species colonized the new suitable habitats from one or several refugia. During the rapid northward expansion, species may have encountered barriers, e.g. mountain ranges and water bodies. These barriers have probably split the species range, not allowing their genomes to mix that eventually lead to genome differentiation between the populations of a single species. It is noteworthy that a species may have already experienced disjunction, separation and genetic differentiation in the southern isolated refugia, maintaining their genetic lineages during and after postglacial migration. During northward colonization after the Last Ice Age, the genetic variants of a single species either maintained their identity and remained separated or became mixed and formed hybrid zones in different parts of the world. Apparently the biological factors, e.g.

dispersal abilities and ecological factors, e.g. environmental requirements of each species differ and subsequently so will the mode of range expansion (Hewitt 1996).

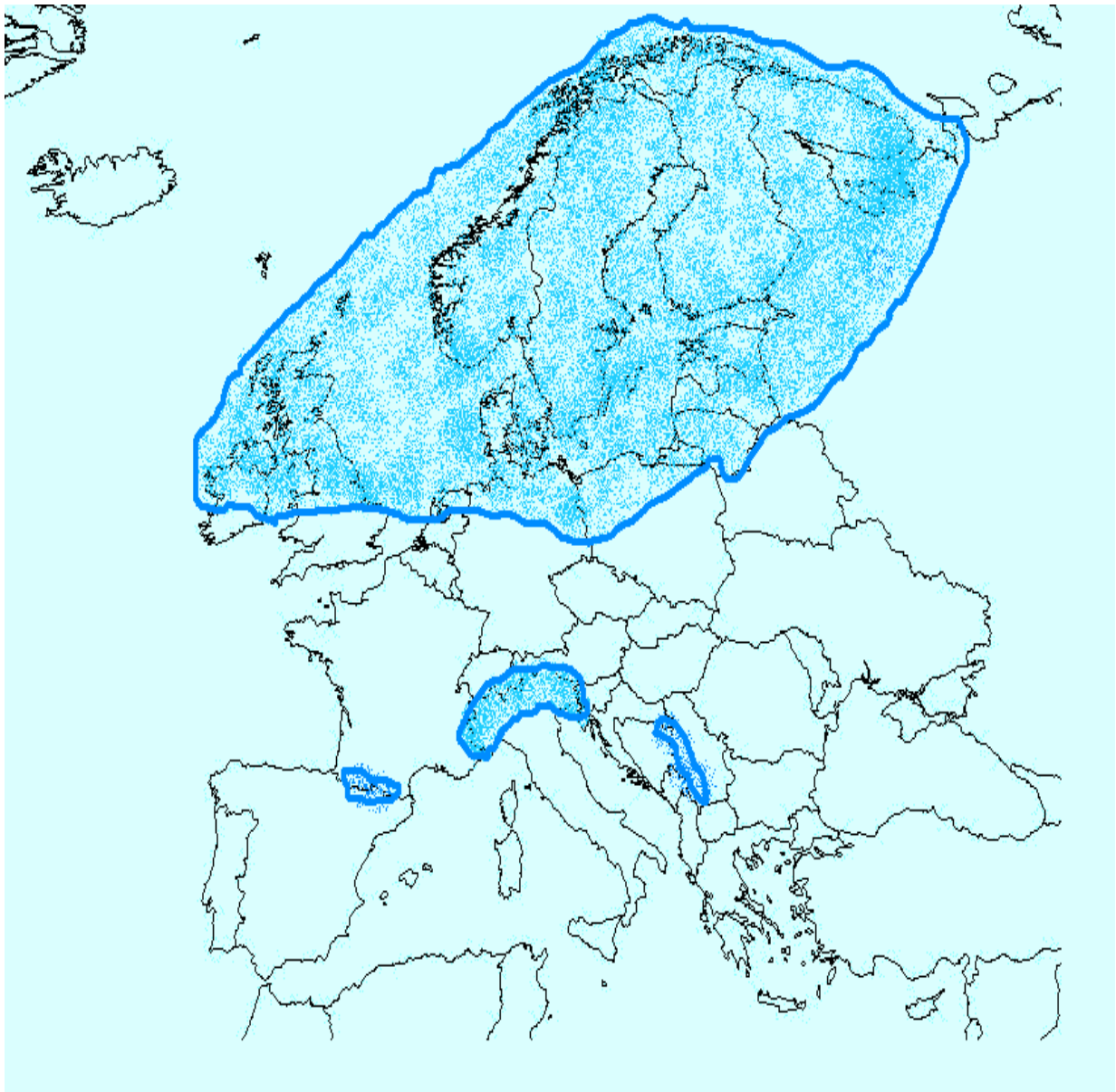


Figure 1. The cover of ice sheets over large parts of northern Europe, including the whole of Scandinavia and northern Britain during the LGM. The major mountain ranges in the south, the Carpathians, Pyrenees and Alps were covered with ice caps.

During the rapid northward expansion, some species may have experienced repeated bottleneck effects and genetic drift. This has led to loss of genetic diversity of the leading populations compared to the larger stable refugial populations in the south. Accordingly, the leading populations would have dominated the colonized areas favoured by natural selection,

and probably might have acted as biological or ecological barriers preventing the later migrants from entering and re-establishing the pre-colonized areas (Hewitt 1996).

However, if the northward expansion was a slow process, one would assume that all the southern refugial populations would have the same opportunity to colonize different areas at the same rate and thus, maintaining their genetic diversity in the newly colonized ranges (Hewitt 1996).

The repeated contraction and expansion of species' ranges induced by a series of advances and retreats of the ice as the climate fluctuated between cold (glacial) and warm (interglacial) periods are probably one of the causes responsible for modification and reorganization of the genome structure within species. A number of empirical studies identify genetic signatures of both animal and plant species that support this scenario of refugia and postglacial expansion.

4. Plant phylogeography

Plant phylogeography has received a lot of attention the past decade due to the development of molecular techniques that can reconstruct the species' history and provide insights into the roles of historical and present events in moulding the species' distribution and differentiation. Many phylogeographic studies based on molecular tools investigate chloroplast polymorphism in different plant species to deduce the geographic structuring of the genetic variation in relation to time and space. Phylogenetic studies are more concerned with the taxonomic structuring of nuclear DNA variation, and how that correlates with other factors such as morphology, ecology and geography. As a result, the geographic mapping of the distribution of chloroplast DNA using both molecular systematics and population genetics reveal two phylogeographical features. First, a phylogenetic tree of cpDNA haplotypes and second, a geographic map describing the distribution of cpDNA polymorphism.

4.1 Applied molecular techniques

Numerous molecular techniques have been acquired to study the intraspecific variation among species at the nuclear and organelle (mtDNA and cpDNA) level. However, not all techniques are capable of providing information about genealogical relationships within species. Thus, the need for phylogenetic relationships between the sampled data, restrict the molecular methods that can be used to study phylogeography.

Isozymes, AFLP (*Amplified fragment length polymorphism*) and RAPD (*Random amplified of polymorphic DNA*) are examples of techniques that produce unordered data, in which the phylogenetic relationship between the sampled data can not be easily deduced (Lowe *et al.* 2004). However, it has been found in some cases where these techniques could infer information about glacial refugia and postglacial lineages, e.g. isoenzymes analysis in *Viola rupestris* detected three distinct haplotypes restricted to three geographical areas: north Scandinavia, north Finland and southern-central parts of Scandinavia (Nordal 1998). RAPD technique used in *Saxifraga oppositifolia* support postglacial immigration into Scandinavia and reject the in situ glacial survival hypothesis (Gabrielsen *et al.* 1997). AFLP analysis of *Ranunculus glacialis* revealed four genetically distinct groups of populations in the Alps (Schönswetter *et al.* 2003). There was a significant genetic divergence between the two western groups (genetically dissimilar) and two eastern groups (genetically similar). This phylogeographic pattern suggests that the western populations were isolated for long periods with restricted gene flow, in contrast to the eastern population that remained in contact and exchanged genetic material. Hence, both the western and eastern populations survived the Ice Ages in non-glaciated peripheral refugia. AFLP analysis of *Trollius europeus* showed that it survived the LGM in southern refugia and recolonized three regions: the Alps, Pyrenees and Fennoscandia (Després *et al.* 2002). The AFLP data distinguished between two opposing evolutionary processes; gene flow and genetic drift that account for the corresponding genetic diversity within and among the three geographically distinct groups. The lack of correlation between genetic and geographical distances in the Alps as revealed by lower between-population differentiation reflects the role of gene flow in shaping the genetic structure of the populations. However, genetic drift is more predominant in the Pyrenees as detected by the high between-population differentiation. By contrast, a correlation between genetic and geographical distances was found in Fennoscandia supporting the isolation by distance model. AFLP analysis of *Saponaria pumila* resulted in four geographically distinct groups of populations in the Alps: East, Centre-east, Centre-west and Dolomites groups (Tribesch *et al.* 2002). The patterns of genetic differentiation of the four groups supports the postglacial immigration from peripheral refugia but does not exclude the possibility of “in situ” survival on isolated islands in the Alps.

DNA sequencing, microsatellite and PCR-RFLP are the most common and popular techniques in phylogeographic studies, since they produce ordered data in which inference about phylogeny can be easily detected. It has been shown that the uniparentally inherited

nonrecombinant cytoplasmic genomes, cpDNA and mtDNA are ideal tools for evolutionary studies. Chloroplast DNA is the smallest genome ranging from 120 to 160 kbp in higher plants. Most studies in plants have focused primarily on cpDNA compared to the other two genomes (mitochondria and nucleus), because it is easy to extract and analyse and does not undergo frequent gene recombination, duplications or major rearrangements (Singh 2004). Since chloroplast DNA is maternally inherited in most flowering plants (usually maternal in angiosperms and parental in gymnosperms), it is predominantly transmitted only through seeds (seed-specific marker) with restricted gene flow and therefore shows higher potential for population differentiation and hence geographic structuring than nuclear genes that are transmitted also through pollen (Cavers *et al.* 2003). Given these characteristics, chloroplast DNA is considered an ideal molecular tool for detecting the effect of both evolutionary processes (genetic drift and gene flow) and postglacial events on shaping the genetic structure within species (Beebee & Rowe 2004). However, the low substitution rates of cpDNA have become a noticeable problem in some intraspecific studies, since it is sometimes quite difficult to obtain sufficient intraspecific variation to reconstruct the past history of a species (Provan *et al.* 2001). For example, in *Erinus alpinus* (Stehlik *et al.* 2002 b), no chloroplast polymorphism was detected in the 22 populations sampled from the Alps. However, the AFLP technique, which investigates nuclear variation, identified three geographically distinct groups (central Switzerland group, Mount Rigi group in Swiss and west-eastern group of the central Swiss). In order to overcome the low polymorphism in chloroplast DNA, several techniques have been recently developed to detect sufficient cpDNA at the intraspecific level.

First, the development of universal primers combined with PCR-RFLP techniques which are highly specific to the non-coding spacers (high substitution rate) of the chloroplast genome. This has revealed neutral intraspecific variation and has proved to be a powerful technique for population genetic studies in plants (Taberlet *et al.* 1991, Dumolin-Lapeque *et al.* 1997 b, Demesure *et al.* 1995). Second, the recent development of chloroplast microsatellites (tandemly repeated mononucleotides in polymorphic loci) has revealed high intraspecific variation (Provan *et al.* 2001). The most common form of microsatellite variation is length polymorphism (insertion / deletion indels), and the least common form is point or substitution mutations. Identifying the microsatellite is possible through screening the database of partially or completely sequencing of cpDNA of particular species. Specific primers can be designed to amplify regions of interest and the polymorphic fragments can be detected (Provan *et al.* 2001). It is worth to note that microsatellite primers are not universal; they can only be

applied to the species of interest and perhaps to the related species. This is due to the highly specific primer-template annealing region and high levels of polymorphism in the flanking regions of cpDNA (Ishii & McCouch 2000).

Plant mtDNA is a poor molecular source (compared to cpDNA) for phylogeographic studies, due to its relative stability and slow rate of molecular evolution at sequence level. On the other hand, mtDNA is an ideal molecule for phylogeographic studies in animals due to its high substitution rate, high copy number, uniparental inheritance, relatively conserved gene sequence and lack of recombination (Avice 2000).

Nuclear DNA sequences have been suggested for phylogeographical studies since it is possible to screen polymorphism at multiple loci and hence investigate complex evolutionary hypotheses. Furthermore, the biparental mode of inheritance in nDNA produces genetic variations that are more representative of the whole population than the cytoplasmic uniparental inherited cpDNA or mtDNA. However, there are some problems associated with nDNA such as recombination and duplication which make it more difficult to interpret complex evolutionary hypotheses (Lowe *et al.* 2004).

Eventually, by studying both nuclear and cpDNA markers, the role of seeds and pollens in shaping the genetic structure of natural populations can be detected (Provan *et al.* 2001).

4.2 Postglacial migration routes and recolonization patterns

As the repeated expansion and contraction of a species range rearrange the distribution of the genomic variants, the different environmental and ecological conditions will eventually select for different genomes. Detailed studies based on morphological, cytological and behavioural data in a wide range of species and species complexes reveal the geographic subdivision of species into lower taxonomic units, e.g. subspecies, varieties, forms and races (Hewitt 1996).

The genetic diversity is usually higher in southern Europe (the Iberian peninsula, Italy and the Balkans) and tends to decrease northward as have been noticed in several plant species using different molecular techniques such as allozymes analysis in the moss *Leucodon sciurides* (Cronberg 2000) and in sedges *Schoenus spp.* (Hedrén 1997). Similar patterns were found in the common beech using allozymes and PCR-RFLPs (Comps *et al.* 1990 and Demesure *et al.* 1996, respectively). This pattern was also detected in white oak *Quercus spp.* (Dumolin-Lapégue *et al.* 1997a), in black alder *Alnus glutinosa* (King & Ferris 1998), in heather

Calluna vulgaris (Rendell & Ennos 2002) and in hornbeam *Carpinus betulus* (Grivet & Petit 2003) using PCR-RFLPs. PCR-RFLPs and microsatellites performed in hazel (Palme & Vendramin 2002) and AFLP used in alpine plant *Trollius europaeus* (Despres *et al.* 2002) revealed similar genetic gradient from south to north.

The greater genetic diversity in the southern Europe may be due to the tendency of southern refugia to accumulate higher genetic diversity owing to their persistence and relative stability. Evolutionary processes such as extensive past gene flow and hybridisation during the glacial cycles may also have contributed to the southern - northern gradient of genetic diversity (Hewitt 1996). The favourable climatic, ecological and environmental conditions in southern Europe have provided suitable habitats not only for plants but also for animals that escaped the glaciers in northern Europe. The southern refugia represent stable environments where the various plant species have met and survived the glacial cycles. Mountain ranges in southern Europe may have acted as barriers or allowed slower latitudinal migration of the species. Populations in the south may also have survived the glacial cycles by ascending the mountains during the warm interglacial periods and descending during the cold glacial periods. The varied topography of the mountain ranges may also split a species range into isolated disjuncts (with restricted gene flow) that will under time diverge and differentiate into unique genetic variants with distinct features in southern refugia (Hewitt 1999).

However, reduction in genetic diversity (in term of e.g. number of species, degree of subspecific division and allelic or cpDNA variation) in northern Europe can be explained by the “leading edge model”. This model implies that the peripheral isolates from larger stable populations (i.e. southern refugia) became separated followed by successive founder events after the Last Ice Age, leading to rapid colonization of ice-free territories (Hewitt 1999, Nordal 1987).

It is important to point out here that a reduction of the neutral chloroplast variation may also be due to selective sweep in coding regions, where a favourable mutation that increase individuals’ fitness in a population becomes dominant. The neutral genetic variation linked to the advantageous mutation becomes fixed and more prevalent, resulting in large reduction of the total genetic variation in the gene pool of the respective population. Selective sweep has more profound effect in smaller recently established populations (due to i.e. bottleneck effect) than in larger stable populations, but it can occur at any time and at any place.

However, there are cases where genetic diversity across a species range was not higher in northern populations than those in southern refugia. This pattern has been noticed in common beech (Comps. *et al.* 2001), *Carex digitata* (Tyler 2002) and in *Lathyrus vernus* (Schiemann *et al.* 2000) based on gene diversity parameter in allozyme data. Similar pattern was also detected in willow *Salix caprea* (Palmé *et al.* 2003) based on PCR-RFLP and microsatellite markers. This phenomenon can be explained by different factors including high mutation rates in the region of interest in the genome, extensive hybridization and introgression within species and rapid northward expansion. Accordingly, the geographic distribution of genetic diversity in species is individualistic, depending on species biological, ecological and evolutionary factors.

4.3 The Nunatak / Tabula rasa controversy

The “Nunatak” or *in situ glacial survival* hypothesis suggests survival of arctic / alpine species that habituated ice-free mountain tops during the Ice Age cycles. Today, these species are endemic and geographically restricted. They did not expand their natural ranges, probably due to repeated ascending and descending the high mountains during the glacial and interglacial cycles. And also due to rapid colonization of the ice-free territories in the surroundings by the neighbouring plants species that blocked the expansion of the arctic / alpine plants after the Last Ice Age (Brochmann *et al.* 2003). There are a few plant studies that suggest the possibility of “Nunatak” survival in the Alps based on AFLP analysis e.g. the Alpine cushion *Saponaria pumila* (Tribisch *et al.* 2002), the *Eritrichum nanum* (Stehlik *et al.* 2001), the *Erinus alpinus* (Stehlik *et al.* 2002 b) and *Rumex nivalis* (Stehlik 2002 a).

The “Tabula rasa” (as described earlier) suggests the survival of organisms in ice free territories in southern refugia such as the Pyrenees, Alps and the Balkans. Most of the northern populations could not survive in the hazard climatic changes and became extinct. As the ice retreated from northern Europe, species from southern refugia migrated towards the north and recolonized new territories where favourable conditions became available.

The “Tabula rasa” and “Nunatak” phenomena exhibit different levels of genetic variation within species. “Tabula rasa” should probably give rise to genetically related populations along the postglacial migration lineages which trace back to the stable populations in the putative refugia. In contrast, “Nunatak” should probably result in disjuncts or isolated islands of closely related populations in central parts of the Alps (Schönswetter *et al.* 2003).

There has been a lot of debate in the past regarding whether or not *in situ glacial survival* of certain alpine species does occur in Scandinavia. Mangrue (1973) suggested that “tabula rasa” is more reasonable in this context, where species recolonized Scandinavia from putative southern refugia, but he does not exclude the “Nunatak” hypothesis. Sollid & Sorbel (1979) suggested the survival of alpine species such as *Antennaria* on ice free refugia on the highest mountains on the Møre coasts in northern Norway. However, Sollid later pointed out that the Møre Mountains peaks were probably covered with ice frost where no plant species could survive due to lack of water (Nordal 1987).

5. Intraspecific phylogeography

Intraspecific phylogeography is concerned with the geographic distribution of genetic variation among subspecies, varieties or populations within a species. The term intraspecific phylogeography implies the connection between systematic biology, population genetics, and biogeography (Avice 1994).

Traditionally, morphological characters have been used to study variation among and within populations of single species. Nowadays, intraspecific phylogeography is mostly associated with the study of molecular markers, especially mt DNA (in animals) and cp DNA (in plants). By determining the phylogenetic relationships of populations within a species, plotting the intraspecific molecular variation on a geographic map, and relating the geographic distribution of molecular markers to geological / climatic events, deductions about the historical events that probably determined the current distribution of species can be made.

Studying the geographic distribution of genetic lineages sampled from different populations has highlighted the factors that influenced population structure and dynamic as well as species divergence (Avice 1994). Plant and animal species exhibit different levels of genetic variation between and within populations. The differences in genetic constitution between subspecies or lower taxa of a species may have evolved long before the Ice Age cycles in their southern refugia, a phenomenon called pre-Pleistocene divergence (Hewitt 1996). Pre-Pleistocene divergence can be detected by estimating time since divergence using molecular techniques as has been done, e.g. in the grasshopper *Corthippus parallelus* (Lunt *et al.* 1998).

6. Interspecific phylogeography

Just as phylogeography investigates the geographic structuring of genetic patterns among the populations within species, it is also concerned with studying genetic patterns at higher taxonomical levels among related groups of plant and animal species.

6.1. Interspecific hybridization

There are two forms of hybridization that may occur between two closely related species, primary and a secondary contact. The primary contact usually takes place between two or more closely related species that exhibit distinct ecological or biological requirements. The species distribution follows a parallel gradient towards each other, allowing these species to meet but not to mix, thus, leading to formation of hybrid or transition zones. The secondary contact involves allopatric speciation, when species differentiated and diverged from the origin due to long distance separation (vicariance), or barrier formation in the past. Consequently, those species meet again due to certain circumstances, e.g. postglacial migration or human activities and hybrid zones are formed, where gene flow and exchange of genetic material occur between the different morphs along the zones (King & Ferris 1999). In Europe, these hybrid zones are being detected along the Alps and Pyrenees, in central Europe and across Scandinavia (Hewitt 2004). There are several animal species that have formed hybrid zones in Europe, e.g. the grasshopper forms hybrid zones along the Pyrenees between French and Spanish populations and in the Balkan Peninsula (Lunt *et al.* 1998). In the hedgehog two parapatric species, *Erinaceus europeus* and *E. concolor* form hybrid zones from the Baltic to Adriatic Sea (Santucci *et al.* 1998). In the brown bear *Ursus arctos* (Taberlet *et al.* 1995) a hybrid zone exist in central Sweden where the western and eastern postglacial lineages meet. The two shrew species *Sorex araneus* and *S. coronatus* form hybrid zones from the western Alps to the Baltic (Taberlet *et al.* 1994). It has also been reported a study based on chromosomal rearrangement in *Sorex araneus* that another hybrid zone is found in central Sweden. The western populations colonized southern Sweden and the eastern populations colonized the northern part after the LGM (Fredga 1996).

6.2. Chloroplast capture

Chloroplast capture is basically concerned with chloroplast allocation and introgression from one species or subspecies into another in the hybrid zones. This phenomenon is strongly correlated with the geographic structuring of a species range. It has been detected in several plant species based on PCR-RFLP, e.g. the english holly *Ilex aquifolium* L. (Rendell & Ennos 2003), two alder species *Alnus cordata* and *A. glutinosa* (King & Ferris 1998, 1999 respectively), white oaks (Dumolin–Lapégué *et al.* 1997a) and common beech (Demesure *et al.* 1996).

In contrast, there is a phylogeographical study done on the willow (Palmé *et al.* 2003), where no correlation was found between cpDNA variation and the geographic distribution of the populations. Accordingly, most of the cpDNA variation was found within the northern populations and that can be interpreted by extensive hybridization between the migrating populations, rapid colonization of new territories or high mutation rates in the genomes.

6.3. Plant vs. animal phylogeography

In plants, molecular data combined with available fossil pollen record elucidate the impact of historical events on modifying species distribution and differentiation. For animals, molecular data are more valuable due to the scarcity or lack of fossil records.

Animal phylogeography is based on mtDNA, while plant phylogeography is primarily based on cpDNA analysis. However, both animal and plants are usually analysed by similar molecular tools: DNA sequences, e.g. ITS (*Internal Transcribed Spacers*) and molecular marker, e.g. AFLP that have contributed greatly to the studies of phylogeography of both kingdoms. Animals are in general mobile; they are able to migrate from one place to another or escape from unfavourable hazard environments. However, the immobility of plants has led to extinction of all populations in northern Europe during the glacial cycles. The biological, ecological and behavioural differences between plants and animals have strongly influenced the survival and extinction of many species during the Ice Age cycles. It is assumed that many animal species survived the ice ages by migrating southward where warm climates and preferable habitats were available, e.g. the Pyrenees, Alps and the Balkans, while plant populations in northern Europe were trapped and could not survive the hazard cold periods. Although, there are differences in the colonization patterns of animals and plants, they do share some common feature in their postglacial migration history (Hewitt 1999):

- The grasshopper (Lunt *et al.* 1998) along with the common beech (Demesure *et al.* 1996) and black alder (King & Ferris 1998) share one main postglacial lineage, where northern Europe was colonized by the Balkan refugium. However, in common beech (Demesure *et al.* 1996), it was concluded that the Iberian Peninsula and Italian populations were inhibited from northward progression due to rapid expansion of the Balkans populations as has been inferred from pollen data and not due to the Pyrenees and Alps barriers as has been detected in grasshopper (Lunt *et al.* 1998) and in black alder (King & Ferris 1998).
- The hedgehog (Santucci *et al.* 1998), white Oak (Dumolin-Lapégue 1997a), common ash (Heuertz *et al.* 2004) and silver fir *Abies alba* (Konnert & Bergmann 1995) colonized most of Europe from at least three putative refugia, the Iberian peninsula, Italy and the Balkans. Apparently, the three major mountain chains: the Alps, Pyrenees and Carpathians did not serve as barriers to migration of these species.

7. Phylogeography vs. Biogeography

Biogeography is the field of study concerned with the geographic distribution of species and higher taxa. It investigates the biodiversity and systematics of different plant and animal species in relation to geology, ecology and paleontology. Some authors prefer to subdivide biogeography into Historical and Ecological biogeography. Historical biogeography deals with the effect of past events on species distribution such as geological (e.g. continental drift) and climatic changes (e.g. Pleistocene). Ecological biogeography addresses the influence of recent events, e.g. migration, competition and adaptation to different habitats. Phylogeography is a new discipline that describes variation at a finer scale, including the geographic structuring of genetic variation among populations within a single species or closely related species (Futuyma 1998).

8. Comparative phylogeography

Comparative phylogeography has recently been developed and only few studies have so far been published in this field. It attempts to search for common postglacial lineages among different taxa, which may then clarify the influence of a common historical event on species distribution such as the Quaternary cold period (Taberlet *et al.* 1998).

The taxa being chosen for comparison should show a broad taxonomical and ecological variation over the geographical range of interest, in order to obtain more realistic and relevant results. In order to analyze as many datasets as possible, different types of molecular tools are being chosen for the multi taxa comparisons, e.g. AFLP and isozymes as well as different genomes, e.g. nuclear, cpDNA and mtDNA. However, it is important to elucidate that those different molecular tools and genomes interpret the patterns of phylogeography in different ways, making these types of studies more complicated. For example, the difference in the modes of gene flow between nuclear (biparental) and cytoplasmic (uniparental) genomes makes the phylogeographic interpretation more difficult.

In a study performed by Taberlet and his colleagues in 1998, ten taxa were investigated for phylogeographic inferences including mammals, plants, amphibians and insects across their geographical ranges. Although major differences were found in the overall phylogeographic patterns among the taxa, most of these taxa share three common southern refugia (the Iberian Peninsula, Italy and the Balkans). This phenomenon can be explained by the fact that each species has its own unique history reflecting its biological (i.e. dispersal ability) features, historical events (number of refugia, mode of recolonization) as well as ecological and environmental requirements (Taberlet *et al.* 1998, Hewitt 2000). Although the different taxa have their own unique phylogeographic histories, they do share some common postglacial migration lineages:

- For example the white oak (Dumalin-Lapégue *et al.* 1997a), *Ilex aquifolium* L. (Rendell & Ennos 2003) and hazel (Palmé & Vendramin 2002) expanded to northern and western Europe after the LGM from at least one potential refuge in the Iberian Peninsula.
- The common beech (Demesure *et al.* 1996), white oak (Dumalin-Lapégue *et al.* 1997a), common ash (Heuertz *et al.* 2004) and silver fir (Konnert & Bergmann 1995) recolonized the eastern part of Europe from a potential refuge in the Balkans.
- The black alder (King & Ferris 1998), hazel (Palmé & Vendramin 2002), hornbeam (Grivet & Petit 2003) and common beech (Demesure *et al.* 1996) exhibit unique genetic signatures confined to the regions surrounding the Alps, in Italy and Austria where the Italian haplotypes did not contribute to the northern expansion.
- It has been detected in the black alder (King & Ferris 1998) and hornbeam (Grivet & Petit 2003), that the Spanish populations did not participate in northward expansion. They

probably survived the successive glaciations south of the Pyrenees and did not progress to the rest of Europe after the LGM due to the presence of the Pyrenees mountain ranges.

A preliminary study done on the Rockrose *Helianthemum oelandicum* complex (Widén Soubani & Hedrén unpublished), revealed a clear picture of the geographical distribution of the cpDNA variation, which share some postglacial lineages and refugia with the plant species mentioned above. Two dominant haplotypes were detected in the complex: *Haplotype one* with a western, central and northern lineage and *Haplotype two* with an eastern, central and northern lineage. Thus, both haplotypes meet in central Europe and also on the Baltic island, Öland. As indicated from *Figure 2*, it is reasonable to suggest that *Haplotype one* expanded to northern and western Europe after the Last Ice Age from at least one potential refuge in north of the Pyrenees / southern France. Similar recolonization pattern based on cpDNA haplotypes has been detected in the white oak (Dumalin-Lapégue *et al.* 1997a). Whereas, *Haplotype two* recolonized the eastern part from another potential refuge in the Balkan and a similar pattern has been found in the Common Beech based on cpDNA polymorphism (Demesure *et al.* 1996) and in the silver fir based on allozymes study (Konnert & Bergmann 1995). The sympatric distribution of the two haplotypes on Öland is of great interest. It may suggest an earlier arrival of *Haplotype one* that colonized the southernmost part of the Great Alvar, followed by a later expansion of *Haplotype two* over the middle and northern part of the Great Alvar. This view is reinforced by the earlier elevation of southernmost part of the island (compared to the northern part) above the sea level after the Last Ice Age (Königsson 1968). *Haplotype three* is confined to the regions surrounding the Alps in Italy and Austria. The sharp northward boundary between *Haplotype three* and the other two haplotypes may suggest an earlier arrival of the two latter haplotypes. Previous studies based on cpDNA polymorphisms in hornbeam (Grivet & Petit 2003), as well in the common beech (Demesure *et al.* 1996) produced similar results where Italian haplotypes did not contribute to the northern expansion. However, *Haplotype two* has been detected in two populations in the Southern Alps, which may suggest a southward expansion from the nearby areas. Two closely related and distinct haplotypes *four* and *five* have been so far found only in Spain in isolated disjuncts. A similar feature where haplotypes were restricted to Spain and did not expand has been observed in black alder (King & Ferris 1998). They probably survived the successive glaciations and did not expand to the rest of Europe after the LGM due to the presence of the Pyrenees mountain ranges. *Haplotype six* is another endemic haplotype found along the west coast of former Yugoslavia that probably remained restricted

to that area during and after Pleistocene ages. Two very rare haplotypes have been detected only once: *Haplotype seven* found in an isolated population in southern France and *Haplotype nine* in northern Italy. It is reasonable to suggest that *Haplotype nine*, which differs in only one polymorphic site (i.e. mutation) evolved from the widespread *Haplotype one* that later became isolated in the southern Alps due to the founder effect. Whereas, *Haplotype seven* is not related to any of the haplotypes, suggesting it to represent an ancient refuge that probably survived the repeated glacial cycles.

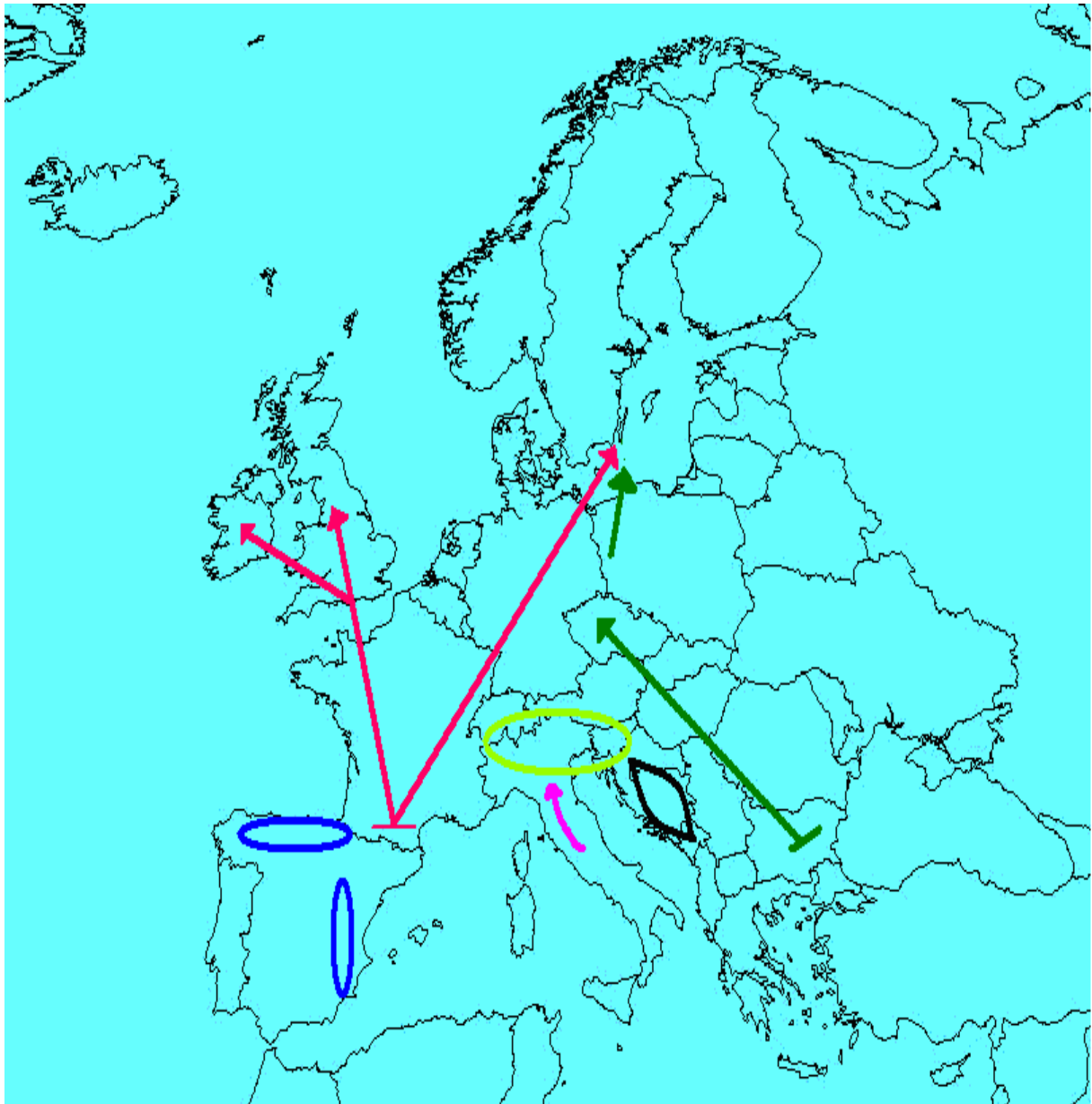


Figure 2. The distribution of the eastern (green) and western (red) postglacial lineages, southern putative refugia (southern France, the Balkan) and the three isolated populations (blue, black and yellow circles) of *H. oelandicum* complex in Europe (Widén, Soubani & Hedrén unpublished).

9. Phylogeography and conservation

“Modern biology has produced a genuinely new way of looking at the world...to the degree that we come to understand other organisms, we will place a greater value on them, and on ourselves”. E.O. Wilson (1984).

Phylogeographic studies detect unique patterns of genetic variation in both animal and plant species. What are thought to be a part of a stable homogenous and widely distributed species turns out to include ancient peculiar morphologically / genetically undefined species or subspecies that are mostly hidden in southern refugia.

Conservation programs main priority is to target unique endangered taxa with restricted geographic distribution and preserve them for future generations, that in order to maintain the evolutionary history of the wild populations. Gene flow, genetic drift, introgression and speciation are examples of dynamic evolutionary processes that contribute greatly to genetic diversity in species. Most of the plant species mentioned previously in this review, have one or several endangered populations with rare genetic markers. These unique genetic signatures are mostly distributed in southern refugia, representing hotspots of genetic diversity within species. A complete understanding of the phylogeographic history of species will allow up-to-date choices in prioritizing areas for species conservation (Avice 1994).

Conservation genetics relies on molecular techniques that offer an objective approach to address the three main issues in the field of phylogeography:

- I. Identify genetically distinct groups within (e.g. population) or between species
- II. Map the specific geographic regions where these genetically distinct groups exist.
- III. Determine the level and distribution of genetic diversity within and between the unique groups of populations in order to preserve the greatest amount of genetic diversity and to maintain the species evolutionary history (Avice 1994).

It is noteworthy that other biological aspects such as morphology – based systematics and behavioural ecology contribute greatly in defining the endemic taxa or organisms. Ultimately, a lot of information needs to be maintained in order to design and perform conservation strategies for wild organisms. Molecular analysis is a powerful tool for generating a great deal of valuable information for the purpose of conservation supplemented by other data sources (Schonewald-Cox *et al.* 1983).

10. Statistical phylogeography

Present phylogeographic studies are currently based on statistical approaches, because it is essential for making accurate estimations about historical events and therefore avoiding over interpretation of the analyzed data (Knowles & Maddison 2002). Phylogeography is concerned with mapping the geographic distribution of population genetic structure within a single species. In order to complete and clarify the historical scenarios of the species, statistical phylogeography is brought in for inference about the history and processes (past & present events) underlying that structure, and to provide facts, rather than *ad hoc* or narrative interpretations of the observed genetic variation (Knowles 2004). Deduction about a species history is based on statistical estimates of historical hypothesis, biogeographical and demographic parameters.

Knowles (2004) divided the statistical phylogeographical methods into two categories: summary statistical methods and likelihood analysis of evolutionary models. The summary statistical approaches perform simplified statistical analyses to summarize data. They are easy to perform, but the interpretation of data is restricted to specific models. Many species from different historical backgrounds can produce similar summary statistics, because these methods do not take into consideration all the information in the data. For example, high F_{st} -values (fraction of genetic diversity between populations) can result due to several events such as the occurrence of successive founder events at the peripheral refugia combined with restricted gene flow after the LGM or long distance isolation of southern refugia separated by barriers with no gene flow. However, likelihood analysis takes into consideration the whole data set (which can be computationally demanding) and estimates the probability of observing the data under alternative models.

Parameter estimation methods test hypotheses such as joint likelihood estimation of divergence times, effective population size and migration rates. They are statistically well developed, but they do not make inference about historical events. However, Templeton's nested clade analysis NCA (2004) aims at reconstructing phylogeographic history by taking into account different historical scenarios. Basically, it is a non-statistical method that does not provide any information regarding the errors being detected, nor does it provide any estimate about alternative historical events other than those tested (Knowles & Maddison 2002).

As the field of statistical phylogeography continues to develop, many difficulties associated with the complexity of testing historical assumptions will continue to evolve. Many events and processes that influence a species history such as gene flow or migration, genetic drift or bottleneck, vary in the time and space of occurrence, as well in their degree of effectiveness. It is also important to point out that not all historical processes may leave a genetic signature in the extant species (Knowles 2004).

Finally, in order to obtain an accurate representation of a species' history, it is essential to choose an appropriate method, irrespective if it is a summary statistic approach or likelihood analysis, for testing the historical hypothesis inferred from molecular data combined with other data sources such as palaeontology.

11. Summary and conclusion

In this paper, I briefly described the impact of the Quaternary cold periods on reorganizing the genetic structure of organisms in Europe. The use of molecular tools in combination with fossil, climatic data and advanced statistical models provide valuable information about the evolutionary history of species and also highlights the role of the past (the Ice Ages) and recent events (gene flow and genetic drift) on shaping species distribution and evolution.

Temperate species generally exhibit low phylogeographical subdivision compared to the southern species probably due to repeated contraction and expansion of species range during the successive Ice Age cycles that have led to extinction of many plant and animal species in the colder latitudes in northern Europe. Southern refugia have been relatively stable and advantageous for a lot of species during the cold periods and have accumulated great genetic diversity where rare genetic signatures can be detected.

Even though plants and animals differ in dispersal abilities and other factors, they do share some characteristics in their postglacial migration history, e.g. lineages and refugia. We can conclude that all organisms were influenced by one dramatic event at that time, the Ice Ages that restructured the genetic constituent of all species. It is noteworthy that although a species share with other species some putative refugia or postglacial routes, it has its own unique history reflecting biological (i.e. dispersal ability) features, historical events (number of refugia, mode of recolonization) and ecological and environmental requirements (Taberlet *et al.* 1998, Hewitt 2000).

Future studies in phylogeography rely on the advances in both molecular techniques and statistical programs that can reconstruct the species history and locate the endangered taxa that represent hotspots of diversity and a part of species range. Conservation priorities should target the populations with rare haplotypes and set up plans to protect and preserve so that we can obtain a complete picture of a species historical background.

12. References

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