

Genetic Differentiation Between and Among Refugia

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Abstract

1. Since the Last Glacial Maximum, the distribution of terrestrial plant and animal taxa in Europe has shifted from their refugia, Northwards in latitude and upwards in altitude.
2. The populations from refugia, mainly the mountain massifs of S Europe (Pyrenees and Alps, and also the Carpathian and Balkan Mountains), harbor a higher genetic diversity than the populations of N Europe.
3. The isolation by distance effect between populations tends to decrease with the increasing distance from the glacial refugia.
4. In areas of admixture from colonization originating in different refugia the heterozygosity may be higher than near the refugia.
5. Numerous thermophilous species have survived in their Mediterranean refugia at low altitude (particularly in islands).
6. Diploid populations or taxa often remained restricted at mid and high altitudes in S Europe, while their polyploid relatives spread in the lowlands.
7. For cold-adapted species, the refugia are mainly located in the S European mountains, and sometimes in N Eurasia, such as in Andøya island or in Taymyr peninsula.
8. The relict populations retaining a high allelic diversity and the endemic diploid taxa are now key elements in the long term conservation.

1 Introduction

Species which are widely distributed do not generally form one panmictic population. The degree of genetic variability varies within the distribution range. The first to formally recognize and search for genetic variability was the Russian scientist

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Nicolaï Ivanovitch Vavilov (1885–1943),¹ who set the foundations of research on the origin of cereals (in particular wheat). He demonstrated that their diploid wild relatives, in their original area, had a much higher variability than their widely cultivated generally polyploid relatives (e.g., Vavilov 1950). These principles were later followed by biologists studying the intraspecific variation of wild plants and animals. This same general principle may be applied to glacial relicts, with the question of the identification of the original area for each concerned species.

Since Forbes (1846) we know that the distribution of plants and animals changes with time, in particular during the Quaternary climatic oscillations. Haeckel formally recognised this when he wrote:

As the glaciation encroached from Northern Europe towards our Alpine chains, the polar inhabitants retreating before it - gentian, saxifrage, polar foxes, and polar hares - must have peopled Germany, in fact all Central Europe. When the temperature again increased, only a portion of these Arctic inhabitants returned with the retreating ice to the Arctic zones. Another portion of them climbed up the mountains of the Alpine chain instead, and there found the cold climate suited to them. The problem is thus solved in a most simple manner (Haeckel 1876, Chap. 14, p. 367).

The ca. 100,000 year cycle of glacial – interglacial periods has had a major impact on the distribution of organisms in the North hemisphere, particularly in Europe because of the East–west orientation of its main mountain massifs (Pyrenees, Alps, Carpathians), leading to a corresponding range shifts (Huntley and Webb 1989). For many cold-adapted species, the latitudinal Northward migration was accompanied by an upward altitudinal migration. These species then ended up with disjunct distribution, often called boreo-mountain or arctic-alpine species, depending on the altitude/latitude of their distribution (de Lattin 1967; Udvardy 1969).

From a genetic point of view, the key factors influencing the genetic structure of the populations of the species with such disjunct distributions are the age of the discontinuity in the distribution and the population size within each of the different distribution patches. For most European species distributed in both Scandinavia and the S European mountains (mostly in the Alps and the Pyrenees), it is assumed that the populations now found in, e.g., the Alps and the ones found in Scandinavia became separated ca. 10,000 years ago, i.e., at the end of the Würm glaciation. However, other scenarios are possible; N Europe may have been colonized from a refugium in Siberia, while Alpine populations were separated from these at a previous colonization cycle (Hewitt 1999). Furthermore, numerous thermophilous species remained at low altitude in the Mediterranean area during glaciations; afterwards some of them remained there while others spread Northwards.

The aim of the present chapter is to briefly discuss: (1) what are the long term consequences of range shifts in terms of endemic and cytotype distributions, and (2) how range shifts influence the genetic structure of populations.

¹The date of death of N.I. Vavilov is usually given as 1942 (e.g. Harland 1954). Details of his tragic death became known only after the demise of the Soviet Union (Azmanov 2002) and its date is 25 January 1943.

2 Karyology as a Marker of Genetic Variation

Chromosomal data are key variables in the genetic makeup of many plants and animals (Dobigny et al. 2004; Mabble 2004). These data accumulated over the last 80 years constitute a valuable material to look for a general framework about the relict cytotype distribution and the genetic differentiation within and among the refugia.

2.1 Endemism in the Mediterranean Basin

The Mediterranean Basin is characterized by its floristic richness (25,000 species) and high rates of endemism (around 12,500 species, i.e., 50%), while there is an obvious South–North gradient of decreasing endemism in Europe (Favarger 1972). The ten Mediterranean hot-spots of plant biodiversity represent privileged endemism areas and the most important refugia: mainly mountains and islands (Médail and Quézel 1997; Médail and Diadema 2008). About two thirds of endemics are perennial taxa that show a clear ability to colonize harsh habitats (coasts, screes, cliffs, rocky places, etc.), at mid and high altitudes (Médail and Verlaque 1997; Verlaque et al. 1997).

The remaining third are lowland endemics and constitute a threatened patrimonial flora (Verlaque 1999). For example, the diploid *Lysimachia minoricensis* of Minorca is now extinct in situ and the plants cultivated in the botanical gardens do not show any genetic variation (Calero et al. 1999). Except in such an extreme case, it is not possible to establish a general pattern of genetic variation according to the restricted or widespread status of taxa (Gitzendanner and Soltis 2000, Thompson 2005). Many rare species show a lower diversity than more common relatives, as theoretically expected, but there are many reverse cases. In the diploid endemic *Cyclamen balearicum*, the threatened populations of S. France (Languedoc) are genetically more differentiated than the healthy and numerous populations of Balearic Islands (Affre et al. 1997). Life history traits (life-form, age, mode of formation etc.; cf. Stearns 1992) and causes of rarity of endemic taxa play a major role in explaining these phenomena (Gitzendanner and Soltis 2000). It should be borne in mind that endemism may have two opposite causes: a small area may be the relict of a former widespread range or the result of a recent speciation. In the latter case, the new taxa may remain restricted neo-endemics, despite some colonization capacities, if they become isolated on an island or at high altitude, or if they are ecologically very specialized in rare habitats, as is the rule for most of the endemics.

In this context, a fourfold classification has been suggested based on the analysis of endemic taxa characters compared to those of their closely related species (Favarger and Contandriopoulos 1961; summarized by Thompson 2005). Proportions of endemic classes calculated in several islands and mainland areas indicate a general Mediterranean framework (Verlaque et al. 1997). The paleo-endemics (relict species morphologically isolated from other taxa) are mainly thermophilous taxa located in the lowlands, and are mostly present on islands. The patro-endemics

(generally diploid cytotype progenitors of polyploid relative taxa) occur at mid to high altitudes and are more present in mainland areas than in islands. The schizo-endemics (vicariant taxa with the same chromosome number) are generally diploid taxa living at high altitude. Lastly, the apo- or neo-endemics (polyploid derived cytotypes, younger than its relatives) mainly occur at low and mid altitudes.

Such a pattern allows the identification of two distinct processes: (1) the “passive” or relictual endemism (including paleo- and patro-endemics) and (2) the “active” or recent endemism (including schizo- and apo-endemics). The relictual endemism matches the refugia, and amounts only to 32% of endemics in Corsica, and 28% in Provence and Balearic Islands (Verlaque et al. 1997). As far as the recent endemism is concerned, the schizo-endemics are dominant (49–55% in W Mediterranean) and can be investigated mainly with genetic markers.

2.2 Polyploidy (i.e. Genome Duplication) in Europe

The occurrence of polyploidy into a group generally corresponds to an expansion phase (Goldblatt 1980; Lewis 1980; Thompson and Lumaret 1992). Conversely to the pattern found in endemism, by analysis of some lowland native floras, Reese (1958) shows a significant South–North gradient of increasing polyploidy, from the S Mediterranean area to N Europe [Table 1]. Brochmann et al. (2004) confirm this phenomenon and sum up the main historical hypotheses. In Europe, the intensity of Quaternary climatic changes has highly favored this polyploidy, due to a combination of phenomena: (1) the successive movements of floras (Huntley and Webb 1989), (2) their intermingling leading to numerous hybrids, and (3) the recurrent colonization of large virgin areas by the neo-polyploids during periods of glacial retreat. To these ecological factors, one must add (4) the fundamental role of genes controlling the meiosis, which can induce 2n-gametes (i.e., diplogametes) production when exposed to temperature changes and other stresses (Lexer and Fay 2005; Wang et al. 2004; Ravi et al. 2008). Thus, the Arctic region, the most affected by the glaciations, was re-colonized by a young flora very rich in allopolyploid taxa with a high heterozygosity (Brochmann et al. 2004).

The colonization power of polyploids can be explained by their wide ecological amplitude and tolerance: they are much more efficient competitors than their diploid

Table 1 Proportions of polyploid perennial taxa from the native lowland flora

Area	Percentages	Latitude
North Sahara, Algeria	42.3	31° N
East Czechoslovakia	56.2	50° N
Sjaelland, Denmark	57.2	56° N
North Sweden	65.5	66° N
Svalbard	78.2	79° N

From Reese (1958)

Table 2 Proportions of diploid species in the perennial taxa from SE France (Provence)

Total native flora	42%
Endemic taxa	52%
Rare non endemic taxa	60%
Rare endemic taxa	70%

progenitors, particularly in unstable conditions (Stebbins 1971; Ehrendorfer 1980; Levin 1983; Thompson and Lumaret 1992; Lumaret et al. 1997, Bretagnolle et al. 1998). Thus in France, the maximum percentage of polyploids is found among alien invaders (77%, Verlaque et al. 2002). Conversely, in native flora the higher rates of diploids reaches 70% in rare endemic taxa (Table 2) and 75% in critically endangered segetal plants (Verlaque and Filosa 1997). There is a strong correlation between the abundance of taxa and their ploidy level: polyploids correspond to the most common taxa, while the rare or extinct species are mainly diploids (Hodgson 1987).

However, the study of polyploids is often very difficult because of their various possible origins: auto- or allo-polyploids from different populations (Levin 2002; Brochmann et al. 2004; Eidesen et al. 2007). A morphological and karyological approach is needed apart from DNA data in studies of plant phylogeny and “it seems advisable to investigate only the diploids (at least at first)” (Stace 2005). Polyploidy is generally a recurrent process followed by quick karyotype reshuffling, leading to a one-way process (Soltis and Soltis 1999; Crawford and Mort 2003). Genome duplication may also alter sexual reproduction and interspecific barriers; thus polyploids often become autogamous or apomictic (Richards 1997), or inter-hybridize (Breton-Sintès 1974; Gadella 1987). The intensive reticulation appears to be the main cause of phylogenetic incongruence because homogenization (of ITS sequences), via concerted evolution, may occur in various descendant lineages (Mansion et al. 2005). Lastly, the redundant structure of the genome in highly polyploid taxa often leads to a buffer effect against arising mutations.

During the glaciation maxima, the thermophilous species were pushed southward, and only the very dynamic taxa and cytotypes could (re)colonize successfully. In the Pteridophytes genus *Asplenium*, the 22 rupicolous diploidized species ($2n=72$) remained mostly confined in the Mediterranean refugia after the Würm glaciation, only five diploid taxa colonized central Europe, the British Isles or Scandinavia. In contrast, the ten widespread taxa up to N Europe are polyploid ($2n=144$). Furthermore, the diploid populations of *A. ruta-muraria* from Gorges du Verdon in SE France show the highest allelic variability known in ferns (Vogel et al. 1999). In Angiosperms, two examples could be cited: (1) the segetal EuroSiberian *Camelina sativa* includes one diploid relict population ($2n=12$) in the SE France (Monts de Vaucluse, Verlaque and Filosa 1992), now critically endangered, one tetraploid in Bulgaria ($2n=26$) and a majority of hexaploid populations ($2n=40$) in Iceland, Poland, China and Russia; (2) the stenoMediterranean *Calicotome spinosa* is tetraploid, except in SE France (Var: Gapeau Valley) where a relict diploid cytotype still survives at low altitude (Aboucaya 1989, Fig. 1).

During the glaciations some diploid species already adapted to cold conditions persisted in refugia, especially in the S European mountains (Favarger 1967, 1972),



Fig. 1 Distribution of *Calicotome spinosa*. Tetraploid populations ($2n=48, 50, 52$) are found from Spain to Italy, while only one population in Var (France) was found to be diploid (*Diamond*: $2n=24$). Distribution map compiled from Aboucaya (1989) and de Bolós and Vigo (1984)

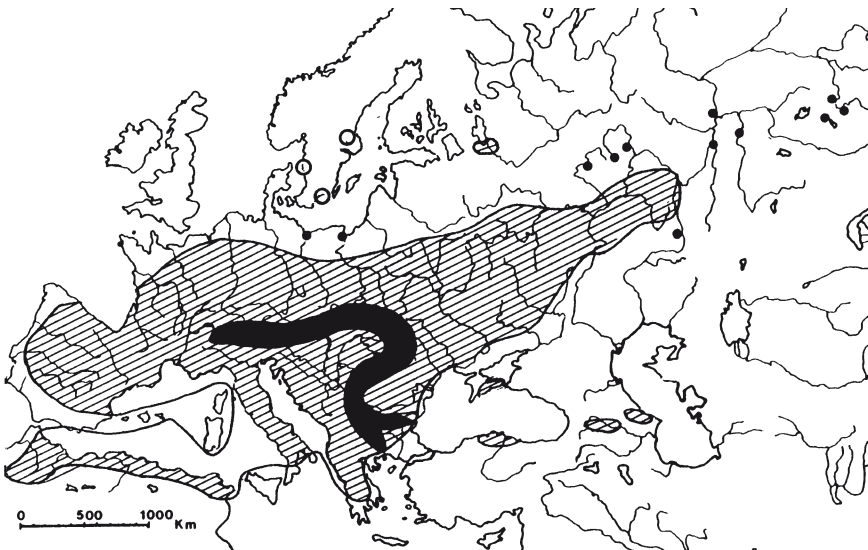


Fig. 2 Distribution of *Tanacetum corymbosum*. The diploid (subsp. *clusii*, $2n=18$, in black) is found only at 1,000–2,000 m altitude in the mountains from the Alps to the Rhodopes, while the tetraploid (subsp. *corymbosum* $2n=36$, hatched) is found in lowlands from Spain to Russia

while only polyploid cytotypes spread widely during the interglacial stages. In *Tanacetum corymbosum*, the diploid subsp. *clusii* is found from the Alps to the Rhodopes (1,000–2,000 m), while the tetraploid subsp. *corymbosum* is widely distributed in the lowlands from N Africa to Russia (Seidenbinder 1989; Verlaque and Contandriopoulos 1990; Fig. 2). In the orophyte *Ranunculus kuepferi* of Corsica and Alps, the diploid sexual relict subsp. *kuepferi* is restricted to the SW margin

(Alpes-Maritimes, Var, Isère), whereas the tetraploid apomictic subsp. *orientalis* is widespread in the rest of the species range (Küpfer 1974).

3 Genetic Structure of Populations

The genetic structure of populations may be studied at different scales. The combination of genetic information from populations through a species' range leads to a knowledge of the spatial aspects of population genetics (Fig. 3). While investigating the population genetics of wild species, several indices may be used (Box 1). The most common for each population are the expected heterozygosity and the allelic richness. When the investigation concerns the structure of the populations, then F statistics and their derivatives are the most common indices (Wallis 1994), but the application of geostatistical techniques indicates that these traditional values might not be the best indicators of population differentiation (Joost et al. 2007, 2008).

Each of these genetic indices has its own objectives. The allelic richness is very sensitive to population bottlenecks and to population admixture. In the diploid beech tree *Fagus sylvatica* (W & C Europe, 0–2,000 m), the area of the highest heterozygosity in Europe corresponds to the area which was colonized between 4,000 and 2,000 years ago, which corresponds to the area of admixture of colonization fronts coming from different glacial refugia (Comps et al. 2001). On the other hand, the highest allelic richness is still found near refugia, i.e., the Balkan Peninsula and S Italy.

The process of colonization leads thus to a decrease of allelic richness, due to multiple foundation events, while colonization from different areas may lead to high heterozygosities, because of the admixture from refugia with different alleles (Widmer and Lexer 2001; Petit et al. 2001). For species which have colonized N Europe from a Southern refugium, their heterozygosity decreases with increasing

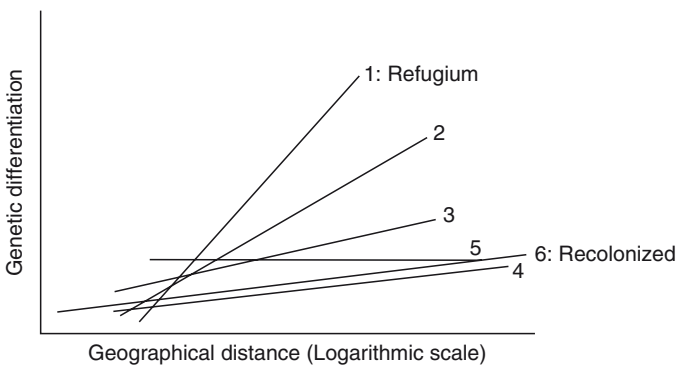


Fig. 3 Isolation by distance effect in *Carabus solieri*. The slope depends on the time since colonization. The northernmost zones (4–6), i.e., the most recently colonized, show a more level slope than the areas close to the refugia (1), with a gradient of slopes for in between zones. Redrawn from Garnier et al. (2004)

Box 1 Indices Used in Population Genetics

H Expected Heterozygosity Probability that two alleles sampled at random from a population are different. It is a function of the allelic frequencies. The expected heterozygosity is basically calculated for each locus, and a mean (or sometimes a median) value is presented for a number of loci. This index is particularly sensitive to bottlenecks having occurred in the population previously. This index is also called the Nei's gene diversity.

Allelic Richness The number of alleles per locus is a good index of diversity. It is highly sensitive to past population bottlenecks (Luikart et al. 1998) and investigated loci. As it depends on the number of sampled individuals, this index has to be normalised to a given sample size (El Mousadik and Petit 1996).

Fixation Index F_{ST} is an index of the way two or more populations are differentiated from each other. It depends on the difference of allele frequencies among the populations. It is usually estimated from allele frequencies in the concerned populations, as follows:

$$F_{ST} = (H_T - \bar{H}_s) / H_T,$$

with \bar{H}_s the mean expected heterozygosity of an individual in each population mating randomly, and H_T the expected heterozygosity of an individual in a total population mating randomly (e.g., Hartl and Clark 1989).

Isolation by distance The strength of any isolation by distance effect is measured as the slope of the regression of an index of genetic distance as a function of the geographic distance, both axes being expressed in logarithmic scale. The genetic distance is expressed either as F_{ST} or as $F_{ST}/(1-F_{ST})$ for each pair of populations. The steeper the slope is, the stronger the isolation by distance effect would be.

Neighborhood Size Individuals often do not reproduce at the location of their parents. The genetic neighborhood has been defined as the area within which most of the individual will reproduce. Dispersal is the movement from the birth or parents' locations to the reproduction location. Wright (1943) defined the genetic neighborhood of an individual as the population of the area within the variance (σ^2) of the dispersal distance. With d the density of individuals, the neighborhood size is given by $N_e = 4\pi d\sigma^2$. As the dispersal distance is usually difficult to measure, the neighborhood size is

(continued)

Box 1 (continued)

often estimated from the differentiation between populations, using F_{ST} values estimated by pairs of populations to estimate the gene flow

$$M_{ST} \approx \frac{1}{4} \left(\frac{1}{F_{ST}} - 1 \right).$$

The y-intercept of the log-log plot of the estimated M_{ST} against geographic distance gives an approximation of the local effective population size (Slatkin 1993; example in Nève et al. 2008).

distances from their refugia. This has been called the “leading edge” effect, where the front (here North) part of the distribution moves, and the northernmost populations are the results of multiple colonization events (Hewitt 1993, 1996; Nichols and Hewitt 1994). The slope of the decrease of this heterozygosity depends on the rate of the loss of genetic diversity through the stepping stone colonization process.

During shifts of species range, the newly occupied area is the result of multiple colonization events. If this colonization is the result of rare events involving individuals only at the front edge of the distribution, a strong isolation by distance will follow together with a reduction of allelic diversity due to the repetitive colonization effect (Ibrahim et al. 1996). This resulted in a series of genetic hotspots distributed in the S Europe. Climate warming led to a gradual upward range shift, with little loss of genetic diversity, while the Northwards range shift involved long distance migration and multiple colonization bottleneck events. In the *Frangula alnus*, Eurosiberian diploid (0–1,400 m), the Iberian Peninsula and Turkey still harbor by far most of the genetic diversity. These probably correspond to the two main refugia for this species, and the colonization of the plains of central Europe was possible only by crossing severe barriers to dispersal (and hence gene flow) caused by the Pyrenees and the Black Sea (Hampe et al. 2003). In the arctic-alpine diploid species *Ranunculus glacialis* (1,700–4,000 m), populations from N Europe show a much lower genetic diversity than Alpine populations, as they resulted from a colonization from E Alps, where populations have retained a high genetic diversity (Schönswetter et al. 2003). In *Gentiana ligustica*, tetraploid endemic to the Ligurian Alps, the Shannon’s Diversity index, computed on AFLP fragments, decreases with increasing distance from putative Würm refugia (Diadema 2006).

The retreating range margin is also subject to important changes in the population structure. As the area becomes generally unsuitable to the species of interest, its local suitable habitat patches become scarce and far between. The populations

which used to be part of large networks of populations exchanging individuals with their neighbors become more isolated. These “trailing edge” populations are then reduced in their effective size, and are subject to drift, independently of other populations (Hampe and Petit 2005). The populations within the different mountain massifs of S Europe have been present in these areas often for several glacial cycles, resulting in levels of population exchanges to have occurred roughly according to the geographic distance. As at the continental scale the population exchanges have been rare, and as this process ended up being leveled over a very long time span (Slatkin 1993), the populations now found in the S European mountains often end up showing a very strong isolation by distance effect. If the North of Europe has been colonized from an Eastern refugium (“*Chorthippus* pattern,” Hewitt 1999), the populations of the mountains of S Europe may have been isolated from the Northern populations at least since the Riss glaciation (ca. 130–240 ky BP). In this case their long isolation resulted in a strong differentiation. The Capercaillie *Tetrao urogallus* of the Cordillera Cantabrica and the Pyrenees is an old lineage different from the rest of the species’ distribution, from the Alps and Scandinavia into Siberia (Duriez et al. 2007), which is the result of populations from the Iberian refugium not colonizing the lowlands North of the Pyrenees.

Within a given mountain massif, populations may also show a high differentiation, as they are separated by strong barriers such as valleys and rivers. If populations survived in nunataks during the last glaciation, the differentiation between these is the result of a pre-glacial isolation. In such cases, the populations now found at high altitudes of the Alps are the result of in situ survival during the Würm glaciation rather than post-glacial recolonization from peripheral areas. The perennial tetraploid cushion plant *Eritrichium nanum* most probably survived in at least three nunatak areas of the Alps (above 2,500 m), as these populations are the most variable (high number of private AFLP fragments) and the whole data set form three distinct clades (Stehlik et al. 2001). In other cases such as the artic-alpine diploid *Saxifraga oppositifolia*, genetics studies do not allow the identification of nunatak survival in the Alps, presumably because any surviving nunatak population would have been swamped by colonization from the main refugia (Holderegger et al. 2002). Sometimes a single pre-glacial population may end up being split: the tetraploid SW Alpine endemic *Senecio halleri* survived in two nunataks in the South part of its distribution, SW and NE of the Aosta valley, Italy. These two populations are not significantly different from each other, resulting probably from a single pre-glaciation colonization event (Bettin et al. 2007). Survival in and around mountain massifs during the last glaciation favoured the development of genetic differentiation between populations. In the widespread diploid *Arabis alpina*, the high among population variation in the mountains of S Europe (Alps, Tatra and Carpathians) suggests several refugia for this species, whereas all over the North of its distribution (Scandinavia, Iceland, Greenland and Newfoundland) it shows very low genetic variation, as these populations probably result from the recolonization from a single refugium closely following the retreating glaciers (Ehrich et al. 2007). Notably the populations in the East African mountains show high inter-populations variation, an evidence of their old age.

The carabid beetle *Carabus solieri* is an endemic species to the SW Alps near the Italian–French border. Garnier et al. (2004) sampled the whole distribution of this species, and performed genotypic identification of ten microsatellite loci in 1,080 individuals. Globally these populations show a strong isolation by distance, which is not surprising for a wingless species living in the mountain forests, in an area with many barriers to dispersal such as non-forested areas, roads, rivers etc. The 41 studied populations were grouped into three clusters, from South to North. Each cluster was then cut into two parts to have a series of six subclusters. Analysing these clusters separately for isolation by distance effect yielded a strong cline in the slope of the relationship between genetic distance and geographic distance. The slope is the steepest nearest to the refugium, and the most gradual in the recolonized area, i.e., northernmost set of populations (Fig. 3).

Other patterns of postglacial colonization have been described, from the refugia in central Asia or N Europe. The rare Alpine populations of the tetraploid *Carex atrofusca* originate from a central Asian refugium, while Siberia and Greenland populations form a sister clade (Schönswetter et al. 2006a). The diploid *Ranunculus pygmaeus* provides even a more extreme case as the Alpine populations probably originate from a refugium on the Taymyr Peninsula, East of the last glacial ice sheet (Schönswetter et al. 2006b). In Norway, the high nucleotide diversity of the populations of the root vole *Microtus oeconomus* from the island of Andøya strongly suggests in situ glacial survival for this species (Brunhoff et al. 2006). These examples show that the recolonization of the European ice sheet, after the last glacial maximum, took part from all directions, even if the contribution of the Southern refugia was prevalent (Fig. 4).



Fig. 4 Map of Eurasia showing the Last Glacial Maximum (LGM; ca. 20 ky BP), with the main identified refugia of European taxa (LGM from Svendsen et al. 2004; Ivy-Ochs et al. 2008). Small glaciated areas in Mediterranean mountains (not shown here) are discussed in Hughes et al. (2006). *Filled circles*: main refugia; *Open circles*: nunatak refugia

4 Isolation by Distance

4.1 Differentiation Among Populations Within a Region

Differentiation among populations within each region is the result of the way individuals move within the region. All species are regionally distributed in an aggregative way: the species is found in its habitat, while it is missing or shows a low density outside its optimal habitats. Some species have a high dispersal power, and their populations are not differentiated within a region. This is the case of many migrating species, where dispersal between the birth location and the reproductive location is common. Many birds and insects (e.g., *Colias* and *Aglais urticae* butterflies; Vandewoestijne et al. 1999; Watt et al. 2003) follow this pattern. Wind pollinated plants also show genetic admixture in large areas, at least as far as the nuclear genome is concerned (e.g., *Quercus* spp., Petit et al. 2002).

The geographical scale of the isolation by distance effect varies with the dispersal scale of the species. In fixed organisms (i.e., plants), the scale of interest may be from a few meters (the distance between two individuals) to the extremes of a species distribution. Depending on the scale and on the dispersal capability, the relationship between geographic and genetic distance may or may not be significant. In the case of the Lycaenid butterfly *Euphilotes enoptes* there is no isolation by distance for distances of less than 30 km, but well in the range 30–400 km (Peterson 1995, 1996). The lack of correlation at small distances was related to the ecological heterogeneity at small scale, such as local topography, which has a profound impact on the species phenology. Isolation by distance at greater distances was attributed to a stepping stone dispersal process. Furthermore, the isolation by distance effect is mainly the result of long-distance migration events, while genetic neighborhood depends chiefly on the rate of movement between neighboring patches. In the butterfly *Proclassiana eunomia* neighborhood size is higher in the mountains than in the lowlands, while isolation by distance is higher in the mountains than in the lowlands (Nève et al. 2008). This shows that dispersal kernels may vary greatly between regions, and that population differentiation patterns may consequently also vary in different ways for short and long distances.

Differences in isolation by distance processes may also go the other way, with significance at the small scale distances of the spectrum, and no significance at a larger scale. In a series of 17 sampled sites for the diploid plant *Rhododendron ferrugineum* in the Alps and Pyrenees, no isolation by distance could be detected for site distances ranging from 4 to 1,000 km while, when a single site was studied in detail, the isolation by distance effect was significant for distances between 10 and 5000 m (Wolf et al. 2004). In this case, short distance isolation by distance was the result of short time scale bio-ecological factors, such as allogamy, pollination and seed dispersal, while the structure of populations on the Alps as a whole resulted from disequilibrium between dispersal and genetic drift, as very long distance dispersal does probably not follow a simple decreasing monotonous curve for this species.

4.2 *Differentiation Among Regions and Long Distance Gene Flow*

Species which were widely distributed during the Würm glaciation may end up having a now disjunct distribution. This may be the result of different processes: the populations of N Europe may have found themselves separated from the populations moving up the S European mountains or from a long distance colonization of newly available habitats such as islands in the high Arctic Ocean.

In arctic-alpine species, colonization may occur at very long distance. Isolated localities such as Bear Island and the Svalbard Archipelago had to be colonized over hundreds of kilometers of sea. Consequently, there is little isolation by distance in species showing such high colonization power. A recent study of nine species found on Svalbard showed that this archipelago was mainly colonized from NW Siberia (Alsos et al. 2007), rather than from the geographically closer Scandinavia and Greenland, because the main polar wind, from East, favored a colonization of high Arctic islands from Siberia (Coulson et al. 2002), and E Siberia (the Beringian area) was not covered by an ice sheet (Brochmann et al. 2004).

Among all the populations of the diploid mountain avens *Dryas octopetala*, Svalbard populations were most closely related to the Siberian ones. This arctic-alpine species displays an East–west differentiation, with five different groups in the Holarctic region (Skrede et al. 2006). The refugium of the European group probably laid in the tundra of C Europe during the Würm glaciation. As the ice retreated, this population split between the parts which migrated northwards to Scandinavia, and the populations which migrated up the mountains of the Alps. Among the European group, the Alpine populations show a much reduced variability ($H < 0.077$) compared to the Arctic ones ($0.101 < H < 0.144$). This unusual pattern is probably due to the widespread distribution of *D. octopetala* in C Europe during the Würm glaciation, which led to a latitudinal range shift involving several large refugia, while the colonization of the Alps involved fewer ones leading to a strong bottleneck effect.

The mating mode of organisms may increase the effect of isolation by distance. The perennial plant *Draba fladnizensis* mainly reproduces by selfing (as numerous arctic plants: Richards 1997). Morphologically, the individuals are homogenous throughout the arctic-alpine range, from N Europe to Alaska. Crossing experiments between specimens from different parts of the range mainly resulted in either no seed, or sterile seeds, while intra-population crossing yielded numerous viable seeds (Grundt et al. 2006). In this diploid species, the differentiation between populations proceeded rapidly, leading to cryptic speciation, even within a limited geographic area: Svalbard archipelago hosts at least three such sibling species. This speciation process (by minor translocation or mutation events, Brochmann et al. 2004) probably occurred in situ during the ca. 10,000 years since the height of the last glaciation was when Svalbard was completely covered by ice. A similar sibling speciation has been found in *Nigella* (Strid 1969) and *Erysimum* (Snogerup 1967) endemic taxa living in harsh habitats in the Aegean islands.

5 Conclusions

Processes of population differentiation had different consequences, according to the spatial and temporal scales at which they occurred. The differentiation between mountain massifs or between North and South Europe is a process due to the occurrence of long distance colonization or lack thereof. Some trends may be suggested: high arctic species tend to have a high colonization power, as their present range had to be efficiently colonized since the Würm glaciation, and also periodically during previous colonization cycles (Huntley and Webb 1989). For genetically spatially structured species, the focus should be on the most variable populations and on the ones which host many private alleles (Diadema et al. 2005). The knowledge of Evolutionary Significant Units (Crandall et al. 2000) taking into account both aspects, would be a valuable tool for conservation.

The postglacial colonization processes have favoured taxa with high vagility of propagules and a great competitive ability. In the latter respect, polyploids generally perform better than their diploid progenitors. However, the conservation of diploids is a key to the future evolution of the flora, their loss would be irreplaceable, as the families with the highest proportion of diploids are generally the ones with the most derived characters. Most of diploids have a better evolutionary potential than polyploids because minor modifications of their genome may alter the species more quickly and deeply. Lastly, harsh habitats, species rarity and endemism are key factors influencing the proportion of diploid in the native flora. Nowadays, disturbances caused by human activities have similar effects as glaciations, with the demise of many diploid stenotopic taxa, and the expansion of a few eurytopic polyploids, leading to an impoverishment and a banalization of the lowland flora.

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