Resistance or emigration? Response of alpine plants to the ice ages

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There is a long-standing debate about the fate of the mountain flora of the European Alps during the Pleistocene ice ages. Two main scenarios of glacial survival of alpine plant taxa have been discussed, namely (1) total extinction within glaciated areas, survival in peripheral refugia, and postglacial re-immigration into vacant areas (tabula rasa hypothesis), and (2) long-term in situ survival within glaciated regions in isolated ice-free areas above the ice-shield (nunatak hypothesis). Four alpine species with differing distributions and ecological demands were investigated to elucidate their glacial history using molecular methods (AFLPs, RFLPs of cpDNA, RAPDs). Their glacial histories are very diverse. Whereas in situ survival in the most intensely glaciated Central Alps played an important role in Eritrichium nanum, the low alpine Erisimum alpinus survived in situ on some mountains of the northern Swiss Prealps, and Rumex nivalis grows at intermediate alpine elevations in snow-beds in both the northern and the Central Swiss Alps. In the common arctic-alpine Saxifraga oppositifolia, the species with the widest distribution and ecological amplitude as compared to the other three species, it is not possible to reconstruct its glacial history. It is probable, therefore, that in the Alps, as in northern Europe, resident genotypes surviving glaciation in situ were integrated into the gene pool of postglacially immigrating periglacial individuals. The size of refugia differed according to species and region. On the one hand, refugia were restricted to individual mountains (E. alpinus, R. nivalis). On the other hand, they spanned several mountain ranges in larger areas (E. nanum, E. alpinus). Postglacial migration over longer distances was inferred for E. alpinus from southern France to northern Switzerland, and, over shorter distances, for R. nivalis from the northern Prealps into the Central Alps in Switzerland. Both postglacial immigration and in situ survival shaped the phyleogeography at least of E. alpinus and R. nivalis. It is likely, therefore, that the nunatak and the tabula rasa hypotheses are too simplistic to describe the rich diversity of glacial and postglacial processes in Alpine plant species. It rather appears that the glacial history of each species is to a certain degree unique and influenced by its ecological demands or breeding systems. Moreover, stochasticity has to be regarded of essential importance, since factors such as preglacial distribution patterns or postglacial dispersal or extinction events should have had effects on the present genetic composition and the distribution of a species.

KEYWORDS: Alps, Erisimum alpinus, Eritrichium nanum, phyleogeography, Pleistocene glaciations, Rumex nivalis, Saxifraga oppositifolia.

INTRODUCTION

Since the end of the 19th century, there has been a long-standing debate about the fate of the mountain flora of the European Alps during the Pleistocene ice ages (reviewed in Stehlik, 2000). Two contrasting scenarios of glacial survival of alpine plant species have been discussed, namely (1) total extinction within glaciated mountain ranges, survival in peripheral refugia, and subsequent re-immigration into vacant areas after the retreat of glaciers (tabula rasa hypothesis), and (2) long-term in situ survival in glaciated regions in isolated ice-free areas above the ice-shield (nunatak hypothesis).

The choice of tools for historical biogeographical investigations was limited before the development of molecular methods. Geographic mapping of macrofossils and pollen deposits documented large-scale postglacial plant migrations (Bennet & al., 1991; Lang, 1994; Burga & Perret, 1998). However, the fossil record is limited in taxonomic resolution (Burga & Perret, 1998). In addition, fossils of herbaceous and/or alpine plants are especially scarce. Earlier studies on alpine plants therefore mainly relied on their distribution patterns (Chodat & Pampanini, 1902; Briquet, 1906; Brockmann-Jerosch & Brockmann-Jerosch, 1926; Merxmüller, 1952, 1953, 1954). In support of the tabula rasa hypothesis, locations of peripheral refugia and postglacial migration routes into the Central Alps had already been proposed more than a century ago (Chodat & Pampanini, 1902; Briquet, 1906). The Grajcz, Cottic and Lepontic Alps were postulated to have acted as important peripheral refugia for the present-day alpine flora of the
Central Alps. Additional peripheral refugia were proposed at the margin of the Northern Alps (Briquet, 1906). However, the present distribution of alpine plant species is often not in accordance with the tabula rasa hypothesis (Brockmann-Jerosch & Brockmann-Jerosch, 1926). For example, central alpine regions sometimes are “hot-spots” in terms of species diversity and even host endemic taxa (Brockmann-Jerosch & Brockmann-Jerosch, 1926; Welten & Sutter, 1982), but, according to the tabula rasa hypothesis, they should have been colonised last, and, hence, show lower diversity. Therefore, it has been proposed that at least high alpine plant species have survived ice ages in situ in regions such as the southern Valais or the Upper Engadine on southerly exposed, windswept cliffs above the ice sheet (nunatak hypothesis; Brockmann-Jerosch & Brockmann-Jerosch, 1926; reviewed in Stehlik, 2000; Fig. 1; red areas).

Historical processes such as bottlenecks, migrations and genetic drift are often reflected in the present-day genetic composition of populations and permit reconstruction of the historical biogeography or phylogeography of extant species (Hewitt, 1996; Taberlet & al., 1998). Molecular methods open the possibility to test the proposed hypotheses on glacial survival of alpine species (Stehlik, 2000) because they should lead to alternative present-day distributions of neutral genetic markers (Stehlik & al., 2000; Widmer & Lexer, 2001). According to the tabula rasa hypothesis, genetic similarity among descendants of populations of particular peripheral refugia along re-immigration routes into the Alps should be comparatively high. Therefore, populations of peripheral refugia and of Central Alps would belong essentially to the same gene pool. As recurrent founder events should have taken place along re-immigration routes (genetic thinning), it is also expected that populations at greater distances from the periphery of the Alps should be genetically less variable than populations closer to peripheral refugia. Expectations according to the nunatak hypothesis are different (Stehlik & al., 2000). High-alpine nunatak populations were probably small and isolated. Long-lasting isolation and inbreeding should have led to relatively low genetic variation within populations and to strong differentiation among populations on different nunataks. Therefore, populations from the Central Alps are expected to be genetically clearly differentiated from those of peripheral refugia. Moreover, discontinuously distributed groups, each characterised by genetically closely related populations, should be discernible.

Four alpine plant species, Eritrichium nanum (L.) Gaudin (Boraginaceae), Erinus alpinus L. (Scrophulariaceae), Rumex nivalis Hegesch. (Polygonaceae) and Saxifraga oppositifolia L. (Saxifragaceae; Fig. 2) with distinctly different ecological demands and distribution patterns were chosen to test explicit phylogeographic hypotheses. They were investigated using several molecular techniques covering uniparental and biparental genetic markers. AFLPs and RAPDs represent biparental and RFLPs of cpDNA uniparental and, supposedly, maternal markers. Additionally, one cpDNA region of E. nanum was further investigated by sequence analysis. For detailed descriptions of the molecular methods and reasons for applying them please refer to the original publications (Stehlik & al., 2001, 2002a, b; Holdereregger & al., 2002; Stehlik, 2002). The aims of this investigation were (1) to infer the mode of glacial survival for each individual species, (2) thereby to resolve the long-standing controversy between the nunatak and the tabula rasa hypotheses and (3) to deduce whether there are common patterns [location of glacial refugia and/or (postglacial) migration routes] among species or whether each species represents its own phylogeographic history influenced by an individual composition of ecological demands, preglacial distribution patterns, and/or stochastic events.

**HIGH-ALPINE ERITRICHIUM NANUM**

The European endemic *Eritrichium nanum* belongs to a group of plant species reaching the highest altitudes in the Alps (2400–3600 m; Lechner-Pock, 1956; Fig. 2A). It occurs mainly in exposed habitats, e.g., on ridges.

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Fig. 1. Postulated nunatak areas and peripheral refugia in the middle part of the Alps during the Pleistocene (Stehlik, 2000). Proposed peripheral refugia are given in dark blue and the corresponding migration routes towards the Central Alps with a transition to light blue. The middle intensity blue shows the main area of re-immigration, but it does not illustrate a defined postglacial point in time. Putative high-alpine nunatak areas within formerly most intensively glaciated regions of the Central Alps are indicated in red (from West to East: southern Valais, Simplon, Val Avers, Upper Engadine). Figure redrawn from Stehlik (2000).
or summits. *Eritrichium nanum* is a perennial cushion plant with short flowering shoots and showy blue flowers pollinated by a wide array of insects ranging from syrphids to bees (Zoller & al., 2001). The species is mainly outcrossing, although it is self-compatible and produces viable seed upon self-pollination (Zoller & al., 2001). In the middle part of the Alps, its distribution covers areas with many postulated high-alpine nunataks (Stehlik, 2000; Fig. 1, red areas). Based on all these facts, it appears possible that the species could have survived *in situ* in the most intensively glaciated regions during the ice ages.

In a first step, 20 mainly Central Alpine populations of *E. nanum* were investigated using AFLPs (Stehlik & al., 2001). Two populations of proposed peripheral refugia in southern France and northern Italy were chosen for comparison. Clear genetic differentiation among these latter two populations and those of the Central Alps was
found. Within the Central Alps, three genetically distinct regions were identified. Two of them correspond to Central Alpine nunatak areas in the southern Valais and in the Upper Engadine (Stehlik, 2000; Fig. 1). The third area is located in northern Tessin. In summary, the results of the AFLP analysis reflect the expectations formulated for the nunatak hypothesis (Stehlik & al., 2000, 2001) as individuals from peripheral refugia appeared to be genetically too distant from the Central Alpine individuals to have acted as sources for postglacial re-colonisation.

The amount of genetic variation either within or among populations as detected with AFLPs is very high (Stehlik & al., 2001). This is most probably caused by considerable levels of random gene flow among populations within the three inferred Central Alpine nunatak regions. If much of this gene flow occurred after the last glaciation, it could have masked originally more distinct patterns of genetic variation. To circumvent this analytic drawback of the highly resolving and recombinant AFLPs, which mostly cover nuclear DNA (Mueller & Wolfenbarger, 1999), chloroplast DNA variation was investigated to search for phylogeographic patterns indicative of glacial refugia in *E. nanum* and to compare it with the one detected with AFLPs (Stehlik & al., 2001, 2002b). As cpDNA is non-recombinant, mostly maternally inherited and has a low mutation rate, it is more likely to retain ancient genetic patterns and to reflect historical processes than biparentally inherited nuclear markers (Comes & Kadereit, 1998). At the same time, the geographic sample size was extended to the total distribution area of *E. nanum*, i.e., the whole Alpine arch with 37 investigated populations (Stehlik & al., 2002b).

One cpDNA haplotype is most common and widespread (Stehlik & al., 2002b; black circles; Fig. 3A). At the same time, it is also genetically basal-most (Fig. 3B). All other haplotypes are inferred to have descended from this haplotype. Possibly, this common haplotype, presumably being the ancestor of all extant individuals of *E. nanum*, colonised the Alps from the East, as the closest relatives of *E. nanum* grow in the Carpathians, Caucasus, Asia, Arctic Europe and North America (Lechner-Pock, 1956). Only after colonization of the Alps by this basal haplotype, the accumulation of mutations in the chloroplast genome and the limited dispersal of seeds resulted

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**Fig. 3.** Thirty-seven populations of *Eritrichium nanum* in the Alps, eleven haplotypes identified, and a minimum spanning tree connecting them (Stehlik & al., 2002b). A, Four individuals have been investigated per population by PCR-RFLPs and sequence analysis of non-coding regions of cpDNA (Stehlik & al., 2002b). Populations were always monomorphic for one haplotype. Dots within the symbols represent the number of mutational steps from the common, black-circled haplotype. Grey-shaded are central Alpine areas (in the West: Southern Valais and southward bordering Aosta; in the East: Upper Engadine and southward bordering Bergamasc Alps) that harbour *in situ* surviving nunatak populations during Pleistocene glaciations. B, The common, black-circled haplotype was inferred to be evolutionarily basal by the inclusion of *Omphalodes verna* and *Myosotis sylvatica* in genetic and statistic analyses (Stehlik & al., 2002b). Bars: indels; crosses: point mutations. Symbols correspond to those used in (A). Figure redrawn from Stehlik & al. (2002b).
in a patchy distribution of the divergent haplotypes. These populations harbouring divergent haplotypes are at the present time embedded as “islands” in a “sea” of the common black-circled haplotype (Fig. 3A). Ten additional haplotypes were identified, differentiated by one to six mutations from the common haplotype (Fig. 3B). Three regions contain more derived haplotypes (Fig. 3A; coloured haplotypes). One of them lies in the Austrian Central Alps of Carinthia and Styria with a haplotype at a maximum genetic distance from the central haplotype (Figs. 3A, B; green haplotype with six dots). From an evolutionary point of view, it can be hypothesised that this genetic subdivision occurred early in the Alpine history of E. nanum (Stehlik et al., 2002b). The other two regions rich in derived haplotypes are situated in the Central Alps and cover two of the three nunatak regions as Southern Valais and Aosta and the Upper Engadine including adjacent Bergamasco Alps (Fig. 3A; grey-shaded areas). The most probable explanation for the cpDNA pattern in E. nanum (Stehlik et al., 2002b), in accordance with AFLPs (Stehlik et al., 2001), implies *in situ* survival in rare and isolated populations on nunataks within the Central Alps (grey-shaded areas; Fig. 3) during Pleistocene glaciations. This result on E. nanum is among the first to show the capacity of alpine plants to resist the hostile climate on nunataks during Pleistocene glaciation (Stehlik et al., 2001, 2002b; see also Füchtner et al., 2001).

**LOW-ALPINE ERINUS ALPINUS**

The perennial herb Erinus alpinus (Fig. 2B) has many contrasting characteristics as compared to *Eritrichium nanum*. It occurs at subalpine elevations, and its present distribution only rarely reaches above the maximum elevation of the ice sheet during the Pleistocene (Hantke & Jäckli, 1978; Welten & Sutter, 1982). The species’ main distribution area lies in southwestern Europe (Meusel et al., 1978). As E. alpinus is restricted to calcareous bedrock (Hartl, 1965), it did not have the potential to survive on the mainly siliceous nunataks within the high Central Alps. There is a nearly continuous band of limestone from southern France to the northern Prealps in Switzerland, also extending further to the East. This almost completely coincides with the present distribution area of E. alpinus in Central Europe, including its occurrence in southern France (Meusel et al., 1978). This distribution pattern corresponds to the theoretically expected postglacial immigration route of E. alpinus from southern France northwards. Brockmann-Jerosch & Brockmann-Jerosch (1926) therefore formulated the hypothesis of glacial survival of E. alpinus in southern France with subsequent (re-)immigration into its present-day Alpine distribution range after the ice ages. Nevertheless, the species also occurs on some proposed northern Alpine peripheral refugia (Stehlik, 2000; Fig. 1), and *in situ* glacial survival cannot be ruled out completely on these latter mountain peaks.

The results of AFLPs gave a strong phylogeographic signal, whereas there was no genetic variation detected by PCR-RFLPs of cpDNA (Stehlik et al., 2002a). Three clearly differentiated genetic groups were found among the 22 investigated populations: (1) central Swiss populations (Fig. 4; blue), (2) individuals from Mt. Rigi (Fig. 4; small arrow), and (3) all other populations southwest and northeast of the central Swiss populations (“west-eastern” populations; Fig. 4; red to yellow). The isolation of the central Swiss populations of E. alpinus is hard to reconcile with a continuous postglacial immigration from a southern refugium. Hence, *in situ* survival in central Switzerland or the nearby Jura mountains is more likely. Individuals from central Switzerland contain a significantly lower number of fragments compared with individuals from west-eastern populations (Stehlik et al., 2002a). This also supports *in situ* survival of the former, since long-term isolation with low numbers of individuals, recurring bottlenecks, inbreeding and genetic drift could have reduced genetic diversity. The geographic locations with the highest probability for *in situ* survival are the northern-most mountain tops of the Prealps, as some of them rose above the ice even during the glacial maximum at 20 to 18 ky BP (Burga & Perret, 1998; Stehlik, 2000; Stehlik et al., 2002a). The second remarkable characteristic within the genetic patterns of E. alpinus is the genetic isolation of the population of Mt. Rigi and, to a lesser extent, of a population in the Säntis area (Fig. 4; small arrows). The persistence of the spatially restricted populations in genetic isolation from the surrounding populations over a long period of time is remarkable. In contrast, Gabrielsen et al. (1997) and Tollefsrud & al. (1998) concluded that potential traces of nunatak survival were wiped out by massively immigrating periglacial genotypes in Nordic Saxifraga oppositifolia and S. cespitosa. The geographic position of Mt. Rigi as a separate and isolated mountain surrounded by lakes on three sides might have prevented a major genetic distortion of its population of E. alpinus.

Most of the genotypes of E. alpinus belong to the west-eastern gene pool (Stehlik et al., 2002a; Fig. 4). As many individuals genetically do not consistently group within their respective populations, the west-eastern populations have to be addressed as one more or less continuous gene pool. A probable postglacial scenario is an immigration of individuals from southern France via the lowlands north of the Alps, as these became ice-free earlier than the Prealps and Alp (Fig. 4). Subsequently,
southwards migration from the prealpine lowlands and recolonization of the Prealps took place resulting in the present distribution of *E. alpinus* (Fig. 4). A slightly, although significantly lower level of AFLP fragments per individual in the east than in the west of the west-eastern group also fits this hypothesis (Stehlik & al., 2002a).

The investigation on *E. alpinus* demonstrates how two different processes acting over different time scales can shape a species’ regional genetic composition. The populations of *E. alpinus* surviving in northern peripheral refugia have to be dated back at least to the last interglacial period (60,000–28,000 y BP), whereas the immigration from southern France took place not before the retreat of the glaciers at 14,000 y BP.

**SNOW-BED SPECIALIST RUMEX NIVALIS**

In the dioecious, wind-pollinated perennial *Rumex nivalis* (Fig. 2C), two aspects of its distribution are conspicuous (Stehlik, 2002). (1) There is a thinning in population density towards the species’ western distribution limit (Welten & Sutter, 1982), which could point to immigration from a north-eastern peripheral refugium (*tabula rasa* hypothesis). (2) Its disjunct, alpine distribution centered in the middle part of the Northern Alps might result from glacial *in situ* survival on high mountain peaks (nunatak hypothesis). The affinity to extreme habitats in snow-beds (Wagenitz, 1981) with short vegetation periods might be a prerequisite for this type of glacial *in situ* survival. Therefore, no clear hypothesis on locations of glacial survival can be formulated for *R. nivalis* (Stehlik, 2002).

The level of geographic structure in the AFLP data in 23 populations of *R. nivalis* is very low (Stehlik, 2002). When compared with that found in other alpine species investigated with AFLPs at similar spatial scales, this low geographic structure in *R. nivalis* becomes even more apparent. The most obvious difference between *R.
nivalis and Eritrichium nanum, Erinus alpinus and Phyteuma globulariifolium is the breeding system (Stehlik et al., 2001, 2002a; Schönswetter et al., 2002), as R. nivalis is the only wind-pollinated species among them. Nearly all populations of R. nivalis are part of one gene pool interconnected via pollen flow (Stehlik, 2002). As the AFLP results do not help to infer glacial refugia or migration routes of R. nivalis, the interpretation relied on variation in cpDNA (Stehlik, 2002). The genetic variation in cpDNA is surprisingly high and resulted in 24 haplotypes (Fig. 5). As mentioned above, in Eritrichium nanum, Stehlik & al. (2002b; Fig. 3A) distinguish 11 haplotypes covering the entire Alps. Holderegger et al. (2002) found only four haplotypes in alpine S. oppositifolia, while Abbott & al. (2000) identified 14 haplotypes in the same species at the circumpolar level. Levels similar to R. nivalis were detected in Dryas integrifolia (20 haplotypes), but this sampling covered the whole of North America (Tremblay & Schoen, 1999). In the present study, the majority of the investigated populations were sampled within a radius of only 150 km, and the high haplotype variation is strongly geographically structured (Stehlik, 2002). Populations at and near the northern border of the Alps are richer in both the total number of haplotypes and the number of private haplotypes, i.e., haplotypes which occur in just one population (Fig. 6A). This pattern is also reflected by a group of haplotypes (B, E, F, and K) occurring in the haplotypically rich populations (Figs. 6A, B). Otherwise these northern Alpine haplotypes only occur in neighbouring populations of these haplotypically rich populations (Figs. 6A, B). The patchy distribution of haplotypes, the high haplotype diversity, and the high number of private haplotypes at the northern periphery of the Alps support glacial survival of R. nivalis in northern peripheral refugia and a colonization of populations towards the Central Alps (Fig. 6D). One group of haplotypes (C, G, and D) is most distant from all other haplotypes (Fig. 5). These three haplotypes are confined to a geographically restricted region in the Central Alps (Fig. 6C). Haplotype A co-occurs with haplotypes C and D in certain populations (Fig. 6C). Haplotype A is also closely related to the C-G-D-group (Fig. 5). Judging from the regional distribution of haplotypes A, C, D, and G, nunatak survival of R. nivalis is suggested in the Central Alpine area containing these derived haplotypes (Fig. 6D). This Central Alpine region was formerly not recognised as a nunatak area (Stehlik, 2000). In concordance with this proposed glacial scenario based on more classical statistical methods (AMOVA, Mantel tests, cluster analyses; Stehlik, 2002), nested clade analysis (Templeton & al., 1987, 1995; Templeton, 1998) applied to the haplotypic data on R. nivalis indicated a high colonising capacity of the Central Alpine haplotypes C, G, and D (as compared to the northern Alpine haplotypes) or a long-lasting range expansion after glaciation (Stehlik, 2002). On the other hand, all haplotypes characterised by restricted gene flow are situated at or near to the periphery of the Alps (northern Alpine haplotypes B, E, F, K; Fig. 6B).

The results of R. nivalis illustrate how strongly dependent the phylogeographic signal is on the type of molecular marker system used (no conclusion based on biparental AFLPs, strong patterns based on uniparental cpDNA). Furthermore, they show how diverse and complicated the Pleistocene history of an alpine species in a comparatively small area can be, and, thus, how important dense sampling is (Stehlik, 2002).

**GENERALIST SAXIFRAGA OPPOSITIFOLIA**

Saxifraga oppositifolia (Fig. 2D) is a long-lived perennial forming loose cushions (Webb & Gornall, 1989). Stems of S. oppositifolia usually bear single, mostly outbred protandrous flowers; selling is, however, possible (Gugerli, 1997). Alpine populations are characterised by high RAPD variation (Gugerli et al., 1999). The species is of arctic-alpine distribution and occurs almost throughout the European mountain ranges as a common, widely distributed plant species. It mainly grows at alpine elevations between 1800 and 3800 m.
Fig. 6. The 23 populations investigated of *Rumex nivalis* in the Alps (Stehlik, 2002). Twenty-two populations were sampled in the western part of the species’ distribution, and one population from the eastern distribution limit in Austria was included for comparison (dotted square in upper right corner of each figure). A, Haplotype richness (four to one haplotypes) per population is indicated by a transition from black to white. The number of private haplotypes per population is given by the number of circles around the populations; B, distribution of “northern Alpine” haplotypes occurring in more than one population (haplotypes B, E, F, and K); C, distribution of “central Alpine” haplotypes occurring in more than one population (A, C, D, and G); D, hypothesised glacial and post-glacial history of *R. nivalis*. Stars represent populations that probably survived glaciations in peripheral refugia at the northern border of the Alps. Diamonds suggest in situ surviving populations in a central alpine nunatak area (surrounded by a broken line). Grey populations were probably postglacially colonised. Bold arrows indicate the inferred migration routes out of peripheral refugia or central alpine nunatak, whereas fine arrows imply mutational changes along with these migrations. No (detectable) dispersal events originated from the three encircled populations. Figure redrawn from Stehlik (2002).

(Kaplan, 1995). *Saxifraga oppositifolia* belongs to those few alpine plants which are able to grow on the highest alpine summits at more than 4000 m. The species’ habitats are diverse, ranging from outcrops, rocks, glacier forefields and moraines to gravel on siliceous and calcareous bedrock (Webb & Gornall, 1989). Several phylogeographic studies have been conducted on this species (Abbott et al., 1995, 2000; Gabrielsen et al., 1997). As in the present compilation, an important question in these studies was whether this species survived glaciation on ice-free nunataks within Pleistocene ice sheets in Scandinavia or whether it re-immigrated from periglacial refugia during the postglacial period. A circumpolar survey of cpDNA haplotypes identified glacial refugia of *S. oppositifolia* in regions that have never been glaciated during Pleistocene ice ages in the Arctic and, potentially, at more southern latitudes (Abbott et al., 2000). The haplotypic diversity in these regions was higher than that of regions that have been covered by ice. However, these reticulate haplotypes hardly dispersed postglacially and, consequently, are regarded of little importance for the postglacial colonisation of newly ice-free areas (Abbott & al., 2000). The same conclusion was made for *S. oppositifolia* in northern Europe (Gabrielsen et al., 1997). If any genotypes did survive in such refugia, they must have been swamped by massive postglacial immigration of common periglacial genotypes (Gabrielsen et al., 1997).

Two widespread, common cpDNA RFLP haplotypes were found in 15 populations of *S. oppositifolia* (black and hatched haplotypes; Fig. 7; Holderegger et al., 2002). Two additional, rare haplotypes also occurred in just three populations (blue and red haplotypes; Fig. 7). In their circumpolar study on *S. oppositifolia*, Abbott &
Nevertheless, potential nunataks or peripheral refugia in the Alps or neighbouring regions (Holderegger & al., 2002).

RAPD analysis revealed a similarly high level of variation and a similar partitioning of genetic variance in former studies on *S. oppositifolia* (Gabrielsen & al., 1997; Gugerli & al., 1999). As in *Rumex nivalis* (Stehlik, 2002), all sampled individuals of *S. oppositifolia* belong to one large nuclear gene pool without a conspicuous grouping according to geographical origin or occurrence in nunatak or peripheral refugial areas (Stehlik, 2000). Nevertheless, some populations from the Central Alpine Valais exhibit some genetic peculiarities, which may be interpreted as weak evidence for *in situ* glacial survival of the species within the Alps (Holderegger & al., 2002). However, these genetically distinct populations as detected with RAPDs are different ones as compared to those harbouring the two rare RFLP-haplotypes (Fig. 7).

Taken together, both sets of molecular markers provide at best weak indications for *in situ* survival of *S. oppositifolia* within the Alps (Holderegger & al., 2002). The best support for such relictual survival is given by an individual occurrence of the blue haplotype otherwise only known from the Siberian Taymyr region (disregarding potential homoplasy; Fig. 7; Abbott & al., 2000). It is thus not possible to reconstruct the glacial history of this common arctic-alpine species, as the hypothesis of *in situ* glacial survival can neither be proven nor falsified (Holderegger & al., 2002). It is therefore probable that in the Alps, as in northern Europe (Gabrielsen & al., 1997; Abbott & al., 2000), resident genotypes surviving glaciation *in situ* were integrated into the gene pool of postglacially immigrating periglacial individuals.
CONCLUSIONS

The phylogeographic Pleistocene history of *Eritrichium nanum*, *Erinus alpinus*, *Rumex nivalis* and *Saxifraga oppositifolia* is very diverse (Stehlik & al. 2001, 2002a, b; Holderegger & al., 2002; Stehlik, 2002). The compiled studies are among the first to demonstrate strong evidence for *in situ* glacial survival in the Central Alps for Alpine species during the Pleistocene (see also Füchter & al., 2001). In the case of *Eritrichium nanum*, the area of *in situ* nunatak survival coincides with a region of intense Pleistocene glaciation (Stehlik, 2000; Stehlik & al., 2002b). The size of these *in situ* glacial refugia differs according to species and region. Some refugia were restricted to individual mountains, i.e., in the case of *Erinus alpinus* and *R. nivalis* (Figs. 4, 6D), whereas others span larger mountain areas as the southern Valais for *Eritrichium nanum* (Fig. 3A), the central part of northern Alps for *Erinus alpinus* (Fig. 4), and a region in the Central Alps for *R. nivalis* (Fig. 6D). No evidence for (or no need to invoke) such Central Alpine nunatak survival was detected in studies with comparable sample sizes, molecular markers; and geographic distributions, e.g., in *Anthyllis montana*, *Saponaria pumila* or *Phyteuma globularifolium* (Kropf & al., 2002; Schönswetter & al., 2002; Tribsch & al., 2002). The genetic patterns of these alpine species could be related to glacial survival in small peripheral refugia in southern and easternmost Alps and postglacial migration towards the species’ present distributions (see also Tribsch & Schönswetter, 2003). Such postglacial migration is also inferred for some of the four species of the present studies reviewed. Over longer distances, *Erinus alpinus* migrated postglacially from Southern France to Northern Switzerland (Fig. 4), or, over shorter distances, *R. nivalis* from the northern Prealps into the Central Alps (Fig. 6D). There is clear evidence that both postglacial immigration and *in situ* survival shaped the genetic constitution and the present distribution pattern of at least *E. alpinus* and *R. nivalis*. It is therefore probable that the nunatak and the *tabula rasa* hypotheses are too simplistic to describe the rich diversity of glacial and postglacial processes. It rather appears that the glacial history of each species is to a certain degree unique and influenced by its composition of ecological demands or breeding systems. Moreover, stochasticity should be regarded of essential importance, since factors such as preglacial distribution patterns or postglacial dispersals and extinctions have clear effects on the present genetic composition and the distribution of species.

While the dichotomy of the nunatak vs. *tabula rasa* hypotheses might lead to effective results when dealing with the massive Arctic ice cap (Gabrielsen & al., 1997; Tollefsrud & al., 1998; Abbott & al., 2000), for the much smaller and more fragmented Alpine ice shield, a greater diversity of processes seems probable. The attempt to explain this diversity of refugia and migration routes under two hypotheses would ignore important phylogeographic signals. A more productive way to infer general patterns in Alpine plants might be to compile the results of future biogeographical investigations with similar sampling designs and to search for repeated patterns, and, thereby refine or reformulate hypotheses for glacial survival in the Alps.

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LITERATURE CITED


