

# Molecular evidence for glacial refugia of mountain plants in the European Alps

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## Abstract

Many mountain ranges have been strongly glaciated during the Quaternary ice ages, and the locations of glacial refugia of mountain plants have been debated for a long time. A series of detailed molecular studies, investigating intraspecific genetic variation of mountain plants in the European Alps, now allows for a first synopsis. A comparison of the phylogeographic patterns with geological and palaeoenvironmental data demonstrates that glacial refugia were located along the southwestern, southern, eastern and northern border of the Alps. Additional glacial refugia were present in central Alpine areas, where high-elevation plants survived the last glaciation on ice-free mountain tops. The observed intraspecific phylogeographies suggest general patterns of glacial survival, which conform to well-known centres of Alpine species diversity and endemism. This implies that evolutionary or biogeographic processes induced by climatic fluctuations act on gene and species diversity in a similar way.

**Keywords:** Alpine plant species, comparative phylogeography, glacial refugia, historical biogeography, Quaternary glaciation

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## Introduction

Quaternary climate oscillations often occurred at a dramatic speed (Dansgaard *et al.* 1993; GRIP Members 1993; Birks & Ammann 2000) and resulted in repeated drastic environmental changes causing massive range shifts of biota (van Andel & Tzedakis 1996). These shifts were traditionally inferred from fossils, including pollen deposits of plants, and from palaeoenvironmental data (Nagl 1972; Burga & Perret 1998; Comes & Kadereit 1998). Recently, molecular biogeography or phylogeography (Comes & Kadereit 1998, 2003; Avise 2000; Hewitt 2000; Stehlik 2003; Tribsch & Schönswetter 2003) has provided new sources of information on the glacial history of species and their range shifts as inferred from the geographical patterns of genetic variation.

Phylogeographic patterns allowed to infer the glacial history of many animals and plants (Comes & Kadereit

1998; Dynesius & Jansson 2000; Hewitt 2000; Davis & Shaw 2001; Kropf *et al.* 2002, 2003; Stehlik 2003; Tribsch & Schönswetter 2003; Bennett 2004) ranging from organisms of tropical rainforests (Schneider *et al.* 1999) and marine species (Hellberg *et al.* 2001) to various temperate mammals, trees (Taberlet *et al.* 1998; Petit *et al.* 2003) and arctic herbs (Abbott *et al.* 2000; Abbott & Brochmann 2003). In Europe, the phylogeographic patterns detected so far suggest that lowland organisms survived in three major glacial refugia in the Iberian Peninsula, Italy and the Balkan Peninsula, from where they migrated northwards after the glaciations (Taberlet *et al.* 1998; Petit *et al.* 2003; Hewitt 2004). The inferred postglacial migration routes of lowland species were often, but not always, blocked by the east–west ranging mountain ranges of the Pyrenees and the European Alps (Taberlet *et al.* 1998; Avise 2000; Mátyás & Sperisen 2001; Petit *et al.* 2002). But what happened to those species for which these mountain ranges were not barriers to migration, but served as their habitats?

With the advance of glaciers during the cold periods of the Quaternary, the habitats of European mountain plants

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became increasingly restricted. Migration to and survival in adjacent lowlands was not as straightforward as it might first appear. A relatively mild climate allowed the growth of trees in the lowlands to the south of the European Alps (Ravazzi 2002). To the north and east of the Alps, vast areas were covered by steppe vegetation adapted to a cold and dry climate (Frenzel 1964; Lang 1994; Burga & Perret 1998). Thus, the lowlands did not provide suitable habitats for many high-elevation plants. For more than a century, biogeographers and ecologists have therefore wondered, where mountain plants could have survived the ice ages (Chodat & Pampanini 1902; Briquet 1906; Brockmann-Jerosch & Brockmann-Jerosch 1926; Merxmüller 1952, 1953, 1954; Simpson 1974).

In the almost complete absence of fossils of mountain plants (Lang 1994; Burga & Perret 1998), molecular investigations can provide data on the locations of glacial refugia (Gugerli & Holderegger 2001; Comes & Kadereit 2003; Stehlik 2003; Tribsch & Schönswetter 2003; Hewitt 2004), i.e. genetic subdivisions within species' ranges provide signals of past biogeographic processes. During the last five years, a number of phylogeographic studies have been carried out, which now allow for a first synthesis on the molecular evidence for glacial refugia of mountain plants in one of the world's best studied 'natural experiments' (Körner 2003), the European Alps.

In this study, we summarize intraspecific phylogeographic data previously published for 12 Alpine plant species. As the phylogenetic relationships of mountain plants of the European mountain ranges were compiled recently (Comes & Kadereit 2003), we only included those studies that explicitly explored intraspecific differentiation. We demonstrate that a comparison of the phylogeographic patterns of 12 Alpine plant species with palaeoenvironmental and geological information on the extent of glaciated

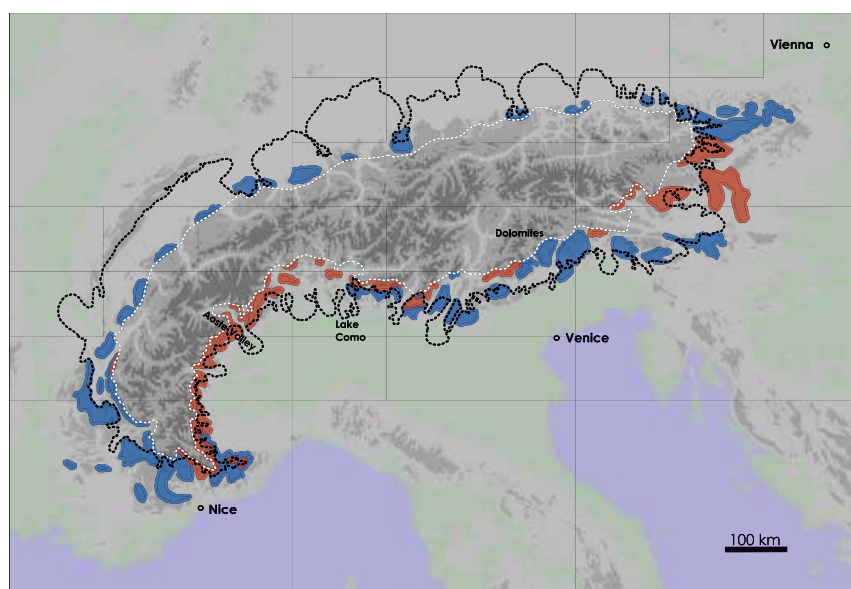
areas allows to locate glacial refugia and to infer general biogeographic patterns.

## Materials and methods

To test falsifiable hypotheses, we defined the locations of potential glacial refugia from a compilation of geological and palaeoenvironmental data (Fig. 1). We used data on bedrock type (Vetters 1933; Möbus 1997), topography, the maximum extent of glaciers during the last glaciation (Penck & Brückner 1909; Jäckli 1970; Nagl 1972; van Husen 1997a, b) and the position of the snowline (i.e. altitude above which snow does not melt in climatically average years; taken from Penck & Brückner 1909 and Nagl 1972) during the last glacial maximum (LGM; 20 000 BP). The snowline during the LGM was at about 1100 m above sea level (a.s.l.) in the northern and at about 2000 m a.s.l. in the central parts of the eastern and southwestern Alps.

Based on these data, potential glacial refugia existed along the border of the European Alps (Fig. 1). Such potential peripheral refugia were widespread for plants of calcareous bedrock, whereas they were more restricted for species of siliceous bedrock. For the latter species, potential peripheral refugia were located at the eastern border of the Alps, where large mountain areas with suitable bedrock remained unglaciated, and along the southwestern and southern border of the Alps. In accordance with Tribsch & Schönswetter (2003), we regarded all mountain areas below the LGM snowline as potential refugia disregarding whether they were outside or within the ice sheet ('peripheral nunataks' according to Schönswetter *et al.* 2004a).

Twelve mountain plants of the Alps, ranging from species with a wide altitudinal range above timberline to specialists of high elevations, have been studied with

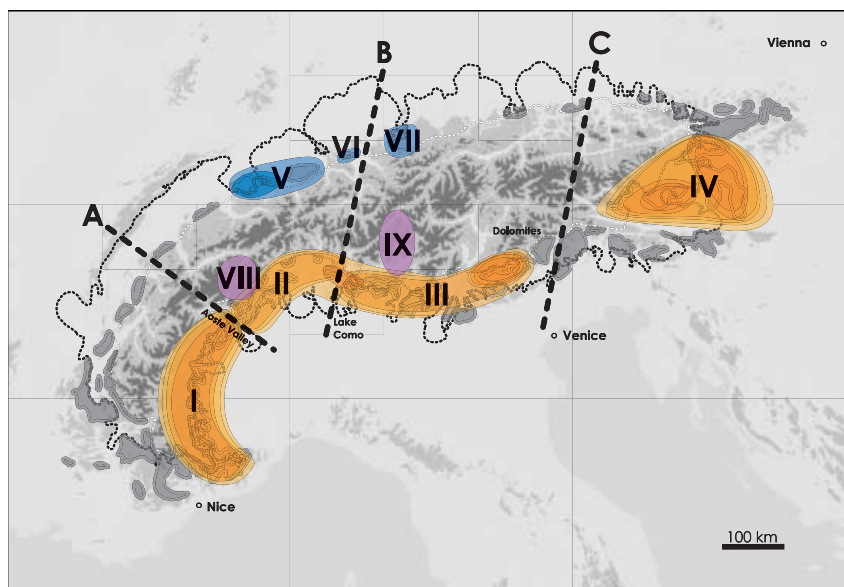


**Fig. 1** Potential peripheral refugia of mountain plants in the European Alps during the last glacial maximum (20 000 BP) based on references given in the Material and methods section. Blue areas refer to glacial refugia on calcareous bedrock and red areas to those on siliceous bedrock. The broken black line gives the maximum extent of the ice sheet, and the white broken line indicates the glacial snow line.

phylogeographic methods (Holderegger *et al.* 2002; Schönswetter *et al.* 2002, 2003a, b, c; 2004a, b, c; Stehlik *et al.* 2001, 2002a, b; Stehlik 2002; Tribsch *et al.* 2002). The corresponding studies are all characterized by a dense sampling of populations, which allows an in-detail assessment of intraspecific genetic subdivisions. Sampling designs either considered the whole distribution of a species in the Alps or were hypothesis-driven with an emphasis on postulated glacial refugia. All studies applied genomewide fingerprinting techniques of neutral DNA [either random amplified polymorphic DNAs (RAPD) or amplified fragment length polymorphisms (AFLP)], and several studies completed these data by investigations of maternally inherited chloroplast DNA [restriction fragment length polymorphisms (RFLP) or sequences]. Details on the sampling design, the molecular methods used, the main statistical analyses performed and the glacial refugia inferred per species are given in the Appendix. Because of the relatively low density of the sampled populations, we were reluctant to include the results of Kropf *et al.* (2002, 2003).

To test for the existence of a general trend in the phylogeographic patterns detected across the species surveyed

(disregarding two species with unresolved phylogeographies, i.e. *Oxytropis campestris* and *Saxifraga oppositifolia*; see below), we used a  $\chi^2$  comparison of observed and expected matrices of glacial refugia. For this aim, two independent matrices were created per species. The columns of the first matrix of observed patterns corresponded to the four major refugial areas observed across the Alps: namely Nice to Aoste, Aoste to Lake Como, Lake Como to Dolomites and Dolomites to Vienna (as separated by the inferred biogeographic lines; see below and Fig. 2). The rows of the first matrix of observed patterns then gave the glacial refugia as identified by molecular analysis. The second matrix of expected patterns per species had the same principal structure, but the entries now referred to a biogeographic situation, where the patterns strictly fitted to the major glacial refugial areas as suggested by the species' current geographical distribution. The principal entries were '2' for an existing or expected refugium and '0' for a missing refugium. The matrix of expected patterns thus only contained entries in the diagonal, while the matrix of observed patterns could also contain entries that deviated from the diagonal in cases where the molecular data indicated



**Fig. 2** Synopsis map of glacial refugia of mountain plants in the European Alps deduced from phylogeographic studies. Different colours refer to different types of refugia, and colour intensities indicate for how many species a particular area has been inferred as a refugium (their number is given in brackets along with a list of the respective species; for details see Appendix). Orange: eastern-, southern-, and southwestern-Alpine peripheral refugia; blue: northern-Alpine peripheral refugia; violet: central-Alpine refugia. I: southwestern-Alpine peripheral refugia between Nice and the Aoste valley (4: *Androsace alpina*, *Comastoma tenellum*, partly *Phyteuma globulariifolium*, *Ranunculus glacialis*); II: southern-Alpine peripheral refugium between the Aoste valley and Lake Como (4: *Androsace alpina*, partly *A. brevis*, partly *Phyteuma globulariifolium*, *Ranunculus glacialis*); III: southern-alpine peripheral refugium between Lake Como and Dolomites (7: *Androsace alpina*, partly *Androsace brevis*, *Androsace wulfeniana*, *Eritrichium nanum*, *Phyteuma globulariifolium*, *Ranunculus glacialis*, *Saponaria pumila*); IV: eastern-Alpine peripheral refugium (6: *A. alpina*, *A. wulfeniana*, *E. nanum*, *P. globulariifolium*, *R. glacialis*, *S. pumila*); V: northern-Alpine peripheral refugium in central Switzerland (2: *Erinus alpinus*, *Rumex nivalis*); VI: northern-Alpine peripheral refugium in eastern Switzerland (1: *R. nivalis*); VII: northern-Alpine peripheral refugium in southern Germany (1: *R. nivalis*); VIII: central-Alpine refugium north of the Aoste valley (1: *E. nanum*); IX: central-Alpine refugium north of Lake Como (1: *E. nanum*). Broken black lines refer to three biogeographic lines (A–C; see text).

**Table 1** Matrices of observed and expected phylogeographic patterns of mountain plants of the European Alps. The table gives an example of the matrices of the observed and expected patterns for one species, *Phyteuma globulariifolium*, which showed glacial refugia that deviated from the generally observed four major geographical areas of glacial survival. The below table gives the matrices of the overall observed and expected patterns summed for ten species. The overall observed and expected matrices did not significantly differ from each other ( $\chi^2 = 3.747$ ,  $P = 0.29$ ). For additional explanations see text

<i>Phyteuma globulariifolium</i>		Nice to Aoste	Aoste to Lake Como	Lake Como to Dolomites	Dolomites to Vienna
Observed	Refugium	3	3		
	Refugium		3	3	
	Refugium			6	
	Refugium				6
Expected	Refugium	6			
	Refugium		6		
	Refugium			6	
	Refugium				6
Summed for ten species		Nice to Aoste	Aoste to Lake Como	Lake Como to Dolomites	Dolomites to Vienna
Observed	Refugium	21	3		
	Refugium		38	8	2
	Refugium		5	47	2
	Refugium		2	2	38
Expected	Refugium	24			
	Refugium		48		
	Refugium			54	
	Refugium				42

refugia that did not correspond to the geographical position of the four major glacial refugial areas. This is illustrated by the example of *Phyteuma globulariifolium* given in Table 1. Based on the species' current distribution, one could assume that it had one glacial refugium in all four major glacial refugial areas. Indeed, the molecular data indicated four different glacial refugia for this species in the Alps. However, the two in the west did not completely fit to the expected major western refugia. This means that each of these two observed refugia in the west could have been located in either of two corresponding expected refugia. Hence, in the matrix of observed patterns in *P. globulariifolium*, the principal entry ('2') for each of the two observed western glacial refugia had to be split and assigned to two expected refugia ('1' per potential refugium). In contrast, in the matrix of expected patterns in *P. globulariifolium*, entries ('2') are found along the diagonal.

The observed and expected matrices were finally summed over all 10 species (Table 1), and a  $\chi^2$ -test was used to detect a potential difference between the two overall matrices. Both matrices contained many zero-cells. These cells were entered as zero addends in the calculation of the  $\chi^2$  statistics. A series of explorative tests (with different matrix sizes) using random entries of observed patterns, for which the entries of the expected matrices were obtained by

permutation, showed that a  $\chi^2$  test is an appropriate approximation of the problem (Christian Hofmann, W.S.L. Birmensdorf, personal communication).

## Results and discussion

Phylogeographic analyses of mountain plants of the Alps identified glacial refugia along the southwestern, southern, eastern, and northern border of the Alps (Fig. 2). This is in congruence with the potential location of glacial refugia given in Fig. 1.

### *Glacial refugia at the southwestern, southern and eastern border of the Alps*

For several plants of siliceous bedrock (*Androsace alpina*, *Androsace wulfeniana*, *Comastoma tenellum*, *Eritrichium nanum*, *Phyteuma globulariifolium*, *Ranunculus glacialis*, *Saponaria pumila*), similar phylogeographic patterns were found (Stehlik *et al.* 2001; Schönswetter *et al.* 2002, 2003b, c, 2004a, c; Tribsch *et al.* 2002). Four peripheral glacial refugia could be delimited (Fig. 2). There was a particularly well-supported eastern-Alpine peripheral refugium (Fig. 2, refugium IV) along the eastern border of the Alps — an area that remained largely unglaciated (Fig. 1). Some species even exhibited evidence for two separate glacial refugia in



this area (Tribisch *et al.* 2002; Schönschwetter *et al.* 2003b). Two southern-Alpine peripheral refugia were detected as well. One refugium spanned from Lake Como eastwards to the Dolomites (Fig. 2, refugium III), but for several species (Schönschwetter *et al.* 2002, 2003c; Stehlik *et al.* 2002a; Tribisch *et al.* 2002), only the restricted area of the Dolomites served as a refugium (Fig. 2). The second southern-Alpine peripheral refugium (Fig. 2, refugium II) was located between the Aoste valley and Lake Como. Genetic data also suggested a well-supported southwestern-Alpine peripheral refugium between Nice and the Aoste valley (Fig. 2, refugium I; Schönschwetter *et al.* 2002, 2003c, 2004a, c).

The southern border of the Alps is well known for its species diversity and high number of endemics (Chodat & Pampanini 1902; Pawłowski 1970; Tribisch & Schönschwetter 2003; Tribisch 2004). One example is *Androsace brevis* with a limited range around Lake Como. No genetic subdivision was found within this species' southern-Alpine distribution (Schönschwetter *et al.* 2003b). Hence, the whole latter area might have served as its glacial refugium, which fitted to the pattern found in other species (see above).

#### *Glacial refugia at the northern border of the Alps*

Glacial survival at the northern border of the Alps, especially in the areas between the major glacier tongues has been invoked to explain disjunct distribution patterns of several plant species that are mainly distributed in the southern or southeastern Alps but have local occurrences near the northern margin of the Alps (Briquet 1906; Merxmüller 1952, 1953, 1954). Two studies found evidence for glacial survival of mountain plants of calcareous bedrock in northern-Alpine peripheral refugia (Stehlik 2002; Stehlik *et al.* 2002b). Three comparably small refugial areas (Fig. 2) could be circumscribed for *Erinus alpinus* and *Rumex nivalis*. One refugium was in central Switzerland (Fig. 2, refugium V) and comprised mountains that had long been postulated to represent glacial refugia (Briquet 1906). The other two refugia were in eastern Switzerland (Fig. 2, refugium VI) and southern Germany (Fig. 2, refugium VII). For plant species of siliceous bedrock, no molecular evidence for northern-Alpine peripheral refugia was found (Fig. 2), which is in concordance with the absence of siliceous bedrock in this area (Fig. 1).

The studies summarized here are biased towards species growing on siliceous bedrock, and taxa growing on calcareous substrate have only been sampled from the middle part of the Alps. It is anticipated that future phylogeographic studies on mountain plants of calcareous bedrock will confirm the importance of refugia in the northeastern, southeastern, southern and southwestern periphery of the Alps for this group of species. In contrast, we regard the array of refugia for plant species of siliceous bedrock presented here as fairly complete.

#### *Glacial survival on nunataks*

Some authors have argued that even within the strongly glaciated central parts of the Alps, high-elevation plants might have survived on ice-free mountain tops, so-called nunataks (Briquet 1906; Brockmann-Jerosch & Brockmann-Jerosch 1926; Gugerli & Holderegger 2001). Recolonization of the Alps would then not only have started from peripheral refugia, but also from source areas within the ice sheet.

It has been argued that it is difficult to prove or reject nunatak survival using genetic data (Hewitt 2004). There was, however, evidence for glacial survival on nunataks for one species of siliceous bedrock. In *E. nanum*, the data suggested that two central-Alpine refugia existed (apart from peripheral refugia; see above; Stehlik *et al.* 2001, 2002a). One was located north of the Aoste valley (Fig. 2, refugium VIII), and the other was north of Lake Como (Fig. 2, refugium IX).

#### *Glacial survival of arctic-alpine plants*

In accordance with the suggestion of Noack (1922) that some plant species with an arctic-alpine distribution immigrated into the Alps after the LGM following the retreating glaciers, the patterns of genetic variation detected in mountain plants could not always be explained by glacial survival in the Alps. In *Saxifraga oppositifolia* (Gabrielsen *et al.* 1997; Abbott *et al.* 2000; Holderegger *et al.* 2002; Holderegger & Abbott 2003) and *Oxytropis campestris* (Schönschwetter *et al.* 2004b), the absence of a clear phylogeographic signal could be caused by postglacial immigration from outside the Alps. Postglacial immigration into the Alps might also explain the genetic patterns found in rare mountain plant species, such as *Saxifraga cernua* (Bauert *et al.* 1998). In addition, there was not only migration of mountain plants to the Alps, but also out of the Alps. Phylogeographic evidence suggested that *R. glacialis* has colonized northern Europe from source populations in the eastern Alps in postglacial times. This is referred to as the 'out of the Alps hypothesis' (Schönschwetter *et al.* 2003a).

#### *Phylogenetic evidence from Anthyllis montana and Pritzelago alpina*

The evolutionary history of two morphologically polymorphic mountain plant species, *Anthyllis montana* and *Pritzelago alpina*, were explored by Kropf *et al.* (2002, 2003). Both taxa have originated from Mediterranean ancestors, and intraspecific diversification was dated based on ITS sequences to be less than 1 million years ago (Ma). It is noteworthy that in *P. alpina* Kropf *et al.* (2003) found weak evidence for a separation of populations from the northeastern and southern Alps. This finding is in good

congruence with a glacial refugium in the northeastern Alps, an area well known both for its high level of endemism (Tribtsch 2004) and the occurrence of many disjunct plant species (Merxmüller 1952, 1953). *A. montana* exhibits a phylogenetic split into a western and an eastern European group with a contact zone in the Maritime Alps (Kropf *et al.* 2002). This species' distribution in the Alps conspicuously overlaps with several potential refugial areas, suggesting that little range expansion has occurred after the Pleistocene. The sampling of populations, however, was not dense enough to infer exact locations of glacial refugia for that species in the Alps.

### *Long-distance dispersal*

One important feature of most phylogeographic studies about Alpine plants in the European Alps conducted so far is the scarcity of proof of long-distance dispersal among regions. This observation might be due to taxon selection in *A. montana* (Kropf *et al.* 2002) and *A. brevis* (Schönswetter *et al.* 2003b), as these species have presumably relic and highly fragmented or very small distribution areas. The other investigated species, in contrast, were selected as study objects because of their wide and often continuous distribution areas in the Alps. We only found evidence for long-distance dispersal from the western Alps to the Pyrenees in *P. globulariifolium* (Schönswetter *et al.* 2002) and from the Dolomites westwards to the mountain range of Adamello in *Saponaria pumila* (Tribtsch *et al.* 2002). Mostly however, we found relatively sharp boundaries with little overlap of gene pools from different refugia (Schönswetter *et al.* 2004a). In *P. globulariifolium* exhibiting an old phylogeographic split into western and eastern population groups (Schönswetter *et al.* 2002), a distribution gap is separating the gene pools. However, the availability of suitable habitats throughout this gap (Harald Niklfeld, University of Vienna, personal communication) strongly suggests incomplete (re)colonization by this species. The obviously slow migration speed is in line with observations of Comes & Kadereit (2003) and might be explained by the rugged topography and the high habitat heterogeneity of the Alps. It stands in marked contrast, however, to surprisingly high migration rates observed in many lowland (especially tree) species (Clark *et al.* 1998) and in arctic plants (Abbott & Brochmann 2003).

### *Intraspecific diversity and species diversity*

The present suite of phylogeographic studies covering 12 mountain species demonstrates that the glacial history of mountain plants is rather complex. Recolonization of the Alps out of one or two southern glacial refugia does not fit the geographical patterns of genetic variation found in molecular analyses. Many species survived in several

peripheral and/or central-Alpine refugia. However, when considering only four main geographical areas of glacial refugia in the European Alps, we detected a more general pattern: the overall matrices of observed and expected patterns of glacial refugia were statistically not significantly different from each other ( $\chi^2 = 3.747$ ,  $P = 0.29$ ; Table 1). This general pattern of glacial survival as suggested by the molecular data can be visualized by the three lines (A–C) given in Fig. 2, which mark the subdivisions between the four major areas of glacial survival. Strikingly, these lines are roughly congruent to known biogeographical boundaries based on floristic evidence. One of these three lines more or less follows the well-known boundary between the western and the eastern Alps (Fig. 2, line B). This boundary has been extensively described and is well supported by patterns of species diversity of plants of bedrock both siliceous and calcareous (Ozenda 1988). Interestingly, line B also designates an important phylogeographic split in the genetic diversity of spruce (*Picea abies*) in the Alps (Gugerli *et al.* 2002). Spruce is the dominant mountain tree in most parts of the Alps and widely forms the timberline. Together with the other two biogeographical lines (Fig. 2, lines A and C), line B divides well-known centres of species diversity and endemism in the Alps (Ozenda 1988; Tribtsch & Schönswetter 2003; Tribtsch 2004). Additionally, even the central-Alpine refugia shown in Fig. 2 coincide with species-rich regions (Wohlgemuth 2002).

In summary, our molecular analyses well conform to known floristic patterns of the Alps (Ozenda 1988). This finding is intriguing, since it suggests a close relationship between genetic differentiation and diversity at the intraspecific level on the one hand and species diversity and endemism on the other hand, two different aspects of biodiversity. If so, evolutionary and biogeographic processes could potentially act on both gene and species diversity in essentially the same way. This has recently been suggested for island biota (Vellend 2003).

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### **References**

- Abbott RJ, Brochmann C (2003) History and evolution of the arctic flora: in the footsteps of Eric Hultén. *Molecular Ecology*, **12**, 299–313.
- Abbott RJ, Smith LC, Milne RI *et al.* (2000) Molecular analysis of plant migration and refugia in the Arctic. *Science*, **289**, 1343–1346.

- van Andel TH, Tzedakis PC (1996) Palaeolithic landscapes of Europe and environs, 150 000–25 000 years ago: an overview. *Quaternary Science Reviews*, **15**, 481–500.
- Avice JC (2000) *Phylogeography: The History and Formation of Species*. Harvard University Press, Cambridge, Massachusetts.
- Bauert MR, Kälén M, Baltisberger M, Edwards PJ (1998) No genetic variation detected within isolated relict populations of *Saxifraga cernua* in the Alps using RAPD markers. *Molecular Ecology*, **7**, 1519–1527.
- Bennett KD (2004) Continuing the debate on the role of Quaternary environmental change for macroevolution. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **359**, 295–303.
- Birks HH, Ammann B (2000) Two terrestrial records of rapid climatic change during the glacial-Holocene transition (14 000–9000 calendar years BP) from Europe. *Proceedings of the National Academy of Sciences, USA*, **97**, 1390–1394.
- Briquet J (1906) Le développement des flores dans les Alpes occidentales, avec aperçu sur les Alpes en général. In: *Résultats Scientifiques du Congrès International de Botanique Vienne 1905* (eds Wettstein R, Wiesner J, Zahlbruckner A), pp. 130–172. Fischer, Jena.
- Brockmann-Jerosch H, Brockmann-Jerosch M (1926) Die Geschichte der Schweizerischen Alpenflora. In: *Das Pflanzenleben der Alpen* (ed. Schröter C), pp. 1110–1215. Raustein, Zürich.
- Burga CA, Perret R (1998) *Vegetation und Klima der Schweiz seit dem Jüngeren Eiszeitalter*. Ott, Thun.
- Chodat R, Pampanini R (1902) Sur la distribution des plantes dans les alpes austro-orientales et plus particulièrement d'un choix de plantes des alpes cadoriques et venitiennes. *Le Globe*, **41**, 1–70.
- Clark JS, Fastie C, Hurr GC *et al.* (1998) Reids paradox of rapid plant migration — dispersal theory and interpretation of paleoecological records. *Bioscience*, **48**, 13–24.
- Comes HP, Kadereit JK (1998) The effects of Quaternary climatic changes on plant distribution and evolution. *Trends in Plant Science*, **3**, 432–438.
- Comes HP, Kadereit JK (2003) Spatial and temporal patterns in the evolution of the flora of the European Alpine system. *Taxon*, **52**, 451–462.
- Dansgaard W, Johnsen SJ, Clausen HB *et al.* (1993) Evidence for general instability of past climate from a 250-kyr ice-core record. *Nature*, **364**, 218–220.
- Davis MB, Shaw RG (2001) Range shifts and adaptive responses to Quaternary climate change. *Science*, **292**, 673–679.
- Dynesius M, Jansson R (2000) Evolutionary consequences of changes in species' geographical distributions driven by Milankovitch climate oscillations. *Proceedings of the National Academy of Sciences, USA*, **97**, 9115–9120.
- Frenzel B (1964) Über die offene Vegetation der letzten Eiszeit am Ostrande der Alpen. *Verhandlungen der Zoologisch-Botanischen Gesellschaft Wien*, **103/104**, 110–137.
- Gabrielsen TM, Bachmann K, Jakobsen KS, Brochmann C (1997) Glacial survival does not matter: RAPD phylogeography of Nordic *Saxifraga oppositifolia*. *Molecular Ecology*, **6**, 831–842.
- GRIP members (1993) Climate instability during the last interglacial period recorded in the GRIP ice core. *Nature*, **364**, 203–207.
- Gugerli F, Holderegger H (2001) Nunatak survival, *tabula rasa* and the influence of the Pleistocene ice-ages on plant evolution in mountain areas. *Trends in Plant Science*, **6**, 397–398.
- Gugerli F, Sperisen C, Mátyás G *et al.* (2002) Molecular markers help to reveal postglacial re-colonisation routes of forest tree species in the alpine region. In: *Interdisciplinary Mountain Research* (eds Bottarin R, Tappeiner U), pp. 156–161. Blackwell, Berlin.
- Hellberg MH, Balch DP, Roy K (2001) Climate-driven range expansion and morphological evolution in a marine gastropod. *Science*, **292**, 1707–1710.
- Hewitt GM (2000) The genetic legacy of the Quaternary ice ages. *Nature*, **405**, 907–913.
- Hewitt GM (2004) Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **359**, 183–195.
- Holderegger R, Abbott RJ (2003) Phylogeography of the arctic-alpine *Saxifraga oppositifolia* (Saxifragaceae) and some related taxa based on cpDNA and ITS sequence variation. *American Journal of Botany*, **90**, 931–936.
- Holderegger R, Stehlik I, Abbott RJ (2002) Molecular analysis of the Pleistocene history of *Saxifraga oppositifolia* in the Alps. *Molecular Ecology*, **11**, 1409–1418.
- van Husen D (1997a) *Die Ostalpen in den Eiszeiten*. Geologische Bundesanstalt, Wien.
- van Husen D (1997b) LGM and late-glacial fluctuations in the Eastern Alps. *Quaternary International*, **38/39**, 1–109.
- Jäckli H (1970) *Die Schweiz zur Letzten Eiszeit 1 : 550 000*. Eidgenössische Landestopographie, Bern.
- Körner C (2003) *Alpine Plant Life*. Springer, Berlin.
- Kropf M, Kadereit JW, Comes HP (2002) Late Quaternary distributional stasis in the submediterranean mountain plant *Anthyllis montana* L. (Fabaceae) inferred from ITS sequences and amplified fragment length polymorphism markers. *Molecular Ecology*, **11**, 447–463.
- Kropf M, Kadereit JW, Comes HP (2003) Differential cycles of range contraction and expansion in European high mountain plants during the Late Quaternary: insights from *Pritzelago alpina* (L.) O. Kuntze (Brassicaceae). *Molecular Ecology*, **12**, 931–949.
- Lang G (1994) *Quartäre Vegetationsgeschichte Europas*. Fischer, Jena.
- Mátyás G, Sperisen C (2001) Chloroplast DNA polymorphisms provide evidence for postglacial recolonisation of oaks (*Quercus* spp.) across the Swiss Alps. *Theoretical and Applied Genetics*, **102**, 12–20.
- Merxmüller H (1952) Untersuchungen zur Sippengliederung und Arealbildung in den Alpen. I. *Jahrbuch des Vereins zum Schutze der Alpenpflanzen und Tiere*, **17**, 96–133.
- Merxmüller H (1953) Untersuchungen zur Sippengliederung und Arealbildung in den Alpen. II. *Jahrbuch des Vereins zum Schutze der Alpenpflanzen und Tiere*, **18**, 138–158.
- Merxmüller H (1954) Untersuchungen zur Sippengliederung und Arealbildung in den Alpen. III. *Jahrbuch des Vereins zum Schutze der Alpenpflanzen und Tiere*, **19**, 97–139.
- Möbus G (1997) *Geologie der Alpen*. Sven von Loga, Köln.
- Nagl H (1972) Zur pleistozänen Vergletscherung Österreichs. *Österreich in Geschichte und Literatur*, **16**, 163–176.
- Noack M (1922) Über die seltenen nordischen Pflanzen in den Alpen. Eine florensgeschichtliche Studie. *Mitteilungen des Botanischen Museums der Universität Zürich*, **95**, 1–288.
- Ozenda P (1988) *Die Vegetation der Alpen im Europäischen Gebirgsraum*. Fischer, Stuttgart.
- Pawlowski B (1970) Remarques sur l'endémisme dans la flore des Alpes et des Carpates. *Vegetatio*, **21**, 181–243.
- Penck A, Brückner E (1909) *Die Alpen im Eiszeitalter*. Tauchnitz, Leipzig.
- Petit RJ, Aguinalde I, de Beaulieu JL *et al.* (2003) Glacial refugia: hotspots but not melting pots of genetic diversity. *Science*, **300**, 1563–1565.

- Petit RJ, Csaikl UM, Bordacs S *et al.* (2002) Chloroplast DNA variation in European white oaks – phylogeography and patterns of diversity based on data from over 2600 populations. *Forest Ecology and Management*, **156**, 5–26.
- Ravazzi C (2002) Late Quaternary history of spruce in southern Europe. *Review of Palaeobotany and Palynology*, **120**, 131–177.
- Schneider C, Smith TB, Larison B, Moritz C (1999) A test of alternative models of diversification in tropical rainforests: Ecological gradients vs. rainforest refugia. *Proceedings of the National Academy of Sciences, USA*, **96**, 13869–13873.
- Schönswetter P, Tribsch A, Barfuss M, Niklfeld N (2002) Several Pleistocene refugia detected in the high alpine plant *Phyteuma globulariifolium* Sternb. & Hoppe (Campanulaceae) in the European Alps. *Molecular Ecology*, **11**, 2637–2647.
- Schönswetter P, Paun O, Tribsch A, Niklfeld H (2003a) Out of the Alps: colonisation of the Arctic by East Alpine populations of *Ranunculus glacialis* (Ranunculaceae). *Molecular Ecology*, **12**, 3373–3381.
- Schönswetter P, Tribsch A, Schnee Weiss GM, Niklfeld H (2003b) Disjunctions in relict alpine plants: phylogeography of *Androsace brevis* and *A. wulfeniana* (Primulaceae). *Botanical Journal of the Linnean Society*, **141**, 437–446.
- Schönswetter P, Tribsch A, Niklfeld H (2003c) Phylogeography of the high alpine cushion-plant *Androsace alpina* (Primulaceae) in the European Alps. *Plant Biology*, **5**, 623–630.
- Schönswetter P, Tribsch A, Stehlik I, Niklfeld H (2004a) Glacial history of high alpine *Ranunculus glacialis* (Ranunculaceae) in the European Alps in a comparative phylogeographical context. *Biological Journal of the Linnean Society*, **81**, 183–195.
- Schönswetter P, Tribsch A, Niklfeld H (2004b) Amplified fragment length polymorphism (AFLP) reveals no genetic divergence of the Eastern Alpine endemic *Oxytropis campestris* subsp. *tirolensis* (Fabaceae) from widespread subsp. *campestris*. *Plant Systematics and Evolution*, **244**, 245–255.
- Schönswetter P, Tribsch A, Niklfeld H (2004c) Amplified fragment length polymorphism (AFLP) suggests old and recent immigration into the Alps by the arctic-alpine annual *Comastoma tenellum* (Gentianaceae). *Journal of Biogeography*, **31**, 1673–1681.
- Simpson BB (1974) Glacial migrations of plants: island biogeographical evidence. *Science*, **185**, 698–700.
- Stehlik I (2002) Glacial history of the alpine herb *Rumex nivalis* (Polygonaceae): a comparison of common phylogeographic methods with nested clade analysis. *American Journal of Botany*, **89**, 2007–2016.
- Stehlik I (2003) Resistance or emigration? Response of alpine plants to the ice ages. *Taxon*, **52**, 499–510.
- Stehlik I, Schneller JJ, Bachmann K (2001) Resistance or emigration: response of the high-alpine plant *Eritrichium nanum* (L.) Gaudin to the ice age within the Central Alps. *Molecular Ecology*, **10**, 357–370.
- Stehlik I, Blattner F, Holderegger R, Bachmann K (2002a) Nunatak survival of the high Alpine plant *Eritrichium nanum* (L.) Gaudin in the central Alps during the ice ages. *Molecular Ecology*, **11**, 2027–2036.
- Stehlik I, Schneller JJ, Bachmann K (2002b) Immigration and *in situ* glacial survival of the low-alpine *Erinus alpinus* (Scrophulariaceae). *Biological Journal of the Linnean Society*, **77**, 87–103.
- Taberlet P, Fumagalli L, Wust-Saucy AG, Cossons F (1998) Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology*, **7**, 453–464.
- Tribsch A (2004) Areas of endemism of vascular plants in the Eastern Alps in relation to Pleistocene glaciation. *Journal of Biogeography*, **31**, 747–760.
- Tribsch A, Schönswetter P (2003) In search for Pleistocene refugia for mountain plants: patterns of endemism and comparative phylogeography confirm palaeo-environmental evidence in the Eastern European Alps. *Taxon*, **52**, 477–497.
- Tribsch A, Schönswetter P, Stuessy TF (2002) *Saponaria pumila* (Caryophyllaceae) and the ice-age in the Eastern Alps. *American Journal of Botany*, **89**, 2024–2033.
- Vellend M (2003) Island biogeography of genes and species. *American Naturalist*, **162**, 358–365.
- Vetters H (1933) *Geologische Karte der Republik Österreich und der Nachbargebiete 1 : 500 000*. Geologische Bundesanstalt, Wien.
- Wohlgemuth T (2002) Environmental determinants of vascular plant species richness in the Swiss alpine zone. In: *Mountain Biodiversity, a Global Assessment* (eds Körner C, Spehn EM), pp. 103–116. Parthenon, Boca Raton, Florida.

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This paper is a synthesis of the PhDs of Peter Schönswetter, Ivana Stehlik and Andreas Tribsch who aimed at elucidating the glacial history of mountain plants of the Alps with molecular markers and species' distribution data. P.S. and A.T. are currently working on migration patterns and evolution of arctic alpine plants in the research group of Christian Brochmann at the National Centre of Biosystematics at the University of Oslo. I.S. is focusing on the ecology and evolution of sex determination in a dioecious alpine plant. Rolf Holderegger is mainly interested in the ecological and conservation genetics of species ranging from lichens and ferns to herbs and trees

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## Appendix

Summary of studies used in the comparative phylogeographic analysis given in Fig. 2. Taxa, references, sampling designs, molecular methods, main statistical analyses and the glacial refugia detected are given. Abbreviations: AFLPs, amplified fragment length polymorphisms; AMOVA, analysis of molecular variance; CA, correspondence analysis; cpDNA, chloroplast DNA; NJ tree, neighbour-joining tree; nrDNA, nuclear ribosomal DNA; PCoA, principal coordinate analysis; PCR, polymerase chain reaction; RAPD, random amplified polymorphic DNAs; RFLP, restriction fragment length polymorphisms; UPGMA tree, unweighted pair group method on arithmetic means tree; I–IX, numbering of glacial refugia is given in Fig. 2

Taxon	References	Distribution	Sampling design	Number of investigated individuals from the Alps (and in total)	Number of investigated populations from the Alps (and in total)	Molecular method	Statistical analyses	Glacial refugia detected
<i>Androsace alpina</i>	Schönschwetter <i>et al.</i> (2003c)	Alps	Distribution-wide	257 (257)	53 (53)	AFLP	AMOVA; PCoA; NJ tree	I, II, III, IV
<i>Androsace brevis</i>	Schönschwetter <i>et al.</i> (2003b)	Around Lago di Como	Distribution-wide	26 (26)	8 (8)	AFLP	AMOVA; NJ tree	II and/or III
<i>Androsace wulfeniana</i>	Schönschwetter <i>et al.</i> (2003b)	Dolomites, eastern central Alps	Distribution-wide	37 (37)	8 (8)	AFLP	AMOVA; PCoA; NJ tree	III, IV (subdivided)
<i>Comastoma tenellum</i>	Schönschwetter <i>et al.</i> (2004c)	Arctic–Alpine	Distribution-wide in the Alps and hypothesis-driven in Europe	132 (167)	30 (36)	AFLP	AMOVA; NJ-tree	I, eastern-Alpine lineage
<i>Erinus alpinus</i>	Stehlik <i>et al.</i> (2002b)	Pyrenees, western Alps	Hypothesis-driven (only middle part of the Alps)	264 (264)	22 (22)	AFLP; cpDNA PCR-RFLP	AMOVA; CA; UPGMA tree; Mantel test	V; re-immigration from a southwestern-Alpine peripheral refugium
<i>Eritrichium nanum</i>	Stehlik <i>et al.</i> (2001, 2002a)	Alps	Hypothesis-driven (only middle part of the Alps); distribution-wide	276 (276)	20 (20)	AFLP, cpDNA PCR-RFLP, cpDNA sequences	AMOVA; CA; UPGMA tree; Mantel test; minimum-spanning network	III, IV, VIII, IX
<i>Oxytropis campestris</i>	Schönschwetter <i>et al.</i> (2004b)	Pyrenees, Alps	Distribution-wide	177 (195)	39 (43)	AFLP	AMOVA; PCoA; NJ tree; Mantel test	Re-immigration from outside of the Alps
<i>Phyteuma globulariifolium</i>	Schönschwetter <i>et al.</i> (2002)	Pyrenees, Alps	Distribution-wide	317 (325)	65 (67)	AFLP	AMOVA; PCoA; NJ tree; Mantel test	I and/or II, II and/or III, III, IV
<i>Ranunculus glacialis</i>	Schönschwetter <i>et al.</i> (2003a, 2004a)	Arctic–Alpine	Distribution-wide in the Alps and hypothesis-driven in Northern Europe	223 (267)	75 (86)	AFLP, cpDNA sequences	AMOVA; PCoA; parsimony phylogeny; Mantel test	I, II, III, IV; colonization of northern Europe out of the Alps
<i>Rumex nivalis</i>	Stehlik (2002)	Middle and eastern Alps, Balkan Peninsula	Hypothesis-driven (only middle part of the Alps)	276 (276)	23 (23)	AFLP, cpDNA PCR-RFLP	AMOVA; CA; minimum-spanning network; Mantel test; nested clade analysis	V, VI, VII
<i>Saponaria pumila</i>	Tribsch <i>et al.</i> (2002)	Eastern Alps, southern Carpathians	Distribution-wide in the Alps	158 (158)	33 (33)	AFLP	AMOVA; PCoA; UPGMA-tree; Mantel test	III, IV (subdivided)
<i>Saxifraga oppositifolia</i>	Holderegger & Abbott (2003); Holderegger <i>et al.</i> (2002)	Arctic–Alpine	Hypothesis-driven (only middle part of the Alps) and Arctic	143 (160)	15 (15)	RAPD; Southern hybridization RFLP, cpDNA and nrDNA sequences	AMOVA; CA; NJ tree; Mantel test; nestedness; parsimony phylogeny	Re-immigration from outside of the Alps

## Erratum

Dr Peter Schoenswetter brought to our attention that the changes he wished to include in Table 1 which appears in the article 'Molecular evidence for glacial refugia of mountain plants in the European Alps' do not appear in the final version. As a result, page 3549 in MEC 14:11 (October 2005) should read:

'The principal entries were "6" for an existing or expected refugium and "0" for a missing refugium.'

Page 3550 should read:

'Hence, in the matrix of observed patterns in *P. globulariifolium*, the principal entry ("6") for each of the two observed western glacial refugia had to be split and assigned to two expected refugia ("3" per potential refugium). In contrast, in the matrix of expected patterns in *P. globulariifolium*, entries ("6") are found along the diagonal.'