Molecular Ecology (2003) **12**, 3373–3381 doi: 10.1046/j.1365-294X.2003.01984.x

Out of the Alps: colonization of Northern Europe by East Alpine populations of the Glacier Buttercup *Ranunculus glacialis* L. (Ranunculaceae)

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Abstract

***Ranunculus glacialis* ssp. *glacialis* is an arctic-alpine plant growing in central and southern European and Scandinavian mountain ranges and the European Arctic. In order to elucidate the taxon's migration history, we applied amplified fragment length polymorphism (AFLP) to populations from the Pyrenees, Tatra mountains and Northern Europe and included data from a previous study on Alpine accessions. Populations from the Alps and the Tatra mountains were genetically highly divergent and harboured many private AFLP fragments, indicating old vicariance. Whereas nearly all Alpine populations of *R. glacialis* were genetically highly variable, the Tatrean population showed only little variation. Our data suggest that the Pyrenees were colonized more recently than the separation of the Tatra from the Alps. Populations in Northern Europe, by contrast, were similar to those of the Eastern Alps but showed only little genetic variation. They harboured no private AFLP fragments and only a subset of East Alpine ones, and they exhibited no phylogeographical structure. It is very likely therefore that *R. glacialis* colonized Northern Europe in post-glacial times from source populations in the Eastern Alps.**

Keywords: amplified fragment length polymorphism, arctic-alpine flora, dispersal, migration, phylogeography, Quaternary biogeography

Received 20 June 2003; revision received 22 August 2003; accepted 22 August 2003

Introduction

Although strong floristic congruence between the Arctic and the Alps was recognized as early as 1768 by the Swiss botanist Albrecht von Haller (cit. in Noack 1922), there is still little knowledge on the causes of these similarities. Only few phylogeographical studies covered both the central and southern European mountain ranges (csEMRs) and Northern Europe. Abbott *et al*. (2000) provided the first full area phylogeography of an arctic-alpine taxon, *Saxifraga oppositifolia*. Després *et al*. (2002) explored the phylogeography of *Trollius europaeus* in the Pyrenees, the Alps & Fennoscandia. Gabrielsen & Brochmann (1998) and Bauert *et al*. (1998) investigated genetic variation in populations of mainly clonally reproducing *Saxifraga cernua* in Scandinavia and the Alps, respectively. This modest level of knowledge of large-scale relationships between the floras of the csEMRs and Northern Europe contrasts the rapidly growing understanding of some of the arctic and alpine species' glacial histories on smaller regional scales, mainly Scandinavia and the European Alps (e.g. Abbott *et al*. 1995; Gabrielsen *et al*. 1997; Hagen *et al*. 2001; Holderegger *et al*. 2002; Schönswetter *et al*. 2002; Stehlik *et al*. 2002; Tribsch *et al*. 2002; Abbott & Brochmann 2003).

The close floristic affinity of the arctic and Alpine floras is hypothesized to have resulted from the predominance of tundra vegetation in the lowlands between the Scandinavian and the Alpine ice shields during the cold stages of the Pleistocene (Frenzel *et al*. 1992), providing a migration corridor between the respective areas (Noack 1922). This hypothesis is supported by fossil evidence for arctic-alpine

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plants from these lowlands (e.g. Lang 1994). Only relatively few arctic-alpine taxa, however, are present in this fossil record, questioning the general significance of such lowland populations as sources for a large-scale colonization of the Arctic after the retreat of the ice shields. Due to the occurrence of steppe communities north of the Alps (as demonstrated by palaeo-vegetational data; Frenzel *et al*. 1992), the existence of a continuous distribution of high-alpine taxa connecting the Alpine populations with, for example, the forelands of the glaciers protruding from the Scandinavian ice sheet, is questionable. This implies that the shared existence of species both in csEMRs and Northern Europe might be due to one of several (post)Pleistocene scenarios: (i) postglacial colonization of formerly glaciated Northern Europe from csEMRs; (ii) glacial survival in Northern European refugia and postglacial colonization of csEMRs; and (iii) glacial survival both in northern refugia and in csEMRs.

Several studies revealed that colonized areas and their respective source populations differ in their genetic characteristics. In most studies exploring genetic differences between colonized areas and their source populations the latter are characterized by higher levels of allelic richness (e.g. Amsellem *et al*. 2000; Comps *et al*. 2001; Widmer & Lexer 2001). Colonized areas are expected to harbour only a subset of their source gene pools (Hewitt 1996; Soltis *et al*. 1997), and genetic diversity often parallels this pattern (e.g. Shapcott 1998). In some studies, however, populations of colonized areas are found to be equally or even more genetically variable than those of refugia, probably due to an amalgamation of immigrants of several different refugia (e.g. Comps *et al*. 2001; Petit *et al*. 2003; Schönswetter *et al*. in press). Furthermore, the pattern of migration has been demonstrated to influence the genetic constitution of populations in colonized areas. In the Alpine cushion plant *Saponaria pumila*, Tribsch *et al*. (2002) detected a gradual decrease of genetic diversity from refugia at the eastern margin of the Alps towards the western limit of the species' continuous distribution. In contrast, some of its isolated, disjunct populations, assumed to result from-long distance dispersals, were strongly effected by founder events.

To test the different (post)Pleistocene scenarios, a study system should ideally be restricted to the csEMRs and the mountainous and arctic regions of Northern Europe in order to exclude a distortion of the present genetic composition due to historical gene flow, e.g. from Central Asian mountain ranges. One of the plants meeting this requirement is *Ranunculus glacialis* L. ssp. *glacialis* (Ranunculaceae). It occurs in csEMRs (Sierra Nevada, Pyrenees, Alps, Carpathians) and the Scandinavian mountains, as well as on the Faeroe Islands, the island of Jan Mayen, Spitzbergen, Iceland and along the eastern coast of Greenland (Hultén & Fries 1986). A closely related, but morphologically divergent taxon, *R. glacialis* ssp. *chamissonis* (= *R. chamissonis*, *Beckwithia chamissonis*), grows in Beringia (Hultén & Fries 1986). *Ranunculus glacialis* is not equally distributed throughout its range, being abundant in large parts of mountainous and arctic regions of Northern Europe and the Alps, but rare in the Sierra Nevada, the Pyrenees and the Carpathians (P. Schönswetter, pers. obs.), where it is confined to the highest elevations.

Schönswetter *et al*. (in press) provided a phylogeographical study of the Alpine populations of *R. glacialis* based on AFLP fingerprinting data of 75 populations. There is strong phylogeographical structure and four population groups are differentiated. Two of them, located in the Western Alps, are genetically isolated from each other and from all other populations, whereas the two Eastern Alpine groups are genetically more similar to each other suggesting longer isolation and/or lower levels of gene flow in the two western groups. As all groups are close to, or overlap with, presumed glacial refugia, invoking glacial survival on nunataks was not needed to explain the present genetic pattern (Schönswetter *et al*. in press).

In this investigation, we report a phylogeographical study of *R. glacialis* ssp. *glacialis* based on previous AFLP data from Schönswetter *et al*. (in press) from the Alps and on new AFLP and *mat*K sequence data covering large portions of the species' total distribution area. We include accessions from the Pyrenees and the Carpathians (Tatra mountains) as well as from Northern Europe and focus on the following questions: (i) What are the genetic relationships among populations of different csEMRs? (ii) Do the Northern European populations and those from csEMRs originate from different glacial refugia? If not, what is the directionality of the migrations; from the csEMRs to Northern Europe or vice versa? (iii) Do genetic data suggest long-distance dispersal or continuous migration within and among the species' extant distribution?

Materials and methods

The study species

Ranunculus glacialis is a very common and abundant element of high alpine to subnival plant communities in csEMRs as well as of arctic scree-fields. It is an alpine pioneer species often growing in unstable habitats such as scree slopes and moraines. Although explicit data on the breeding system of *R. glacialis* are lacking, it is presumably outcrossing as are other alpine *Ranunculus* species, e.g. *R. alpestris* (Müller & Baltisberger 1984). The amount of vegetative propagation is very limited, as the species has a short rhizome.

Sampling

Seventy-three populations of *R. glacialis* from the European Alps already investigated by Schönswetter *et al*. (in press)

Table 1 Acronyms of populations, numbers of investigated individuals, locations, co-ordinates, numbers of fragments per population and Shannon Diversity Indices (H_{Sh}) of the 11 investigated populations of *Ranunculus glacialis* ssp. *glacialis* which were investigated in addition to the 75 populations in Schönswetter *et al*. (in press)

Acronym	$N_{ind.}$	Location	Co-ordinates	$N_{fragments}$	H_{Sh}
PYR	3	Spain, Huesca, Collado del Cao	0.27° E/42.65° N	28	2.08
TATRA	4	Poland, Kraków, Tatry Wysokie, Zawrat	20.00° E/49.22° N	43	0.37
SCAND-1	3	Norway, Tromsø, Krokdalen	19.11° E/69.66° N	34	0.81
SCAND-2	3	Sweden, Norrbotten, Tjievrra	17.92° E/66.90° N	37	0.64
SCAND-3	5	Norway, Sør-Trøndelag, Oppdal	9.73° E/62.44° N	36	1.27
SCAND-4	3	Norway, Sør-Trøndelag, Knudshøa	9.50° E/62.28° N	36	1.64
SCAND-5	3	Norway, Oppland, Galdhøpiggen	8.33° E/61.63° N	37	1.37
SCAND-6	5	Norway, Hordaland, Finse	7.53° E/60.61° N	35	1.00
ICEL-1	5	Iceland, Akureyri	18.20° W/65.62° N	35	0.64
ICEL-2	5	Iceland, Holmavik, near road 61	22.12° W/65.74° N	34	0.27
JM	5	Norway, Jan Mayen, Blinddalen	8.00° W/71.00° N	35	0.27

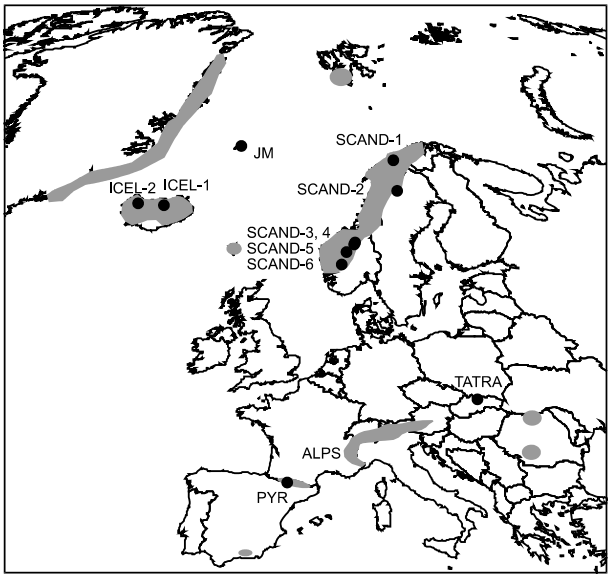

Fig. 1 Distribution area of *Ranunculus glacialis* ssp. *glacialis*. Sampled populations are indicated with details given in Table 1. Except for the specified populations, this study covers 73 populations from the Alps already analysed in Schönswetter *et al*. (in press).

were included in the present study. Populations 9 and 39 from Schönswetter *et al*. (in press) with only two investigated individuals were excluded. We sampled one population of *R. glacialis* ssp. *glacialis* from each of the Pyrenees (PYR) and the Tatra mountains (TATRA), six from Scandinavia (SCAND-1 to SCAND-6), two from Iceland (ICEL-1, ICEL-2) and one from Jan Mayen (JM; Table 1, Fig. 1). Three individuals per population were analysed in the Alpine populations and PYR and in populations SCAND-1, SCAND-2, and SCAND-4, four in TATRA and five in JM, ICEL-1, ICEL-2 and SCAND-3. Voucher specimens are deposited in the herbaria of the Institute of Botany of the University of Vienna (WU) and the Botanical Museum in Oslo (O).

DNA isolation and AFLP fingerprinting

Total genomic DNA was extracted from similar amounts of dried tissue following a CTAB-protocol (Doyle & Doyle 1987) with the modifications described in Schönswetter *et al*. (2002). The quality of the extracted DNA was checked on 1% TAE-agarose gels. Approximately 10% of the extracts were quantified photometrically (UV 160 A Spectrophotometer, Shimadzu) and subsequently the average DNA concentration was estimated. The AFLP procedure followed Vos *et al*. (1995) with the modifications described in Schönswetter *et al*. (in press). Two primer combinations using *Mse*I primers with four selective nucleotides were chosen: *Eco*RI ACC (NED)-*Mse*I CATA and *Eco*RI ACT (6-FAM)-*Mse*I CTCG. The fluorescence-labelled selective amplification-products were separated on a 5% polyacrylamide gel with an internal size standard (GeneScan-500 [ROX], PE Applied Biosystems) on an automated sequencer (ABI 377, Perkin Elmer). Raw data were collected and aligned with the internal size standard using the ABI Prism GENESCAN Analysis Software (PE Applied Biosystems). Subsequently, the GENESCAN-files were imported into GENOGRAPHER (version 1.6.0, Montana State University 1998©; http://hordeum.msu.montana.edu/genographer/) for scoring of the fragments. Individuals already analysed in Schönswetter *et al*. (in press) were re-analysed together with the new samples. AFLP-fragments that exhibited ambiguous peaks were excluded from the analysis. Peaks of low intensity were included in the analysis when unambiguous scoring was possible. As the homology of some fragments from Alpine individuals with the new accessions was doubtful due to strongly

different intensities or minor length differences, these fragments were excluded from the analysis. This introduced minor differences, e.g. in genetic diversity measures, of the Alpine individuals as compared to Schönswetter *et al*. (in press). The results of the scoring were exported as a presence/absence matrix and used for further analysis.

Sequencing of mat*K*

In a taxonomic study of the genus *Ranunculus* (Paun *et al*. unpubl.), intraspecific variation in the *mat*K region of cpDNA was detected in some of the investigated taxa. Therefore, that region was amplified and sequenced for one individual of populations 19 (Western Alps), 57 and 71 (Eastern Alps) from Schönswetter *et al*. (in press) and from TATRA, PYR, and SCAND-5 according to the protocol described by Müllner *et al*. (2003) except that the following primers were used: *trn*K3AF (5′-CGKAAACACAAAA-GTASTGTACG-3′) and *trn*K3R (5′-GATTCGAACCCG-GAACTAGTCGG-3′). Primer sequences were kindly provided by Peter Lockhart (Allan Wilson Center, Massey University, New Zealand). Genebank accession numbers are AY312239, AY312240, AY312237 (populations 19, 57 and 71, respectively, in Schönswetter *et al*. (in press), AY312238 (SCAND-5), AY312241 (PYR) and AY312242 (TATRA).

Data Analysis

We computed the number of AFLP fragments per population. Shannon Diversity $H_{Sh} = -\Sigma(p_j \ln p_j)$, where p_j is the relative frequency of the *j*-th fragment (Legendre & Legendre 1998), was calculated for three randomly selected individuals per population. Only the presence of markers was considered. The index was calculated for each putative locus and then summed without averaging by the number of loci. The numbers of private and fixed private fragments were estimated for each population. In order to trace the origin of the Northern European populations (i.e. SCAND-1–6, ICEL-1, ICEL-2, JM; Table 1), the number of fragments that were shared between the pooled AFLP-profile of all Northern European individuals (i.e. the combination of presences of AFLP markers found in the single individuals) and the Alpine populations was estimated.

We performed a parsimony analysis with PAUP 4.0 b10 (Swofford 1998). Heuristic searches were done using addition sequence set at 1000 random additions of taxa, tree bisection-reconnection (TBR) branch swapping, and 'MulTrees' on (keeping multiple, shortest trees), but keeping only 10 trees per replicate to reduce time spent in swapping on large number of trees. After these 1000 replicates, we used the shortest trees found as new starting trees for a swapping-to-completion. An unrooted 95% majority rule consensus tree of 10 000 equally most parsimonious trees was constructed. Robustness of clades was estimated using the bootstrap approach (Felsenstein 1985) with 1000 replicates with simple sequence addition, TBR branch swapping, and 'MulTrees' on, but holding 10 trees per replicate to reduce time spent on each replicate.

A principal coordinate analysis (PCoA) based on a matrix of between-individual Jaccard similarities obtained from AFLP data, was calculated and plotted with SPSS 8.0.0 (SPSS Inc. 1989–97). The goodness of fit of the genetic interindividual Jaccard distances ($1–C_J$; $C_J = a/a + b + c$, where a is the number of fragments shared between two populations and *b* and *c* are the numbers of fragments present in only one population) and geographical distances of SCAND-1 to SCAND-6 was assessed with a Mantel test (Mantel 1967) as implemented in R-PACKAGE 4.0 (Casgrain & Legendre 1999). We only included the mainland Scandinavian populations, as these were sampled in a comparable density as the Alpine ones.

Results

AFLP

For the combined data set of Alpine populations [i.e. in populations 1–75 from Schönswetter *et al*. (in press)

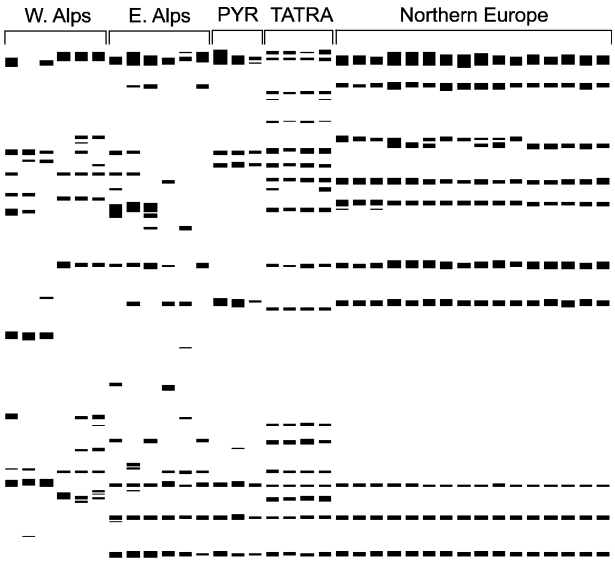

Fig. 2 Comparison of AFLP profiles generated with primer pair *Eco*RI-ACT *Mse*I-CTCG in *Ranunculus glacialis* ssp. *glacialis*. The lengths of the visualized fragments range from 150 to 300 bp. AFLP profiles were aligned with GENOGRAPHER 1.6.0. W.Alps, populations 1 and 20; E.Alps, populations 27 and 74 from Schönswetter *et al*. (in press); Northern Europe, populations SCAND-1, SCAND-2, SCAND-4, SCAND-5, ICEL-1 from the present study.

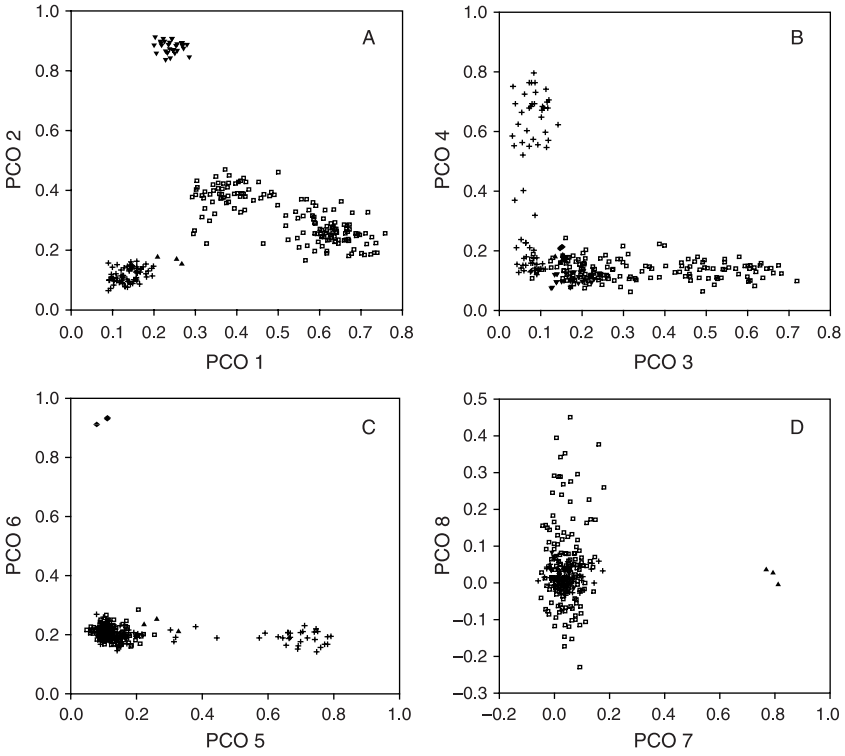

Fig. 3 Principal coordinate analysis (PCoA) of Alpine, Pyrenean, Carpathian and Northern European individuals of *Ranunculus glacialis* ssp. *glacialis*. To enforce legibility, we combined population groups C and E from the Eastern Alps and SW and W from the Western Alps (Schönswetter *et al*. in press). Open squares, Eastern Alps; crosses, Western Alps; upturned triangles, Northern Europe; upright triangles, Pyrenees; filled rhombi, Tatra mountains. The eight axes explain 18.77%, 11.17%, 9.05%, 7.52%, 6.81%, 1.53%, 1.01% and 0.96% of the overall variation.

with the exception of populations 9 and 39] and the new populations (Table 1), the two primer combinations yielded 202 unambiguously scorable fragments, 200 (99.0%) of which were polymorphic. Of the 40 fragments found in the Northern European populations, only 11 (27.5%) were polymorphic. A comparison of aligned AFLP profiles generated with primer pair *Eco*RI ACT-*Mse*I CTCG is given in Fig. 2. The length of the fragments varied from 55 to 439 bp. We detected two pairs of identical genotypes in the Alpine populations and in TATRA, respectively, but none in PYR. In the Northern European populations, three identical AFLP profiles occurred in ICEL-2 and four in ICEL-1. In JM, we found one triplet and a pair of identical individuals. Two individuals of populations SCAND-2 and SCAND-5 also shared one genotype. The number of fragments per population varied from 28 in PYR to 43 in TATRA (Table 1). In the Alpine populations, the mean was 42.45 (SD = 5.71). The H_{Sh} of the new accessions not included in Schönswetter *et al*. (in press) varied between 0.27 in JM and ICEL-2 and 2.08 in PYR (Table 1). On a regional basis, H_{Sh} was highest in the Alps (mean 6.73 ± 1.73), followed by PYR (2.08), Northern Europe (0.88 ± 0.48) and TATRA (0.37).

We detected 117 private fragments in the Alpine populations (none of them fixed), whereas there were three private fragments in PYR and 11 in TATRA, all of which were fixed. No private fragments occurred in the Northern European populations. A comparison of a pooled AFLP profile of all Northern European individuals with populations from the Alps revealed that populations 65, 68, 71 and 75 from population group E from Schönswetter *et al*. (in press) shared most fragments (> 75%) with the Northern European populations.

In the PCoA (Fig. 3), the East Alpine populations (groups C and E from Schönswetter *et al*. in press) were separated from all others along the first factor explaining 18.77% of the overall variation (Fig. 3A). The Northern European populations were separated along the second factor (11.17%; Fig. 3A). The third (9.05%; Fig. 3B), fourth (7.52%; Fig. 3B) and fifth (6.81%; Fig. 3C) factors revealed internal structure of the East and West Alpine populations. The sixth factor (1.53%; Fig. 3C) separated TATRA, the seventh (1.01%; Fig. 3D) PYR and from the eighth factor (0.96%; Fig. 3D) onwards there was no interpretable pattern.

The unrooted 95% majority rule consensus tree of 1000 equally most parsimonious trees (Fig. 4) differentiated the following clades. The Western Alpine population groups SW and W and the Eastern Alpine C and E as detected by Schönswetter *et al*. (in press) were again present, but had no bootstrap support above 50. Additionally, TATRA and PYR constituted well-supported lineages. TATRA was similar to four individuals from the Eastern Alps. The Northern European populations also formed a group with high bootstrap support but were nested within E. The assignment of a few Alpine individuals differed in the

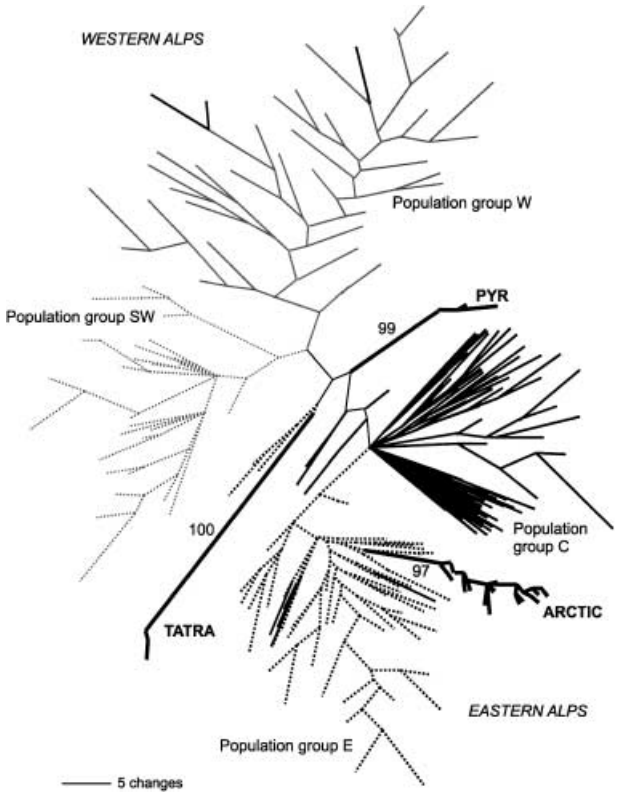

Fig. 4 Unrooted 95% majority rule consensus tree of 10 000 equally most parsimonious trees of 268 Alpine, Pyrenean, Carpathian and Northern European individuals of *Ranunculus glacialis* ssp. *glacialis*. The numbers are bootstrap values above 50 (1000 replicates). Population groups differentiated in Schönswetter *et al*. (in press) with phenetic methods (i.e. SW and W from the Western Alps and C and E from the Eastern Alps) are indicated with different branch styles. The new accessions are given in bold. For details, see text.

parsimony analysis (Fig. 4) as compared to the phenetically defined population groups from Schönswetter *et al*. (in press). All three individuals of population 23 from group W in Schönswetter *et al*. (in press) were nested in C in the present study, and one individual of population 55 and three individuals of population 56 appeared in E instead of C. All of the mentioned differences in the classification refer to populations from the borders of the population groups. Furthermore, one individual of population 58 and three individuals of population 74, both from group E in Schönswetter *et al*. (in press), were associated with individuals of TATRA.

As an estimate of isolation by distance, the Mantel test comparing genetic and geographical distances for the mainland Scandinavian populations (SCAND-1 to SCAND-6) detected only weak isolation by distance ($R_M = 0.16$, $P = 0.034$). In the Alpine populations, it was 0.67 ($P = 0.001$; Schönswetter *et al*. in press)

mat*K sequences*

The sequences obtained from PYR, SCAND-5 and the Alpine populations 19, 57 and 71 from Schönswetter *et al*. (in press) were identical, but TATRA differed in three point mutations (substitutions) out of 402 bp at positions 56, 220 and 228.

Discussion

Phylogeographical pattern

Both principal coordinate analysis (PCoA) and maximum parsimony (MP) analysis revealed a strong structure among the 84 investigated populations of *Ranunculus glacialis* in central and southern European mountain ranges (csEMRs) and Northern Europe (Figs 3 and 4). Within the Alpine populations, there was a differentiation into two Western and two Eastern Alpine population groups as already identified by Schönswetter *et al*. (in press) applying phenetic methods with only few individuals being differently placed in geographically adjacent groups. The Northern European populations were separated along the second factor in the PCoA, presumably due to the shared absence of many fragments present in Alpine populations and the relatively large size of the Northern European group. In the MP analysis, by contrast, the Northern European populations were nested within the easternmost population group E from the Alps (Schönswetter *et al*. in press). Due to the uneven group size, TATRA and PYR were separated from the rest of the data set containing the Alpine and Northern European populations along axes with low explanatory values in the PCoAs. In the MP analysis, however, both populations exhibited high bootstrap supports. Population PYR was characterized by three fixed private fragments and an overall low number of markers. TATRA was highly divergent from all other investigated populations with 11 fixed private fragments (Table 1; Fig. 2) and an overall high number of fragments. Sequences of the chloroplast *mat*K region revealed that TATRA differed markedly from the other accessions that shared a chlorotype.

Genetic diversity differed strongly among the population groups. By far the highest average value of H_{Sh} was found in the Alpine populations. Only a single population from the Alps [population 12 from Schönswetter *et al*. (in press)] was characterized by a lower H_{Sh} than PYR. The Scandinavian populations and especially TATRA were genetically even less diverse than PYR. Due to the strong genetic divergence, the low degree of genetic variation of TATRA is most probably not attributable to a recent long distance dispersal but rather to one or more bottlenecks during the Pleistocene. Alternatively, due to the small size of the high-alpine parts of Tatra mountains TATRA might

have experienced a post-Pleistocene bottleneck. Altogether, the strong divergence of PYR and especially of TATRA as compared with the Alpine and Northern European populations indicates that the Pyrenees and Tatra mountains can be excluded as source populations for the colonization of Northern Europe.

Fast, recent colonization of Northern Europe from the Eastern Alps

The most recent event in the phylogeographical history of *R. glacialis* was probably the colonization of Northern Europe from source populations in the Eastern Alps. This is supported by several lines of evidence. In the parsimony analysis (Fig. 4), the Northern European individuals are nested as a group within population group E from the Eastern Alps (Schönswetter *et al*. in press). However, presumably due to the high degree of genetic variation within the four populations groups, none of them had high bootstrap support. Additionally, the Northern European populations shared most fragments with populations from E. Preliminary results of a restriction fragment length polymorphism (RFLP) analysis of cpDNA (Schönswetter *et al*. unpublished) revealed the cpDNA haplotype found in the eastern part of the Alps to be identical to that in Northern Europe. The alternative scenario, the colonization of Northern Europe from a northern refugium, e.g. southerly adjacent to the Scandinavian ice sheet, is not supported by our data, because, if *R. glacialis* survived the glaciations in a northern refugium, the lack of divergence of the Northern European populations would be difficult to explain.

Colonization of Northern Europe from the Alps was not supported in previous phylogeographical studies on plants. Rather, Bauert *et al*. (1998) invoked immigration from Scandinavia to the Alps in *Saxifraga cernua*. In Alpine populations of *Saxifraga oppositifolia*, Abbott *et al*. (2000) detected only cpDNA haplotypes that are widespread in the Arctic and Després *et al*. (2002) found evidence for colonization of Fennoscandia from Carpathian source populations in *Trollius europaeus*. Thus, to our knowledge, our study is the first to support postglacial northward expansion of an arctic-alpine plant from source populations in the Alps.

The Northern European populations of *R. glacialis* were genetically much less variable as compared to the Alpine ones. Interestingly, morphological variability parallels the pattern of genetic diversity (Böcher 1972). Whereas the Alpine populations exhibit strong differences in leaf shape and habitus, the northern populations are morphologically very uniform and represent only a small part of the variability in the Alpine populations (Böcher 1972). As *R. glacialis*, similarly as in the Alps, is widespread and inhabits common habitats in Northern Europe (e.g. Lid & Lid 1998), the low genetic variation of the Northern European populations is most likely a consequence of its immigration history. The species probably did not reach Northern Europe by a phalanx expansion in the sense of Hewitt (1996), as such a migration is expected to result in higher levels of genetic diversity. A more probable immigration pathway involves a single postglacial long distance dispersal event (or multiple dispersal from the same gene pool) or a stepping stone migration that led to severe bottlenecks and strong genetic drift but not to genetic divergence. Whereas long distance dispersals were formerly considered to be highly unlikely, recent genetic data have shown that they occurred among csEMRs (Schönswetter *et al*. 2002) as well as between eastern North America and Eurasia crossing the Atlantic ocean (e.g. Hagen *et al*. 2001; reviewed in Abbott & Brochmann 2003). The current distribution of *R. glacialis* with its isolated occurrences in, e.g. Sierra Nevada or on arctic islands indicates that the species is obviously able to undergo long distance dispersals.

Low levels of genetic diversity in Northern European populations were already reported for other plant taxa (but see, e.g. Abbott *et al*. 1995 and Gabrielsen *et al*. 1997). In *Carex capitata*, which is widespread in the northern hemisphere, and the closely related exclusively arctic *C. arctogena*, Reinhammar (1999) found significantly lower levels of isozyme variation in the latter. The genetic pattern detected in the present study is similar to that of *Trollius europaeus* (Després *et al*. 2002), which is suggested to have survived glaciations in interconnected populations in southern Europe represented nowadays by relictual occurrences in the Pyrenees, Alps and Carpathians. The genetically little variable arctic populations originated from immigration from a Carpathian refugium accompanied by a past founder effect during northward recolonization.

The spread of *R. glacialis* in Northern Europe was probably rapid, as there was nearly no genetic divergence among populations or groups of populations and only weak isolation by distance compared to that found in the Alpine populations (Schönswetter *et al*. in press). Similarly low genetic structuring, but combined with higher levels of genetic diversity, was detected in arctic *Saxifraga cespitosa* (Tollefsrud *et al*. 1998) and arctic-alpine *S. oppositifolia* (Gabrielsen *et al*. 1998) in Scandinavia. The only observable trend in the distribution of genetic diversity of *R. glacialis* in Northern Europe was the relatively higher diversity in mainland Scandinavia as compared to Iceland and Jan Mayen, where nearly fixed populations occur (Table 1).

Conclusion

Our study shows that the arctic-alpine plant *Ranunculus glacialis* experienced old vicariance in the Alps and Tatra mountains. The Pyrenees were apparently more recently colonized, but are still characterized by private AFLP

markers. Populations in Northern Europe, by contrast, are similar to those from the Eastern Alps but genetically much less variable. It is thus feasible to conclude that *R. glacialis* colonized Northern Europe fairly recently, accompanied by a strong founder effect. Thus, *R. glacialis*, as well as the previously studied *Trollius europaeus* (Després *et al*. 2002), illustrate the general importance of central and southern European mountain ranges as source regions for certain components of the extant arctic flora.

A recent study on the phylogeography of Alpine populations (Schönswetter *et al*. in press) showed that *R. glacialis* is genetically highly variable in the Alps. As a consequence, we regard the species as a very suitable study system for future combined ecological and genetic studies exploring the effects of limited genetic variation on the fitness of natural populations.

Acknowledgements

Funding by the Austrian Science Foundation (FWF, P13874-Bio) is highly acknowledged. The administrations of the Hohe Tauern National Park (Austria) in Carinthia and Salzburg and the Bezirkshauptmannschaft (district administration) Lienz (Austria) as well as the Tatrzański National Park (Poland) are thanked for issuing collection permits. We thank Michael Barfuß for efficient technical support in the lab. Christopher Dixon improved the grammar and Ivana Stehlik provided helpful comments on the manuscript. Thanks to Alexandra Müllner and Gerald M. Schneeweiss for help with PAUP. Special thanks also go to all people (Inger Alsos, Pernille Bronken, Reidar Elven, Marte Holten Jørgensen, Gro H. Jacobsen, Inger Skrede and Anders Wollan) who collected plant material for us from Northern Europe and to Magdalena Wiedermann and Gerald M. Schneeweiss, who accompanied us during much of our collection trips in the Alps and Pyrenees.

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This work is an extension of a comparative phylogeographical project focusing on the European Alps to the European Arctic. Peter Schönswetter and Andreas Tribsch are working on the synthesis of distribution patterns of endemic taxa of the European Alps with phylogeographical data. Ovidiu Paun is a PhD student investigating the phylogeny of the genus *Ranunculus* in general and the evolution of apomixis in the *R. auricomus* group in detail. Harald Niklfeld's interests focus on comparative interpretation of distribution patterns of vascular plants in Central Europe.
