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# Rare arctic-alpine plants of the European Alps have different immigration histories: the snow bed species *Minuartia biflora* and *Ranunculus pygmaeus*

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**Abstract**

***Minuartia biflora* and *Ranunculus pygmaeus* are circumarctic plants with a few isolated occurrences in the European Alps. We analysed amplified fragment length polymorphism (AFLP) and chloroplast DNA sequence data to unravel the history of their immigration into the Alps and to provide data on their circumpolar phylogeography. In spite of the similar ecological requirements of the two species, they exhibit strikingly different immigration histories into the Alps. In *M. biflora*, the Alpine populations are most probably derived from source populations located between the Alpine and Scandinavian ice sheets, in accordance with the traditional biogeographic hypothesis. In contrast, the Alpine populations of *R. pygmaeus* cluster with those from the Tatra Mountains and the Taymyr region in northern Siberia, indicating that the distant Taymyr area served as source for the Alpine populations. Both species showed different levels of genetic diversity in formerly glaciated areas. In contrast to the considerable AFLP diversity observed in *M. biflora*, *R. pygmaeus* was virtually nonvariable over vast areas, with a single phenotype dominating all over the Alps and another, distantly related one dominating the North Atlantic area from Greenland over Svalbard to Scandinavia. The same pattern was observed in chloroplast DNA sequence data. Thus, postglacial colonization of *R. pygmaeus* was accompanied by extreme founder events.**

*Keywords*: arctic-alpine, comparative phylogeography, European Alps, founder event, plant migrations, Quaternary glaciations

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## Introduction

A considerable number of plant species of predominantly arctic distribution grow in nonarctic mountain ranges as well (e.g. Hultén & Fries 1986). This distribution type is referred to as arctic-alpine. There are large differences between taxa, however, as regards the extent of their distribution areas outside the Arctic. Whereas some arctic-alpine species are common in many mountain ranges of the Northern Hemisphere, others are very rare, and in some cases only a few populations are known outside the Arctic and the directly connected mountain ranges (Hultén & Fries 1986).

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The European Alps, although separated from the Arctic and the adjacent Scandinavian mountains by a broad belt of forested lowlands, harbour a high number of arctic-alpine plant species of which many are rare in the Alps (*c.* 90 rare taxa; Noack 1922), more than any other central or southern European mountain range. Whereas the distribution patterns of most of the endemic plant species of the Alps are highly congruent with unglaciated or weakly glaciated presumed Pleistocene refugia at the southern and eastern margin of the Alps (Merxmüller 1952, 1953, 1954; Pawlowski 1970; Tribsch & Schönswetter 2003), most of the rare arctic-alpine plant species are confined to the central-most and highest parts of the Alps (Noack 1922; Welten & Sutter 1982), areas that were strongly glaciated during the Pleistocene.

This restriction of the rare arctic-alpine plants to the centres of the Pleistocene glaciations in the Alps was recognized

fairly early and gave rise to a controversy about the location of their glacial refugia. Survival on ice-free mountaintops in the central parts of the Alps was suggested (Brockmann-Jerosch & Brockmann-Jerosch 1926); alternatively, the species could have immigrated into the Alps from refugia in the lowlands north of the Alps (Noack 1922). In both scenarios, however, a close relationship between Alpine and Scandinavian populations was assumed because of the geographic proximity of the two areas. Recent molecular studies have challenged such views by revealing that long-distance dispersal of arctic-alpine plant species has been much more important for postglacial recolonization of the North Atlantic areas than traditionally believed (Abbott & Brochmann 2003; Brochmann *et al*. 2003). Thus, the possibility of immigration into the Alps from more remote arctic areas that remained unglaciated during the Pleistocene, such as northern Siberia and Beringia, should not be neglected.

Only a few studies have, partly very peripherally, addressed the genetic background of the arctic-alpine disjunctions in Europe. In *Ranunculus glacialis*, which is common in the Alps, postglacial immigration into Scandinavia and Iceland from source populations in the Eastern Alps was invoked based on amplified fragment length polymorphism (AFLP) analysis (Schönswetter *et al*. 2003). This scenario was suggested because of the strong genetic depauperation of the arctic populations relative to the Alpine ones, and because the arctic populations nested within the Eastern Alpine ones and only contained a subset of their AFLP fragments. In contrast, for *Saxifraga cernua*, one of the rare arctic-alpine species in the Alps, immigration from Scandinavia into the Alps was suggested based on RAPD (random amplified polymorphic DNA) analysis, but that study included only two Scandinavian samples (Bauert *et al*. 1998). Based on AFLP evidence, Després *et al*. (2002) invoked colonization of Fennoscandia from Carpathian source populations of *Trollius europaeus*, whereas a Western Alpine population was sister to a population in Germany. The Alpine populations did not show close relationships to the Fennoscandian ones. In Alpine populations of *Saxifraga oppositifolia*, Abbott *et al*. (2000) detected only two chloroplast DNA (cpDNA) haplotypes, which also were widespread in the Arctic. In a study focusing on the Alpine populations of the same species, Holderegger *et al*. (2002) detected two additional, rare cpDNA haplotypes in the Alps. One of these was previously known only from the Taymyr area in northern Siberia, and the other, detected in a single individual, probably represents a local, postglacial length mutant of a widespread Eurasian haplotype. In *Vaccinium uliginosum sensu lato*, two of the three cpDNA lineages identified occur in the European Alps and the Pyrenees (Alsos *et al*. 2005). The 'Boreal Amphi-Atlantic Lineage' of *V. uliginosum* (corresponding to ssp. *uliginosum*) is represented by the common Eurasian haplotype, whereas the 'Arctic-Alpine Lineage' (corresponding to ssp. *microphyllum sensu lato*) is represented by three local, closely related haplotypes, suggesting isolation in this region (i.e. the Alps and the Pyrenees) at least since before the last glaciation.

This study focuses mainly on identification of the source areas for the immigration of rare arctic-alpine plants into the Alps and tests the classical hypothesis of a close relationship between Alpine and Scandinavian populations. We selected two arctic-alpine plant species with similar habitat preferences, *Minuartia biflora* and *Ranunculus pygmaeus*. Both have wide circumpolar distributions in the Arctic and are occasionally found in more southern mountain ranges such as the Alps, the Central Asian mountains and the Rocky Mountains. In middle and southern Europe, *M. biflora* is restricted to the middle and Eastern Alps (Switzerland, Italy, Austria), whereas *R. pygmaeus* occurs in the Eastern Alps (Switzerland, Italy, Austria) and the High Tatra Mountains (Slovakia, Poland). In contrast to the Arctic, where both species are common and nearly ubiquitously distributed over large areas, they are extremely rare in the Alps. Although they occur in a large portion of that mountain range, they cannot be regularly found in any area. The Alpine populations are typically highly disjunct, cover less than a few square metres, and usually comprise less than 100 individuals (personal observation; Table 1).

## Materials and methods

### *The study species*

*Minuartia biflora* (L.) Schinz and Thellung (Caryophyllaceae) and *Ranunculus pygmaeus* Wahlenb. (Ranunculaceae) are diploid ($2n = 26$ and $2n = 16$, respectively; Löve & Löve 1975), perennial herbs lacking any means of clonal propagation. Allozyme evidence suggests that *M. biflora* exhibits a mixed mating system of selfing and outcrossing (Borgen 1998), whereas pollinator-exclusion experiments suggest that *R. pygmaeus* is strongly autogamous (Tikhmenev 1985). Both species are typically restricted to habitats with short growing seasons due to extended snow cover. *Ranunculus pygmaeus* is restricted to acidic bedrock, and in the Alps it typically occurs close to permanent snow-fields providing continuous water supply. *Minuartia biflora* has a broader amplitude. It grows on acidic as well as base-rich bedrock, and although it usually occurs in snow beds, it can be found on ridges as well given that there is permanent snow cover in winter (P. Schönswetter, personal observation).

### *Sampling*

Twenty-two populations of *M. biflora* and 33 populations of *R. pygmaeus* were sampled in 2003 and 2004 covering the entire ranges of the species, but focusing on the Alps and Scandinavia (Table 1). As both species grow in patchy

**Table 1** Geographic origin, size, and average gene diversity over loci in the investigated populations of *Minuartia biflora* (M1 to M22) and *Ranunculus pygmaeus* (R1 to R33)

| | Country | Sampling locality | Long/Lat | *n* | Pop. size | Average gene diversity ± SD |
|---|---|---|---|---|---|---|
| M1 | Switzerland | Fuorcla Valetta | 9.83°/46.50° | 10 | 2 | 0.0012 ± 0.0018 |
| M2 | Italy | Cima di Pozzin | 10.20°/46.54° | 10 | 1 | 0.0062 ± 0.0051 |
| M3 | Switzerland | Ils Chalchogns | 10.28°/46.90° | 10 | 3 | 0.0000 ± 0.0000 |
| M4 | Austria | Oberlahmspitze | 10.49°/47.21° | 10 | 2 | 0.0088 ± 0.0065 |
| M5 | Italy | Pfelderer Tal | 11.04°/46.76° | 9 | 1 | 0.0048 ± 0.0042 |
| M6 | Austria | Glungezer | 11.53°/47.21° | 8 | 2 | 0.0113 ± 0.0081 |
| M7 | Austria | Grünbergspitze | 11.55°/47.18° | 10 | 2 | 0.0105 ± 0.0075 |
| M8 | Italy | Lenkstein | 12.16°/46.94° | 10 | 2 | 0.0121 ± 0.0083 |
| M9 | Austria | Schönleitenspitze | 12.69°/46.99° | 10 | 2 | 0.0027 ± 0.0029 |
| M10 | Norway | Torfinnsdalen | 8.58°/61.38° | 10 | — | 0.0217 ± 0.0135 |
| M11 | Norway | Røros | 11.82°/62.56° | 10 | — | 0.0053 ± 0.0045 |
| M12 | Norway | Leirtjønnkollen | 9.75°/62.44° | 5 | — | 0.0187 ± 0.0135 |
| M13 | Norway | Lávkaslubbu | 20.43°/69.23° | 10 | — | 0.0263 ± 0.0160 |
| M14 | Norway, Svalbard | Dickson Land | 16.25°/78.66° | 10 | — | 0.0114 ± 0.0080 |
| M15 | Russia | Polar Urals: Chernaya Mountains | 65.45°/66.84° | 9 | — | 0.0088 ± 0.0066 |
| M16 | Russia | Taymyr: Lake Taymyr | 99.73°/74.45° | 8 | — | 0.0000 ± 0.0000 |
| M17 | Russia | Taymyr: Khatanga | 102.57°/71.97° | 9 | — | 0.0078 ± 0.0060 |
| M18 | Russia | Taymyr: Ary-Mas | 101.84°/72.40° | 10 | — | 0.0023 ± 0.0027 |
| M19 | Russia | Altai: valley of Zhumaly | 88.04°/49.42° | 2 | — | 0.1053 ± 0.1081 |
| M20 | Canada | Richardson Mountains, Wright Pass | –136.25°/67.05° | 5 | — | 0.0023 ± 0.0030 |
| M21 | Canada | Newfoundland: White Hills | –55.67°/51.37° | 10 | — | 0.0000 ± 0.0000 |
| M22 | Greenland | Ilulissat | –51.96°/70.01° | 9 | — | 0.0149 ± 0.0100 |
| R1 | Italy | Pfelderer Tal | 11.03°/46.76° | 5 | 2 | 0.0027 ± 0.0034* |
| R2 | Austria | Kirchenkogel | 11.07°/46.90° | 5 | 2 | 0.0000 ± 0.0000* |
| R3 | Austria | Grünbergspitze | 11.55°/47.18° | 5 | 2 | 0.0000 ± 0.0000 |
| R4 | Italy | Geltal | 12.07°/46.90° | 5 | 2 | 0.0000 ± 0.0000* |
| R5 | Austria | Umbaltal | 12.22°/47.04° | 5 | 3 | 0.0000 ± 0.0000* |
| R6 | Austria | Felber Tauern | 12.50°/47.16° | 5 | 1 | 0.0000 ± 0.0000* |
| R7 | Austria | Hochegg | 12.51°/46.86° | 5 | 3 | 0.0038 ± 0.0038 |
| R8 | Austria | Ebeneck | 13.11°/47.03° | 5 | 2 | 0.0000 ± 0.0000* |
| R9 | Austria | Altenbergscharte | 13.43°/47.08° | 5 | 3 | 0.0000 ± 0.0000* |
| R10 | Slovakia | Mlynicka Dolina | 20.03°/49.17° | 8 | — | 0.0012 ± 0.0017 |
| R11 | Slovakia | Mala Studena Dolina | 20.20°/49.18° | 8 | — | 0.0000 ± 0.0000 |
| R12 | Norway | Ulvik | 7.55°/60.60° | 10 | — | 0.0027 ± 0.0030* |
| R13 | Norway | Hurrungane | 7.75°/61.48° | 10 | — | 0.0000 ± 0.0000* |
| R14 | Norway | Torfinnsdalen | 8.58°/61.38° | 10 | — | 0.0000 ± 0.0000* |
| R15 | Norway | Leirtjønnkollen | 9.75°/62.43° | 10 | — | 0.0013 ± 0.0020* |
| R16 | Norway | Røros | 11.44°/62.75° | 10 | — | 0.0000 ± 0.0000* |
| R17 | Sweden | Latnjachorru | 18.50°/68.33° | 10 | — | 0.0013 ± 0.0020* |
| R18 | Sweden | Gearggevaggi | 18.50°/68.33° | 5 | — | 0.0000 ± 0.0000 |
| R19 | Sweden | Njulla | 18.70°/68.37° | 10 | — | 0.0000 ± 0.0000* |
| R20 | Norway | Lávkaslubbu | 20.43°/69.23° | 10 | — | 0.0061 ± 0.0052* |
| R21 | Norway, Svalbard | Wedel Jarlsberg Land | 15.53°/77.01° | 5 | — | 0.0000 ± 0.0000* |
| R22 | Norway, Svalbard | Dickson Land | 16.00°/78.51° | 4 | — | 0.0000 ± 0.0000* |
| R23 | Norway, Svalbard | Haakon VII Land | 11.98°/78.99° | 10 | — | 0.0000 ± 0.0000* |
| R24 | Russia | Polar Urals: Chernaya Mountains | 65.45°/66.84° | 9 | — | 0.0075 ± 0.0056 |
| R25 | Russia | Polar Urals: Slantzevaga | 65.80°/66.92° | 9 | — | 0.0280 ± 0.0168 |
| R26 | Russia | Taymyr: Lake Taymyr | 99.73°/74.45° | 9 | — | 0.0462 ± 0.0266 |
| R27 | Russia | Taymyr: Ary-Mas | 101.84°/72.40° | 6 | — | 0.0446 ± 0.0276 |
| R28 | Russia | Taymyr: Ary-Mas | 101.85°/72.44° | 10 | — | 0.0254 ± 0.0152 |
| R29 | Canada | Richardson Mountains, Wright Pass | –136.25°/67.05° | 10 | — | 0.0375 ± 0.0246 |
| R30 | Canada | High Tuya | –130.52°/59.25° | 2 | — | 0.0529 ± 0.0552 |
| R31 | Greenland | Siorapaluk | –70.70°/77.78° | 5 | — | 0.0000 ± 0.0000* |
| R32 | Greenland | Saqqaq | –51.96°/70.01° | 9 | — | 0.0000 ± 0.0000* |
| R33 | Greenland | Cape Dalton | –24.10°/69.48° | 5 | — | 0.0000 ± 0.0000* |

*n*, number of individuals investigated with AFLP. Population size was estimated in three size classes (1, < 25 individuals; 2, 25–100 individuals; 3, 100 individuals). Populations of *R. pygmaeus* analysed with only three of the four primer combinations are marked with an asterisk.

habitats, the circumscription of populations was most often straightforward. Individuals were sampled randomly at distances that generally depended upon the spatial extent of the populations and ranged from few decimetres in very small Alpine populations to few metres in large populations. If possible, leaf material of 10 plants per population was collected. For the Alpine populations, population size was estimated using three size classes (< 25 individuals, 25–100 individuals, > 100 individuals; Table 1). Voucher specimens are deposited at the Institute of Botany, University of Vienna (WU), or in the Botanical Museum, University of Oslo (O).

### *DNA extraction, AFLP fingerprinting, and DNA sequencing*

Total genomic DNA was extracted from silica gel-dried leaf material (except that only air-dried herbarium specimens collected in 2003 were available of *M. biflora* from the Altai Mountains) following the 2 × cetyltrimethyl ammonium bromide (CTAB) method (Doyle & Doyle 1987) with minor modifications (Schönswetter *et al*. 2002). The AFLP procedure followed Gaudeul *et al*. (2000), but reaction volumes in the polymerase chain reactions (PCR) were reduced by 50%. The following three selective primer combinations were selected for both species (fluorescent dye in brackets): *Eco*RI AGT (6-FAM)-*Mse*I CAA; *Eco*RI ACC (NED)-*Mse*I CAT; *Eco*RI AAG (VIC)-*Mse*I CAA. As the lack of genetic variation in the Alpine populations of *R. pygmaeus* became apparent, only five individuals per population from that region were analysed. Because of the low overall level of AFLP variation we observed in *R. pygmaeu*s, a subset of the individuals was also analysed with a fourth primer combination [*Eco*RI ACT (6-FAM)-*Mse*I CTA]. From the Alps and the North Atlantic region, where virtually no variation was detected in *R. pygmaeus* with the first three primer combinations, we included only a few representative individuals for analysis with the fourth primer combination. For each individual, 2 μL 6-FAM, 2 μL VIC and 3 μL NED labelled selective PCR products were combined with 0.3 μL GeneScan ROX 500 (Applied Biosystems) and 11.7 μL formamide and run on a capillary sequencer ABI 3100 (Applied Biosystems). Blind samples and replicates were routinely included to test for contamination and reproducibility. Raw data were collected and aligned with the internal size standard using the ABI PRISM GENESCAN version 3.7. analysis software (PE Applied Biosystems). The GENESCAN files were imported into GENOGRAPHER (version 1.6, available at http://hordeum.oscs.montana.edu/genographer) for scoring. Fragments in the size range of 60–500 bp were scored. The data were exported as a presence/absence matrix.

Five individuals from both species were screened for DNA sequence variation. Six cpDNA regions and the nuclear rDNA ITS1-5.8S-ITS2 region were amplified and sequenced using the following primers for both PCR and cycle sequencing: the *rps16* intron using rpsF and rpsR2R (Oxelman *et al*. 1997), the *trnD-trnT* region using trnD$^{GUC}$F and trnT$^{GGU}$ (Demesure *et al*. 1995), and trnE and trnY (Shaw *et al*. 2005) as internal sequencing primers, the *trnS$^{UGA}$-trnfM$^{CAU}$* region using trnS$^{UGA}$ and trnfM$^{CAU}$ (Demesure *et al*. 1995), *trnS$^{GCU}$-trnG$^{UUC}$-trnG$^{UUC}$* region using trnS$^{GCU}$ and 3'trnG$^{UUC}$ (Shaw *et al*. 2005), *rpoB-trnC$^{GCA}$* (only *R. pygmaeus*), the *trnT-trnL* region (only *M. biflora*) using trnA2 (Cronn *et al*. 2002) and tabD (Taberlet *et al*. 1991) and tabB as internal sequencing primer (Taberlet *et al*. 1991), and the nuclear ribosomal DNA ITS1-5.8S-ITS2 region using ITS4 and ITS5 (White *et al*. 1990).

Two cpDNA regions that exhibited intraspecfic variability were chosen for each species [the *trnD-trnT* spacer, *trnT-trnL* (only *M. biflora*), and the *trnS-trnfM* (only *R. pygmaeus*)] and sequenced in one individual from every population except the Scandinavian and European Alps populations, which were represented by two populations each. Population R29 (Yukon) was not included in the *R. pygmeus* data set.

PCR conditions were 0.4 U *Taq* (ABgene, Epsom) per 10-μL reaction, buffer supplied with the enzyme, 2.5 mM $Mg^{2+}$, 0.4 μM of each primer, 1 mM of each dNTP (Applied Biosystems), 0.04% BSA, and 3 μL template DNA of unknown concentration. PCR cycling was performed with a GeneAmp 3700 (Applied Biosystems), PTC100 or PTC200 (MJ Research) with the following parameters: initial denaturation for 5 min at 95 °C followed by 35 cycles of 30 s at 95 °C, 1 min at 52–55 °C, and 2 min at 72 °C. A two-step protocol (5 min at 95 °C followed by 35 cycles of 30 s at 95 °C and annealing/extension at 66 °C for 4 min) was used to amplify the *trnS$^{UGA}$-trnG$^{UUCA}$-trnG$^{UUC}$* region using the trnS$^{GCU}$ and 3'trnG$^{UUC}$ primers. Both protocols ended with 10-min extension. Sequencing was performed using BigDye V 1.1 (Applied Biosystems) according to the manufacturer's manual except for using 10-μL reaction volumes, and visualized with an ABI 3100 capillary sequencer (Applied Biosystems).

### *Data analysis*

Average gene diversity over loci (and its standard deviation for both sampling and stochastic processes) was calculated using ARLEQUIN 2.0 (Schneider *et al*. 2000). We performed neighbour-joining analyses of Nei & Li (1979) genetic distance matrices with TREECON 1.3b (Van de Peer & De Wachter 1997). The trees were midpoint rooted. Branch support was estimated with 1000 bootstrap replicates. Equally weighted parsimony analyses were conducted with PAUP 4.0b10 for Windows (Swofford 2002) on data sets including exclusively nonidentical AFLP phenotypes, which, in the case of *R. pygmaeus*, have been obtained from

all four primer combinations. The settings for the heuristic search were random sequence addition with 1000 replicates and not more than 500 trees saved per replicate, MULTREES on, steepest descent option not in effect, TBR (tree-bisection–reconnection) branch swapping and ACCTRAN (accelerated transformation) optimization. Branch support was estimated via nonparametric bootstrap using 10 000 bootstrap replicates with the same heuristic search settings as above with the exception that only 10 random sequence addition replicates were used. Analyses of molecular variance (AMOVAS) were computed with ARLEQUIN 2.0. To explore the origin of the Alpine populations, we performed assignment tests based on the multilocus genetic data following Duchesne & Bernatchez (2002), using their AFLPOP version 1.0 program with the following settings: marker frequencies of zero were replaced by (1/number of sample size + 1); the minimal log likelihood difference to assign an individual was set to 2, i.e. it was only assigned if the allocation to a certain population was 100 times more probable than to another population; and the number of artificial (simulated) genotypes to compute *P* values was set to 500.

The cpDNA sequences were edited using STADEN version 1.5.3 (https://sourceforge.net/projects/staden) and manually aligned using SE-AL version 2.0a11 (Rambaut 1996). Inferred insertions/deletions (indels) were coded using Simple Gap Coding (Simmons & Ochoterena 2000) as implemented in GAPCODER (Young & Healy 2003). Phylogenetic analyses were made using PAUP* (version 4.0b10) for Macintosh (Swofford 2002). Maximum-parsimony analyses of the combined matrices including coded indels were performed using a branch-and-bound-search strategy with MULTREES option on. Maximum-parsimony bootstrap analyses were carried out with full heuristics, 1000 replicates, TBR branch swapping, the MULTREES option off, and random addition of sequences with four replicates. The *M. biflora* tree was rooted with populations 18 and 20, and the *R. pygmaeus* tree was rooted using midpoint rooting.

## Results

### Minuartia biflora

In *Minuartia. biflora*, 171 AFLP fragments were scored, of which 117 (68.4%) were polymorphic. Average gene diversity over AFLP loci varied from zero in populations M3, M16 and M21 to 0.105 in population M19 (Table 1). The average gene diversity over loci was significantly lower in the Alps (0.0064, SD = 0.0045) than in Scandinavia including Svalbard (0.0167, SD = 0.0094; $t = -3.04$, $P = 0.01$). Based on the maximum extent of the ice sheets during the last glaciation given in Brochmann *et al*. (2003), we found no significant difference in the genetic diversity between areas that were glaciated during the last glacial maximum (populations 1–14, 21, 22) and those that were not (populations 15–20; $t = 1.088$, $P = 0.289$).

In the neighbour-joining analysis of AFLP phenotypes in *M. biflora* (Fig. 1), the populations from Taymyr and Yukon formed a well-supported sister group to the remaining populations. The relationships within the latter group were weakly supported, with sequentially branching groups consisting of the populations from Altai, Taymyr, Newfoundland and western Greenland. The monophyly of the Taymyr, Newfoundland, and western Greenland populations, respectively, was moderately to strongly supported. The western Greenland populations were sister to a weakly supported but unresolved group consisting of the populations from the Alps, Scandinavia including Svalbard, and the Urals.

The parsimony analysis (Fig. 1) yielded a similar tree topology as the neighbour-joining analysis with respect to the major branches. The sole exception was population 19 from Altai, which was resolved as paraphyletic to the remainder. Bootstrap values for the major branches are given in Fig. 1.

We detected seven private AFLP fragments in the Alpine populations of *M. biflora*, of which six were rare (found in 1–6 individuals) and one was found in 40 individuals. The Scandinavian populations also had seven private fragments, but all were rare (found in less than five individuals). The population from the Urals shared all markers with the Scandinavian populations.

In separate AMOVAS, 73.3% of the variation was found among the Alpine populations and 45.3% among the Scandinavian (including Svalbard) populations (Table 2). In a nested AMOVA, the variation between the Alps and Scandinavia/Svalbard was 50.2%.

In the assignment test, the 87 Alpine individuals of *M. biflora* were assigned to the Scandinavia/Svalbard group ($P < 0.01$, except for one individual of population M6 that was assigned with $P = 0.014$).

The *M. biflora* matrix consisted of concatenated sequences from the *trnD-trnT* and the *trnS-trnG* regions from 12 individuals. Three substitutions and five coded indels of in total 2360 aligned positions were parsimony informative. A single most parsimonious tree (11 steps) was found (Fig. 2) with consistency index (CI) and retention index (RI) 1. The analyses resolved a sister-group relationship between Taymyr/Yukon and the rest of the populations (86% bootstrap support). Within the latter clade, Taymyr/Altai, western Greenland/Newfoundland, and the European Alps/Svalbard/Scandinavia were supported as monophyletic (87%, 86%, and 65% bootstrap support, respectively) but formed a trichotomy. The sequences are available at EMBL/GenBank with accession nos AM085625–AM085629, AM085642–AM085646, AM085652–AM085675, AM085693–AM085696, AM085702–AM085706.

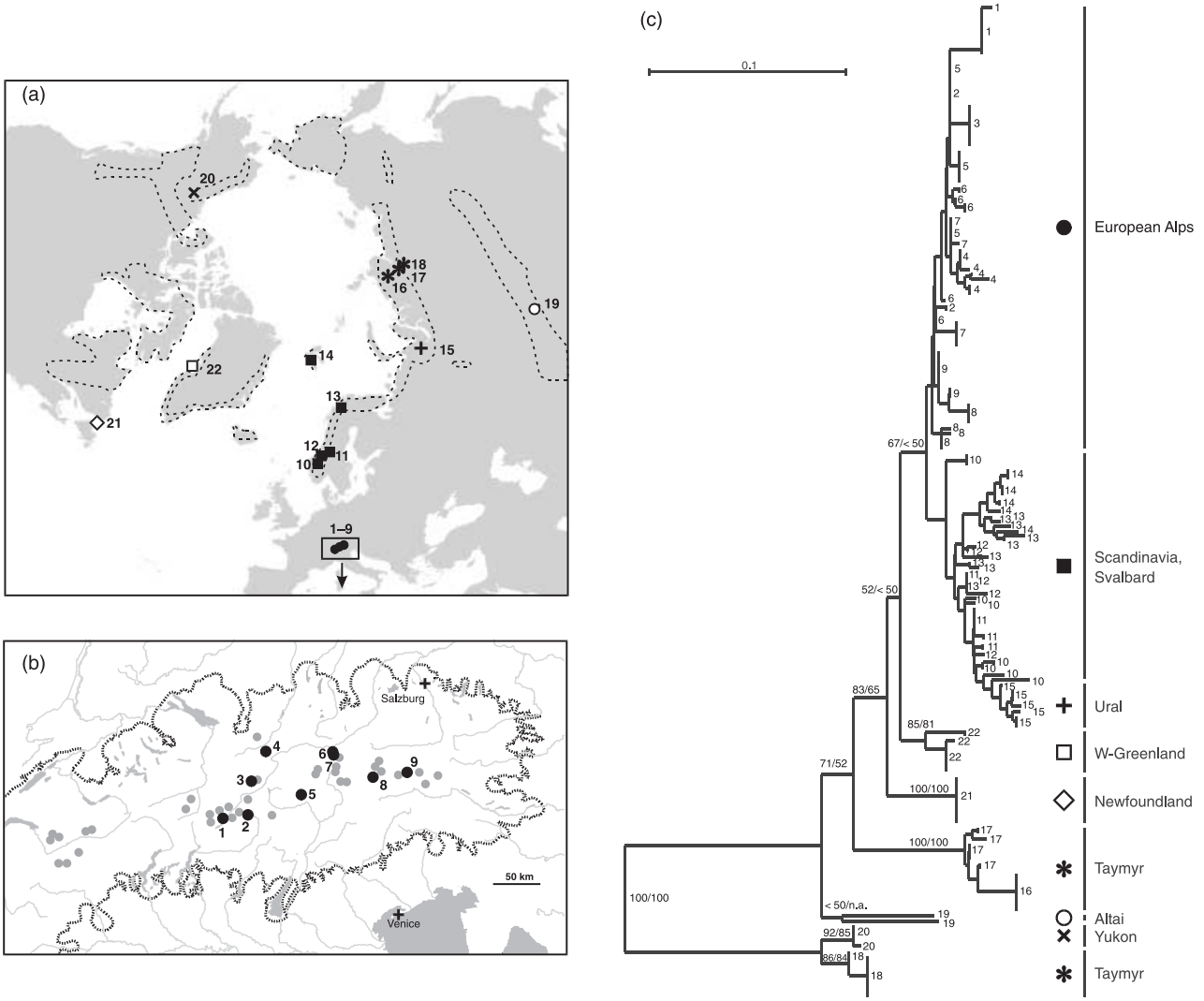


**Fig. 1** Distribution, sampled populations and neighbour-joining tree of AFLP phenotypes in *Minuartia biflora*. (a) Geographic distribution (broken line) and sampled populations labelled with population numbers (cf. Table 1). (b) Distribution of *M. biflora* in the Alps (grey dots) and sampled populations (black dots). The broken line shows the maximum extent of the ice sheet during the last glacial maximum *c.* 18 000 BP. (c) Neighbour-joining tree based on Nei & Li's (1979) genetic distances. Numbers above major branches are neighbour-joining bootstrap values higher than 50% (before slashes). Additionally, maximum-parsimony bootstrap values of major branches are given (after slashes). Numbers at the tips of branches are population numbers. Individuals with identical multilocus phenotypes are labelled only once, if they belong to the same population.

**Table 2** Analyses of molecular variance (AMOVA) for AFLP phenotypes in *Minuartia biflora*

| Source of variation | d.f. | Sum of squares | Variance components | % total variance | $F_{ST}$* |
|---|---|---|---|---|---|
| Among Alpine populations | 8 | 119.67 | 1.49 | 73.27 | 0.73 |
| Within Alpine populations | 78 | 42.45 | 0.54 | 26.73 | |
| Among Scandinavian and Svalbard populations | 4 | 47.04 | 1.17 | 45.33 | 0.45 |
| Within Scandinavian and Svalbard populations | 40 | 56.20 | 1.41 | 54.67 | |
| Among Alps and Scandinavia, Svalbard | 1 | 146.97 | 2.24 | 50.18 | |
| Among populations | 12 | 166.71 | 1.39 | 31.10 | 0.81 |
| Within populations | 118 | 98.65 | 0.84 | 18.72 | |

*All $P$ values were < 0.001.

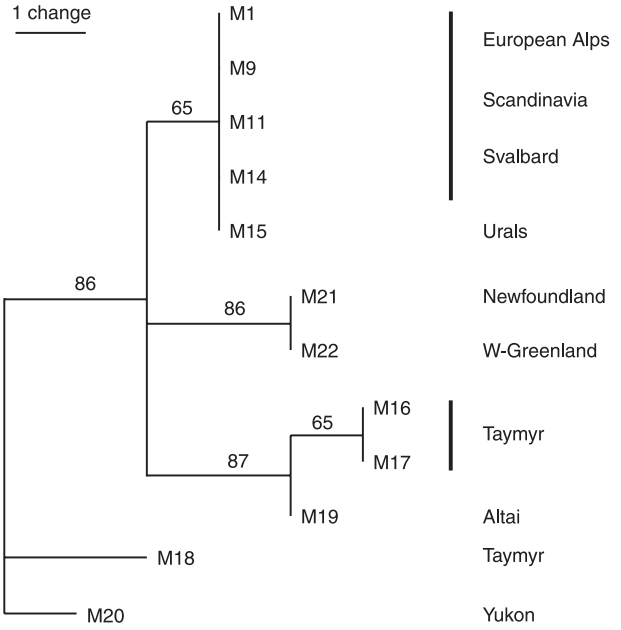


**Fig. 2** The single most parsimonious tree of 11 steps (CI and RI = 1) found in the analysis of the concatenated *trnD-trnT* and the *trnS-trnG* regions from *Minuartia biflora*. Numbers at the tips of branches are population numbers. Numbers associated with nodes are maximum-parsimony bootstrap percentages above 50%. The tree was rooted with populations M18 and M20. Branch lengths are proportional to the number of changes.

### Ranunculus pygmaeus

In *Ranunculus pygmaeus*, 207 fragments were scored, of which 82 (39.6%) were polymorphic. In the Alpine populations (45 investigated individuals), we found only four AFLP phenotypes, whereas five AFLP phenotypes could be differentiated in the North Atlantic region (123 investigated individuals). Average gene diversity over loci ranged from zero in 19 populations to 0.053 in population R30 (Table 1). The average gene diversity over loci was significantly lower in the Alps (0.0007, SD = 0.0015) and the North Atlantic region (0.0008, SD = 0.0018) than in western North America (0.0452, SD = 0.0109) or Siberia (0.0303, SD = 0.0159). In accordance with this result, we found a significant difference in levels of genetic diversity between formerly glaciated (populations 1–23, 31–33; mean 0.0007 ± 0.0014) and nonglaciated (populations 24–30; mean 0.0346 ± 0.0155) areas ($t = 11.438$, $P < 0.001$).

The neighbour-joining analysis of *R. pygmaeus* (Fig. 3) revealed two main groups. One group consisted of the populations from Taymyr, the Tatra Mountains and the European Alps, with the Tatra/Alps populations forming a well-supported subgroup. One of the Tatra populations (R11) was sister to all Alpine populations. The second main group comprised the populations from Scandinavia, Svalbard, Greenland, the Urals and northwestern North America. Within this group, one of the Urals populations (R24) was resolved as sister to the rest, followed by the Yukon population as sister to a clade consisting of the British Columbia population and a group formed by the second Urals population (R25) and the five multilocus genotypes from the North Atlantic region.

Virtually all markers observed in the Alpine populations of *R. pygmaeus* were shared with both the Tatra Mountains and Taymyr; only one marker was exclusively shared with Taymyr, and two markers were exclusively shared with the Tatra mountains.

The assignment test corroborated the pattern revealed by the neighbour-joining analyses (all $P < 0.001$). The four Alpine multilocus phenotypes were assigned to the accessions from the Tatra Mountains. If the latter were removed, all Alpine individuals were assigned to the three populations from Taymyr. The North Atlantic phenotypes were assigned to the Urals (R24, R25). If the two populations from the Urals were separated, the North Atlantic phenotypes were assigned to population R25.

The *R. pygmeaus* matrix consisted of concatenated sequences from the *trnD-trnT* and the *trnS-trnfM* regions from 12 individuals. Five substitutions and one coded indel of in total 2517 aligned positions were parsimony informative. Only two cpDNA haplotypes were found and the analyses resulted in a single most parsimonious trees (6 steps; Fig. 4) with consistency index (CI) and retention index (RI) 1. One group consisted of representatives of the populations from the European Alps, Tatras and Taymyr, whereas the British Columbia, Urals, Scandinavia, Svalbard, and Greenland populations were confined to the other group with a bootstrap support of 100%. The sequences are available at EMBL/GenBank with accession nos AM085630–AM085641, AM085647–AM085651, AM085676–AM085692, AM085697–AM085701, AM085707–AM085711.

## Discussion

### *Immigration into the Alps*

Our two model species *Minuartia biflora* and *Ranunculus pygmaeus* exemplify contrasting immigration pathways into the Alps. Given the weak differentiation among the Alpine populations as compared to the strong phylogeographic signal outside that area, an Alpine origin such as suggested for the arctic-alpine *Ranunculus glacialis* (Schönswetter *et al*. 2003) can be excluded for both taxa.

In *M. biflora*, the populations from the Alps were closely related to those from Scandinavia, Svalbard and the Urals, in agreement with the classical hypothesis. This was not only apparent from the neighbour-joining and maximum-parsimony analyses of AFLP data (Fig. 1) and cpDNA data (Fig. 2) but also from the allocation tests, where nearly all Alpine individuals were assigned with

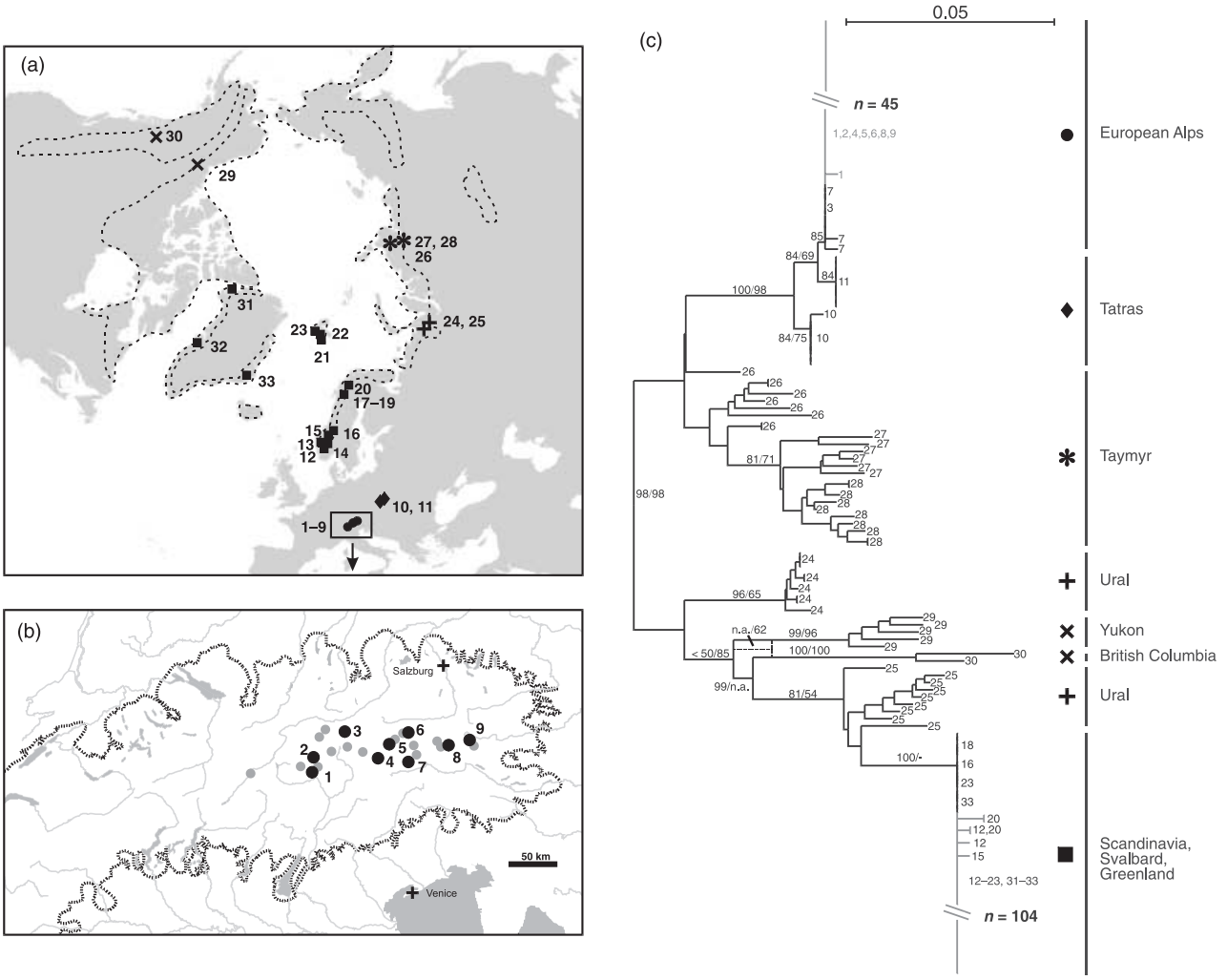


**Fig. 3** Distribution, sampled populations and neighbour-joining tree of AFLP phenotypes in *Ranunculus pygmaeus*. (a) Geographic distribution (broken line) and sampled populations labelled with population number (cf. Table 1). (b) Distribution of *R. pygmaeus* in the Alps (grey dots) and sampled populations (black dots). The broken line shows the maximum extent of the ice sheet during the last glacial maximum *c*. 18 000 BP. (c) Neighbour-joining tree based on Nei & Li's (1979) genetic distances. Numbers above major branches are neighbour-joining bootstrap values higher than 50% (before slashes). Additionally, maximum-parsimony bootstrap values of major branches are given (after slashes). For the clade including the accessions from Scandinavia, Svalbard and Greenland only one individual was analysed (see Materials and methods) and therefore no bootstrap value is available. Numbers at the tips of branches are population numbers. Individuals with identical multilocus phenotypes are labelled only once, if they belong to the same population. Individuals only analysed with three of the four primer combinations are indicated in grey.

high likelihood difference to Scandinavia. The differentiation between the northern European and the Alpine populations appears, however, to be too strong (as evidenced by hierarchical AMOVA; Table 2) to be explained by recent immigration of *M. biflora* into the Alps via long-distance dispersal from Scandinavia. As there is fossil evidence of ecologically similar species such as *Salix herbacea* from the lowlands between the Scandinavian and the Alpine ice sheets (Tralau 1963; Lang 1994), it is probable that *M. biflora* also was present in that area during cold periods of the last glaciation, and that populations in this area served as sources for postglacial colonization of Scandinavia as well as the Alps. When the climate ameliorated, the continuity between northern and southern populations was disrupted. An alternative scenario of northward range expansion from the Alps, as hypothesized for *R. glacialis* (Schönswetter *et al*. 2003), is not supported by our data as we detected significantly lower genetic variation in the Alps than in Scandinavia.

In strong contrast with the close relationship between the Alpine and Scandinavian populations of *M. biflora*, the Alpine populations of *Ranunculus pygmaeus* are only very distantly related to those from Scandinavia, and probably

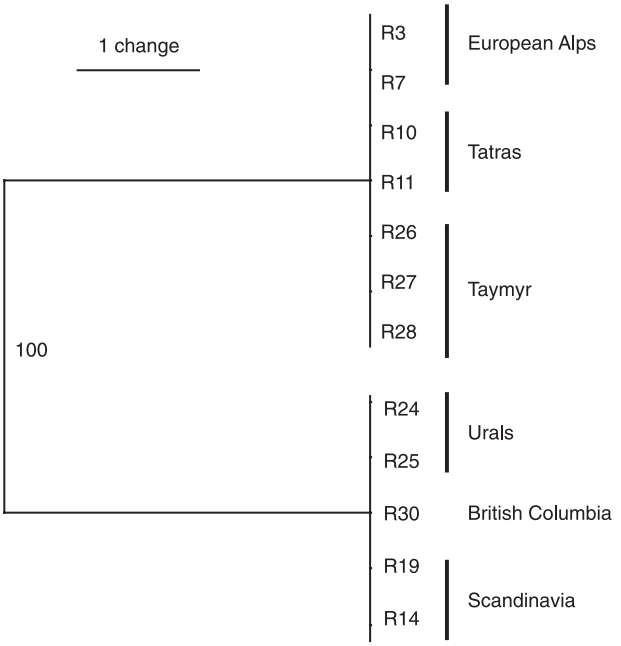


**Fig. 4** The single most parsimonious tree of 6 steps (CI and RI = 1) found in the analysis of the concatenated *trnD-trnT* and the *trnS-trnfM* regions from *Ranunculus pygmaeus*. Only two haplotypes were found, and the tree was midpoint rooted. Numbers at the tips of branches are population numbers. The number associated with the node is the maximum-parsimony bootstrap percentage. Branch length is proportional to the number of changes.

immigrated into the Alps from northern Siberia, most likely via the Tatra. This hypothesis is strongly supported by the following findings. (i) The Alpine and the Tatra populations formed a strongly supported group together with those from Taymyr in the neighbour-joining and maximum-parsimony analyses of the AFLP data (Fig. 3). The fact that the two cpDNA haplotypes found differ in as much as six characters provides very robust support for a much closer relationship between the Alpine and the Tatras/Taymyr populations than with the Scandinavian populations (Fig. 4). (ii) In the assignment tests, the individuals from the Alps were assigned to the populations from the Tatra, or, if the latter were removed, they were assigned to Taymyr. (iii) The two investigated populations from the Tatra differed from the dominant Alpine phenotype in only one AFLP fragment, which was fixed in the Taymyr populations and probably lost by drift in the Tatra. (iv) In contrast to the virtual absence of genetic variation in the Alps, the two Tatra populations analysed were more divergent, fitting a pattern of successively stronger bottlenecks in stepwise colonization events from Taymyr via the Tatra to the Alps. Additionally, immigration from the east is still mirrored by the restriction of *R. pygmaeus* to the eastern part of the Alps. It is noteworthy that Holderegger *et al*. (2002) detected a rare cpDNA haplotype that was previously known only from Taymyr in Alpine populations of *Saxifraga oppositifolia*.

### *Circumpolar phylogeography*

In *M. biflora*, we inferred two very divergent, but taxonomically unrecognized lineages from the AFLP data (Fig. 1). The two lineages can also be inferred from midpoint rooting of the cpDNA tree (not shown). One of these appears to be broadly amphi-Pacific, the other broadly amphi-Atlantic/Eurasian, a pattern frequently encountered in arctic plants (Hultén & Fries 1986). The two lineages of *M. biflora* co-occur within short distance on the Taymyr Peninsula in northern central Siberia. Taymyr has never been identified as a contact region in animals (Hewitt 2004), but in the arctic herb *S. oppositifolia* haplotypes of both the Eurasian and the North American clades were found on Taymyr (Abbott *et al*. 2000). Within the amphi-Atlantic/Eurasian lineage of *M. biflora* the populations from Taymyr were the most divergent ones according to the branch lengths in the AFLP and cpDNA trees (Figs 1 and 2). There was also strong differentiation between populations from different sides of the Atlantic in both data sets (two mutational steps in the cpDNA analysis). This finding stands in contrast to most previous studies, which have not identified the North Atlantic as a strong barrier to gene flow in plants (reviewed in Abbott & Brochmann 2003; but see Aares *et al*. 2000), but we did not include populations from east Greenland in our study. The populations from the Alps, Scandinavia, Svalbard and the Urals formed a strictly European group with no supported internal pattern and had an identical cpDNA haplotype. In line with the results presented here, a previous allozyme study (Borgen 1998) identified a close relationship between populations from Svalbard and Scandinavia and failed to find geographic structuring of genetic variation within Scandinavia.

Although irrelevant for the conclusions of this study, the apparent incongruence between the AFLP and the sequence data deserves attention. The Taymyr/Altai and Newfoundland/western Greenland cpDNA groups are contradicted in the AFLP data but with poor bootstrap support (71/52% and 52/< 50%, respectively; Fig. 1), whereas the cpDNA support is stronger (87% and 86%, respectively, Fig. 2). It should also be noted that the cpDNA phylogeny is completely free from homoplasy (CI = RI = 1), whereas that is not the case with the AFLP tree. Although the bootstrap support for the cpDNA groups can be considered moderate in absolute numbers, the percentages are at their maximum given the number and distribution of parsimony-informative characters.

In *R. pygmaeus*, the neighbour-joining and maximum-parsimony analyses of the AFLP data (Fig. 3) revealed two

main results. In addition to the identification of a sister relationship between the populations from Taymyr and those from the Tatra and the Alps (cf. above), the individuals from the North Atlantic region were nested within a population from the Urals. Thus, whereas the Alps probably were colonized from northern Siberia via the Tatra, the North Atlantic region was probably colonized from source populations in the Urals. As the Urals remained largely unglaciated during the last glaciation, the strong divergence between the two investigated geographically close populations (Fig. 3) may indicate long-term population differentiation in the Urals. We cannot, however, offer a sound explanation for the apparently close relationship between the western North American populations and those from the Urals, as our sampling from North America is too incomplete.

### *Historical and contemporary determinants of genetic diversity*

Genetic diversity is influenced by historical processes (isolation in Pleistocene refugia, postglacial migrations and accompanying bottlenecks; Hewitt 2000), as well as by contemporary factors acting on the populations (e.g. the breeding system, the effective population size and the level of gene exchange with other populations). In the following, we attempt to evaluate the historical component of genetic diversity by comparing formerly glaciated with nonglaciated areas. Contemporary factors are being addressed by contrasting Scandinavian and Alpine populations of *M. biflora*.

*Ranunculus pygmaeus* exhibited significantly lower genetic diversity in areas that were glaciated during the last glaciation than in regions that remained largely unglaciated. More explicitly, the species virtually lacks genetic variation throughout the Alps and the entire North Atlantic region. Among 128 investigated individuals from the latter area, all were identical except for five that lacked a particular AFLP fragment and two in which one fragment was shifted for 1 bp, most probably due to an insertion. To our knowledge, our study is the first to unravel the virtually exclusive existence of a single multilocus AFLP phenotype in such vast areas. This is especially remarkable as *R. pygmaeus* is a common plant in the North Atlantic region. We interpret the observed pronounced reduction of genetic diversity in the Alps and the North Atlantic region as compared to formerly unglaciated areas (e.g. northern Siberia) as the result of extreme bottlenecks during early phases of colonization. As *R. pygmaeus* is strongly autogamous (Tikhmenev 1985), single individuals could have founded strongly depauperated populations at the leading edge (Hewitt 2000) of colonization. Pronounced reduction of genetic diversity presumably caused by a strong bottleneck during postglacial range expansion has previously been identified in *R. glacialis* (Schönswetter *et al*. 2003).

In contrast, in *M. biflora* which exhibits a mixed mating system (Borgen 1998) we did not observe a relationship between levels of genetic diversity and Pleistocene glaciation, suggesting that this species was able to retain much of its variation during the postglacial migrations. This finding is in accordance with some previous studies on arctic or arctic-alpine plant species that have detected high levels of genetic variation in northern Europe (e.g. Gabrielsen *et al*. 1997; Tollefsrud *et al*. 1998).

Comparing contemporary factors acting on the populations is only meaningful in areas that have similar glacial histories, such as the central parts of the Alps and Scandinavia. We anticipated that the typically small population size and the isolation among the Alpine populations of both *M. biflora* and *R. pygmaeus* should have led to differentiation among populations through genetic drift along with low genetic diversity within populations. In contrast, the generally larger and more closely connected Scandinavian populations were expected to be genetically more variable, but less differentiated. The virtual absence of genetic variation in *R. pygmaeus* both in the Alps and in Scandinavia impedes any conclusions in this respect, but in *M. biflora*, the intrapopulational genetic diversity in the Alps is significantly lower than in Scandinavia. Although some AFLP phenotypes were shared between populations (populations M2 and M5; populations M5 and M7; Fig. 1), the differentiation among populations as described by nonhierarchical AMOVA was stronger in the Alps than in Scandinavia (73% vs. 45%; Table 2). Thus, the Alpine populations of *M. biflora* fit the theoretical expectations of genetic drift acting on small and isolated populations fairly well.

## Conclusions

In conclusion, the two investigated taxa *Minuartia biflora* and *Ranunculus pygmaeus*, which have similar geographic distributions as well as similar habitat preferences, exhibit highly incongruent phylogeographic patterns, on the circumpolar scale as well as regarding their history of immigration into the Alps. The source populations for the Alpine populations of *M. biflora* were most probably located somewhere between the Scandinavian and the Alpine ice sheets, whereas northern Siberia is suggested as source area for Alpine *R. pygmaeus*. The pattern in *M. biflora* is in line with the classical hypothesis of colonization of the Alps from refugia south of the Scandinavian ice sheet, whereas the identification of northern Siberia as source area for the Alpine populations of *R. pygmaeus* clearly adds a new dimension to the debate.

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