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SAPONARIA PUMILA (CARYOPHYLLACEAE) AND THE ICE AGE IN THE EUROPEAN ALPS[1]

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The polymerase chain reaction (PCR)-based amplified fragment length polymorphism (AFLP) technique was applied to elucidate the glacial history of the alpine cushion plant *Saponaria pumila* in the European Alps. Special emphasis was given to a dense sampling of populations. Our data support a survival of *S. pumila* during the last ice age in at least three refugia, which are characterized by unique marker sets. Patterns of genetic diversity and divergence can be explained by survival in peripheral refugia and additional in situ survival within the ice sheet on peripheral nunataks. A nunatak survival in interior parts of the Alps needs not be postulated to explain our results. The level of genetic diversity is dramatically different between populations (Shannon's diversity index: 0.87–19.86). Some peripheral populations are characterized by a high number of rare fragments indicating long isolation, but not necessarily by a high level of genetic diversity. Parts of the present distributional area were recolonized via recent long-distance dispersal, leading to severely bottlenecked populations lacking private or rare fragments. The combination of our data with palaeogeological and palaeoclimatological evidence allows us to confine Pleistocene refugia to certain regions and to draw a detailed scenario of the glacial and postglacial history of *S. pumila*.

Key words: alpine plants; amplified fragment length polymorphism (AFLP); Caryophyllaceae; ice age refugia; long-distance dispersal; phylogeography; Pleistocene; *Saponaria pumila*.

Climatic changes during the Pleistocene ice ages had a dramatic influence on biota (Dynesius and Jansson, 2000) causing separation, migration, and extinction of populations (Bennett, 1997; Taberlet et al., 1998) as well as accelerating evolution (Comes and Kadereit, 1998; Hewitt, 2000). The European Alps offer an excellent model to elucidate such processes. Taxa that did not expand their ranges after deglaciation are often confined to peripheral, formerly unglaciated, areas of the Alps resulting in hotspots of endemism (Pawlowski, 1970; Harold and Mooi, 1994; Ozenda, 1995). If taxa survived in more than one refugium, the same lack of range expansion led to infraspecific disjunctions (Merxmüller, 1952, 1953, 1954). Nunataks within the ice sheet may also have supported populations during the glaciations, but this assertion is still controversial (Nordal, 1987; Brochmann et al., 1996; Stehlik, 2000; Stehlik, Tribsch, and Schönswetter, 2001).

The climatic situation during the coldest stages of the ice ages was very harsh in the Alps (Lister et al., 1998). Large areas were covered by a continuous ice sheet, with only nunataks protruding from the glaciers. Outside the ice sheet, the temperature decrease was also dramatic. Whereas north of the Alps discontinuous permafrost with tundra-like vegetation predominated (Huijzer and Vandenberghe, 1998), south of the Alps, under less severe climatic conditions, even oak forest survived in sheltered places (Paganelli, 1996). In Central Europe mean annual temperatures are estimated to have been around 13–17°C lower than at present (Frenzel, 1992a, b). As a consequence, the snowline decreased approximately 1200 m in the Eastern Alps (Nagl, 1972). A shift of the vegetation belts of the same extent can be expected. During the Holocene there were also stages slightly warmer (~2°C) than at present (Prentice et al., 1998), moving the timberline and thus vegetation belts 100–300 m higher (Bortenschlager, 1991; Burga, 1993; Lang, 1993).

The dramatic climate and habitat changes during the ice ages have caused diverging evolutionary lineages within species (Hewitt, 2000). Various molecular methods offer the possibility to track these diverging tendencies (Schaal et al., 1998; Newton et al., 1999). Genetic structure, as revealed in phylogeographic studies in plants in recent years (reviewed in, e.g., Comes and Kadereit, 1998; Schaal et al., 1998), can be very different, ranging from high geographic variance within small areas (e.g., Travis, Maschinski, and Keim, 1996; Bauert et al., 1998; Stehlik, Schneller, and Bachmann, 2001) to weak structural patterns in large areas due to high levels of (long-distance) migration and gene flow (e.g., Gabrielsen et al., 1997; Hagen, Giese, and Brochmann, 2001).

We selected *Saponaria pumila* (Caryophyllaceae) as a system of choice to track the impact of the ice ages in Europe on the fate of an alpine plant species. It is taxonomically isolated and occurs disjunctly in the Eastern Alps (Austria, Italy) and in the southern parts of the Eastern Carpathians in Romania (Simmler, 1910; Meusel and Mühlberg, 1979). *Saponaria pumila* is a perennial herb forming dense, large cushions only a few centimeters high. Flowers are large and pink with a balloon-like synsepalous calyx. As typical for Caryophyllaceae, they are proterandrous. Seeds are quite large (1.0–1.5 mm diameter) and have no adaptations for dispersal over long distances. After seed ripening, the capsules open only under dry conditions and sometimes the seeds remain in the capsule for several months (A. Tribsch, personal observation). It is likely that the balloon-like calyx containing capsule and seeds

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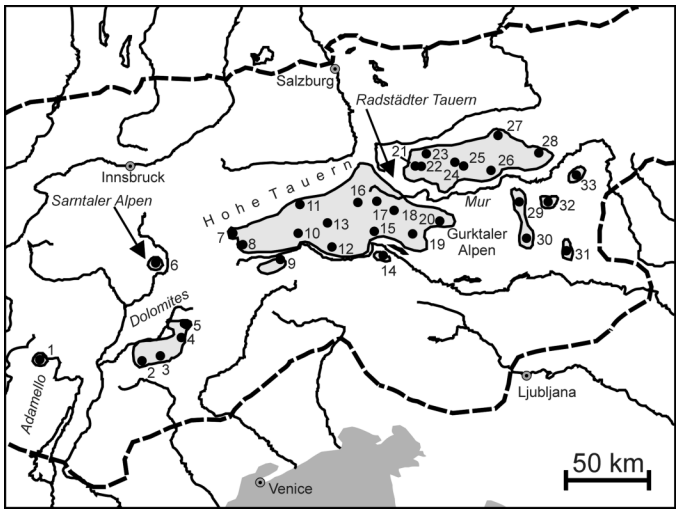

Fig. 1. Distribution of *Saponaria pumila* in the Eastern Alps (shaded), sampled populations (dots; numbers refer to populations in Table 1), and border of the Alps (dashed line).

functions as a diaspore with good dispersal abilities, especially during periods of continuous snow cover (late autumn, winter), but that has not been tested experimentally.

Within the Alps, the distribution of *Saponaria pumila* shows strong affinities to presumptive refugial areas. The species' range includes formerly unglaciated easternmost parts of the central Eastern Alps (Fig. 1), where it is very abundant, and extends to adjacent, formerly glaciated, ranges. *Saponaria pumila* is restricted to acidic, siliceous bedrock and exposed, open, but stable habitats within alpine grassland communities. Disjunct populations are found in the Sarntaler Alpen, in siliceous parts of the Southern Alps (southern Dolomites: Lagorai, Cima d'Asta), and in Adamello (Pedrotti, 1988). The altitudinal distribution ranges from 1800 to 2800 m.

The main focus of this study is the localization of glacial refugia of *Saponaria pumila* during the last ice age as detailed as possible. We want to assess whether *S. pumila* survived in only one eastern refugium or also in other regions and whether the presently isolated easternmost and westernmost populations are products of long-term isolation or of recent long-distance dispersal. Another important aspect is the source of recolonization of the once-glaciated parts of the Alps and whether it is mirrored by genetic depauperation of the newly established populations or if bottlenecks caused genetic depauperation in refugial populations. A dense sampling of populations covering the entire distributional area of this species in the Alps combined with the application of highly polymorphic amplified fragment length polymorphism (AFLP) fingerprinting (Vos et al., 1995) should give sufficient resolution. Amplified fragment length polymorphisms have proven reliable and efficient for obtaining a high number of molecular markers from many individuals (Mueller and Wolfenbarger, 1999).

MATERIALS AND METHODS

Sampling—Thirty-three populations (Table 1, Fig. 1) throughout the Alpic distributional range of *Saponaria pumila* were sampled during the summers of 1999 and 2000. Special emphasis was placed on a dense sampling of populations, whereby five individuals per population were usually collected. Leaf material (flowers and buds were removed) from young shoots was dried in small plastic bags with silica gel. Herbarium specimens of all sampled populations are deposited in the herbarium of the Institute of Botany of the University of Vienna (WU). The maximum distance between two populations is ca. 350 km (Adamello, population 1; Gleinalpe, population 33).

DNA isolation and AFLP fingerprinting—Total genomic DNA was extracted following the cetyl-trimethylammonium bromide (CTAB) protocol (Doyle and Doyle, 1987) with minor modifications. The quality of the extracted DNA was checked on 1% TAE-agarose gels. The amount of DNA was estimated photometrically (UV-160A, Shimadzu, Tokyo, Japan) at 260 nm. Genomic DNA (~500 ng) was digested with Mse I (New England BioLabs, Beverly, Massachusetts, USA) and EcoR I (Promega, Madison, Wisconsin, USA) and ligated (T4 DNA-Ligase; Promega, Madison, Wisconsin, USA) to double-stranded adapters and preamplified using the AFLP ligation and preselective amplification module for regular genomes following the manufacturer's instructions (PE Applied Biosystems, 1996). The incubation of the restriction-ligation reactions (2 h at 37°C) as well as the polymerase chain reactions (PCRs) were performed on a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, California, USA). Deviating from the manufacturer's instructions, the PCRs were run in a reaction volume of 5 μL. An initial screening using nine selective primer combinations was performed on three individuals from two populations using the Selective Amplification Start-up Module for Regular Genomes (PE Applied Biosystems, Foster City, California, USA). Three primer combinations that gave clear and reproducible bands and showed variation within and between populations were chosen for further analysis (Table 2). On several samples independent AFLP reactions were performed for internal control. The fluorescence-labeled selective amplification products were separated on a 5% polyacrylamide gel together with an internal size standard (GeneScan-500 [ROX], PE Applied Biosystems) on an automated sequencer (ABI 377). 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 1.1.0, J. Benham, Montana State University, 1998; available at http://hordeum.msu.montana.edu/genographer/) for scoring of the fragments. Each AFLP fragment was scored using the ''thumbnail'' option of the program that allows comparison of fragments per locus over all samples. Amplified fragment length polymorphism loci that exhibited ambiguous peaks were excluded from the analysis. When unambiguous scoring was possible, peaks of low intensity were also included in the analysis. Results of the scoring were exported as a presence/absence matrix and used for further manipulation.

Data analysis—The geographic distribution of all fragments was evaluated using a Geographic Information System (ArcView GIS 3.1 for Windows, ESRI). The same program was used to evaluate the various statistical parameters on a geographic background. Shannon's diversity index, $H_{Sh} = -\Sigma(p_i \ln[p_i])$, where p_i is the relative frequency of the ith fragment (Legendre and Legendre, 1998), and the percentage of AFLP fragments that are polymorphic within each population ($\%P_{pop}$) were calculated for all populations. Two additional diversity markers, the number of rare (f_r) and private (unique) fragments (f_u) were estimated per population. Fragments were treated as ''rare'' when they occurred in less than 20 of the 158 investigated individuals. Private fragments were confined to a single population. An unweighted pair group method with arithmetic mean (UPGMA) analysis based on Nei's distance measure (Nei, 1972) was calculated for populations to search for groupings with Popgene (Yeh and Boyle, 1997). A neighbor-joining tree of all individuals based on the distance measure by Nei and Li (1979) was constructed with TreeCon 1.3b (Van de Peer and De Wachter, 1994) using the bootstrapping option with 1000 replicates. A principal coordinates analysis (PCoA) based on a matrix of inter-individual Jaccard similarities ($C_J = a/a + b + c$, where a is the number of fragments shared by two compared individuals and b and c are the numbers of fragments present in only one of them) was calculated and plotted with SPSS 8.0 (Norusis, 1992). This analysis was applied to the total data set and to subsets. Mantel permutation tests were applied in two different ways (Stehlik, Schneller, and Bachmann, 2001). The matrix of genetic inter-individual Jaccard distances ($1 - C_J$) was compared with a matrix of geographic distances in kilometers. As a second test to assess re-

TABLE 1. Numbering of populations, location name, coordinates, number of investigated individuals (*N*), and affiliation to genetically defined groups of populations (E = East, CE = Center-East, CW = Center-West, D = Dolomites) of the 33 investigated populations of *Saponaria pumila*. Shannon's diversity index (H_{Sh}), percentage of polymorphic loci ($\%P_{pop}$), number of unique fragments (f_u), and of rare fragments (f_r) are given.

Population	Location	Coordinates	*N*	Group	H_{Sh}	$\%P_{pop}$	f_u	f_r
1	Folgorida-Adamello	10°36′45″E 46°10′10″N	5	D	1.23	3.6	0	0
2	Ziolera	11°27′00″E 46°10′00″N	5	D	7.76	22.5	2	6
3	Cima d'Asta	11°36′00″E 46°11′00″N	5	D	9.34	26.9	0	4
4	Cavalazza	11°47′20″E 46°17′45″N	5	D	8.40	21.8	0	9
5	Col Margherita	11°48′00″E 46°22′45″N	5	D	3.98	10.8	0	5
6	Schrotthorn	11°33′05″E 46°44′10″N	5	CW	1.05	3.5	0	5
7	Staller Sattel	12°12′30″E 46°53′30″N	5	CW	0.87	2.6	0	0
8	Kalksteinjöchl	12°17′20″E 46°49′20″N	5	CW	1.88	5.1	0	1
9	Golzentipp	12°36′20″E 46°44′00″N	5	CW	3.94	11.6	0	0
10	Schleinitz	12°45′00″E 46°53′45″N	5	CE	11.87	29.1	0	18
11	Leiterkögel	12°46′00″E 47°03′20″N	3	CE	3.36	9.6	0	10
12	Sadnig	12°59′30″E 46°56′30″N	5	CE	11.08	29.4	2	9
13	Scharnik	13°02′30″E 46°48′00″N	5	CE	11.97	29.1	0	15
14	Goldeck	13°27′30″E 46°45′30″N	5	CE	10.93	27.2	2	15
15	Hocheck	13°24′00″E 46°53′00″N	5	CE	17.18	36.2	1	21
16	Ankogel	13°15′00″E 47°03′00″N	5	CE	12.45	30.4	1	9
17	Hafner	13°24′00″E 47°04′15″N	5	CE	11.55	27.9	0	15
18	Wandspitze	13°32′00″E 47°01′00″N	5	CE	11.41	28.6	0	9
19	Rosennock	13°43′00″E 46°52′35″N	4	CE	12.51	29.3	0	12
20	Eisenhut	13°56′00″E 46°57′15″N	4	CE	13.03	28.5	0	8
21	Samspitze	13°43′50″E 47°16′40″N	3	E	11.28	25.5	0	9
22	Hochgolling	13°45′20″E 47°16′15″N	5	E	17.06	39.7	0	17
23	Hochwildstelle	13°49′45″E 47°20′10″N	5	E	17.72	40.0	1	18
24	Deneck	14°03′20″E 47°17′00″N	5	E	17.38	38.0	2	17
25	Rettlkirchspitze	14°08′00″E 47°15′45″N	5	E	19.86	40.6	0	29
26	Schießeck	14°20′00″E 47°14′15″N	5	E	16.84	37.8	0	21
27	Bösenstein	14°25′00″E 47°26′30″N	5	E	15.39	35.9	2	17
28	Seckauer Zinken	14°44′45″E 47°20′35″N	4	E	16.54	36.1	2	16
29	Zirbitzkogel	14°34′00″E 47°04′00″N	5	E	15.41	36.3	4	16
30	Saualpe	14°39′00″E 46°51′00″N	5	E	11.02	28.8	1	10
31	Koralpe	14°58′45″E 46°47′30″N	5	E	8.69	21.4	1	20
32	Ameringkogel	14°49′00″E 47°04′00″N	5	E	15.81	36.1	2	18
33	Gleinalpe	15°03′00″E 47°13′30″N	5	E	13.04	30.5	2	36

gion-wise spatial genetic relationships, the genetic distance matrix was tested against a model matrix where all pairwise comparisons between the population groups where coded. All Mantel R_M values were calculated and Bonferroni-corrected using the R-Package 4.0 (Casgrain and Legendre, 2001).

RESULTS

The AFLP results—From the three selective primer combinations used (Table 2), 223 fragments were scored, ranging from 60 to 494 base pairs (bp). Sixty-four bands (28.7%) were present in all individuals. The number of fragments per individual was between 107 and 127 (mean 116.5). In populations 1, 6, and 7 identical genotypes were found. One hundred fifty-eight individuals were included in the analysis.

TABLE 2. Primers, fluorescent dye labels, total number of fragments, and number and percentage of monomorphic fragments in the amplified fragment length polymorphism (AFLP) analysis of *Saponaria pumila*.

AFLP primer	Color dye	Total number of fragments	Monomorphic fragments
MseI-CAC EcoRI-ACA	5-FAM	82	14 (22.6%)
MseI-CAG EcoRI-ACC	NED	62	29 (35.4%)
MseI-CTG EcoRI-AGG	JOE	79	21 (26.6%)
Pooled	—	223	64 (28.7%)

Genetic variation—The intrapopulational diversity expressed in the Shannon diversity index (H_{Sh}) and in the percentage of polymorphic fragments ($\%P_{pop}$) increases from west to east (Table 1, Fig. 2). Extremely low diversity (H_{Sh}, $\%P_{pop}$, f_r) is found only in populations in the western and northwestern portions of the distributional range (populations 1, 4, 5, 6, 7, 8, 9, 11). Private fragments (1–4 per population, see Table 1) and rare fragments (Fig. 3) are present especially in populations in the eastern part of the range. The number of fragments private to one of the four regions or shared by only two regions is given in Table 3.

Geographic groups—The UPGMA of the populations (Fig. 4), and the PCoA (Fig. 5) allow a grouping of the populations into four units. These correspond with geographic regions (Table 1, Fig. 6): East (E), the easternmost siliceous Alps including also the region north of Mur valley; Center-East (CE), south of Mur valley westwards; Center-West (CW), adjacent to the west; and Dolomites (D), a small siliceous region in the southern Dolomites (Lagorai, Cima d'Asta) including the disjunct Adamello population. In the UPGMA analysis of the populations (Fig. 4), these groups form clusters. Only population 33 (Gleinalpe) is isolated at a high level.

The neighbor-joining tree of all individuals (data not shown) divides the data set into E on the one hand and the rest on the other. The CW region forms one group, but three individuals

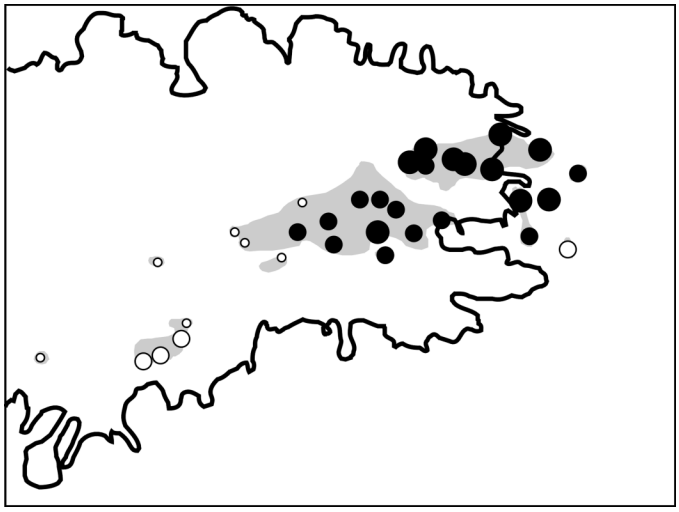

Fig. 2. Genetic variability of populations of *Saponaria pumila* (H_{Sh}, Shannon's diversity index, Table 1) in relation to the maximum extent of the Würm ice shield (black line). Large dots, $20 > H_{Sh} > 15$; medium dots, $15 > H_{Sh} > 10$; medium circles, $10 > H_{Sh} > 5$; small circles, $5 > H_{Sh} > 0$.

from population 15 (Hocheck, CE) fall into the D group. Thus, a separation into four groups is unsupported.

The PCoA of all individuals (Fig. 5) allows definition of E, CE, D, and CW as more or less discontinuous groups. Some individuals of population 15 (Hocheck, CE) are close to D (Fig. 5A). A PCoA with populations from E only (data not shown) shows no clear structure within this group along the first axis. Along axes 2, 3, and 4 (the four axes explain 15.4, 13.6, 13.4, and 13.3% of the overall variation), populations 30 (Saualpe), 33 (Gleinalpe), and 31 (Koralpe), respectively, are clearly separated.

Geographic relationships—Analyses of molecular variance (AMOVA) were assessed with different groupings of populations (2 to 5 groups, see Table 4). In all combinations about

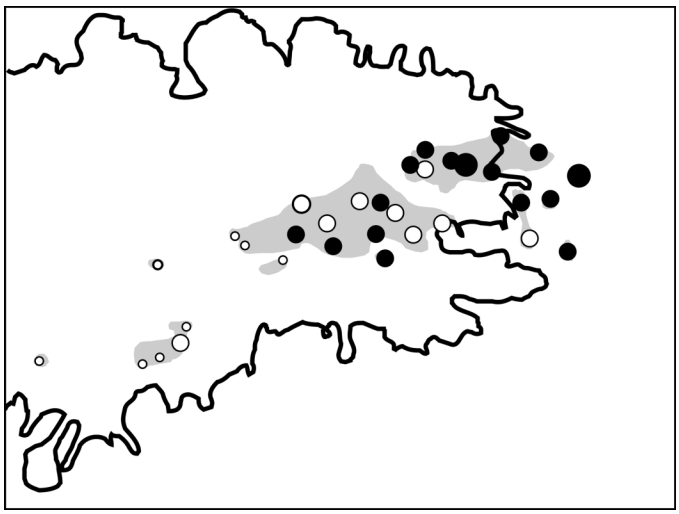

Fig. 3. Number of rare fragments (f_r, Table 1) in *Saponaria pumila* populations in relation to the maximum extent of the Würm ice shield (black line). Large dots, $f_r \geq 22$; medium dots, $21 \geq f_r \geq 15$; medium circles, $14 \geq f_r \geq 8$; small circles, $7 \geq f_r \geq 0$.

TABLE 3. Number of fragments private to one region or shared by only two regions.

Region	E	CE	CW	D
East (E)	—	34	0	1
Center-East (CE)		—	2	0
Center-West (CW)			—	0
Dolomites (D)				—
Number of private regional fragments	33	9	0	4

50% of the overall diversity is assigned to variation within populations. When four regional groups (E, CE, D, CW) of populations are considered, 22.6% of the overall genetic diversity is found among populations within regions and 27.2% among regions.

The Mantel R_M value calculated with the matrix of inter-individual Jaccard distances and a matrix of geographic distances in kilometers was 0.44, hence indicating a good congruence between genetic and geographic distances (''isolation by distance''). With the four groups obtained by the preceding methods, significantly negative correlation was obtained between E/CE, E/CW, and E/D (Fig. 7). There is no significant correlation between E/E, CE/D, and CE/CW. Significantly pos-

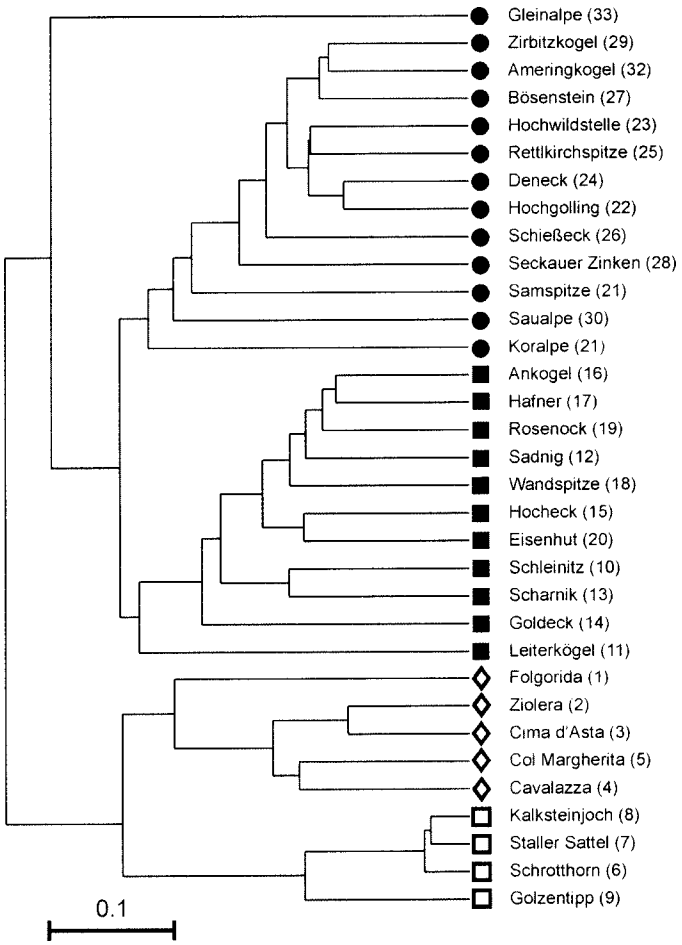

Fig. 4. The UPGMA tree of 53 populations of *Saponaria pumila* (Nei's [1972] genetic distance). Black dots, E = East; black squares, CE = Center-East; diamonds, D = Dolomites; open squares, CW = Center-West; population numbers are given in brackets.

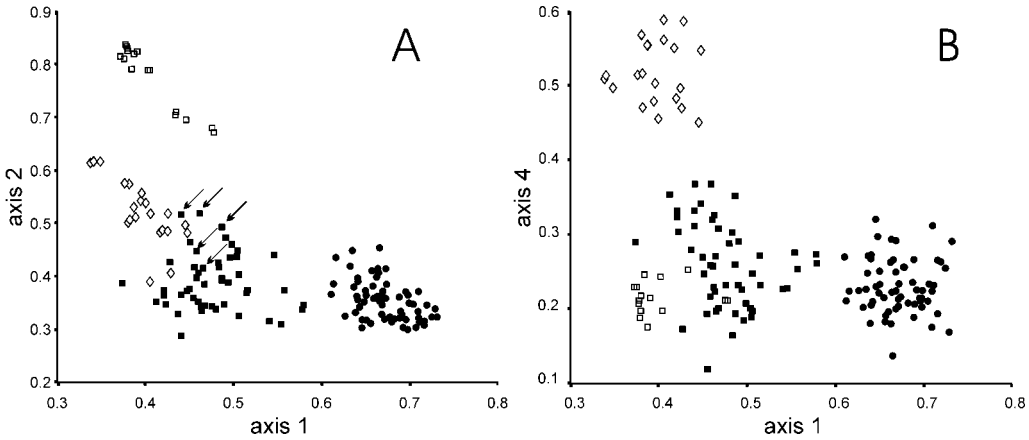

Fig. 5. Principal coordinates analysis (PCoA) of all investigated individuals of *Saponaria pumila*. (A) Ordination 1 vs. 2 and (B) 1 vs. 4. (These three axes explain 29.6, 22.1, and 9.4% of the overall variation, respectively.) Black dots, E = East; black squares, CE = Center-East; diamonds, D = Dolomites; open squares, CW = Center-West. Arrows mark individuals of population 15 (Hocheck, CE).

itively correlated are CE/CE, CW/CW, and D/D. The only significantly positive correlation between regions is between D and CW. This means that a differentiation supported by Mantel tests only exists between E and the other groups.

The numbers of markers found in each region are visualised on a geographic background in Fig. 8, which also shows the uneven distribution of markers. In region E, 92% of the 223 AFLP markers are found while E + CE contain 98%, and E + CE + D 100%.

DISCUSSION

Geographic pattern and Pleistocene refugia—The results of the UPGMA and PCoA allow the differentiation of four geographically separated groups of populations (E, CE, D, CW) in *Saponaria pumila*. But does this mean that there were four isolated glacial refugia for this species? During the last cold period of the Pleistocene most of the recent distributional area was glaciated (Voges, 1995; see Fig. 6). In the most heavily glaciated parts, the surface of the ice sheet ranged down to more than 2500 m above sea level (asl) (van Husen, 1987). The snow line was between 1500 and 1900 m asl (Nagl, 1972). Region E was only partly covered with ice and thus, from a geological point of view, it can be regarded as a presumptive refugium. Within the ice shield, less severe conditions can be expected on peripheral nunataks than on those in more central parts. The former are mountains situated in comparatively weakly glaciated areas, protruding from valleys filled with large glaciers but not from ice domes. They could have provided habitats at or even below the Pleistocene snowline especially on south-exposed ridges, e.g., in the southern part of CE (western Gurktaler Alpen, southeastern Hohe Tauern; for geographic localization of toponyms see Fig. 1) and in the southern Dolomites. The decrease in summer temperatures was less pronounced than in winter temperatures (Frenzel, 1992a, b) making survival of higher plant life within the ice sheet more likely. In contrast to regions E, CE, and D, region CW was completely glaciated. This means there is no clear correlation between the groups found and the possibility of glacial survival in four independent refugia.

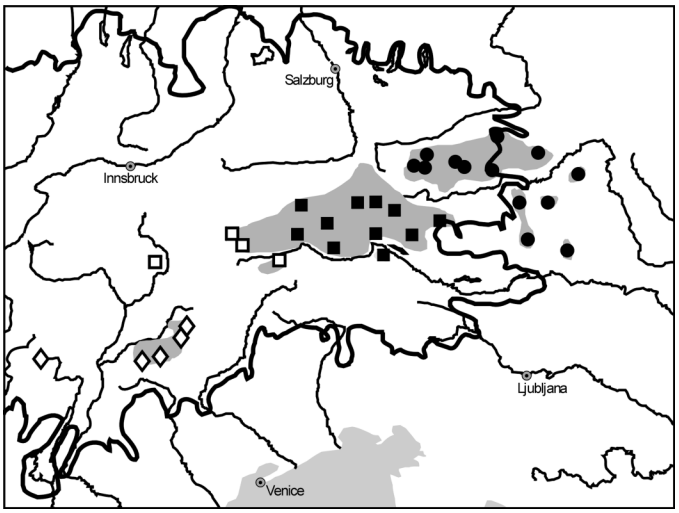

Fig. 6. Geographic distribution of the populational groups of *Saponaria pumila* defined by UPGMA (Fig. 4), and PCoA (Fig. 8A, B). Black dots, E = East; black squares, CE = Center-East; diamonds, D = Dolomites; open squares, CW = Center-West. The maximum extent of the Würm ice shield is given by the black line.

The deepest split in the phylogeography of *S. pumila* is found *within* the large unglaciated area in the easternmost Central Alps. Mantel Tests (Fig. 7) support only this splitting (E/CE + D + CW). Even if the geographic distances between E and CE are very short, there are several possible geological as well as biological causes that might have prevented the amalgamation of the two groups. (1) Limestone, on which the species does not grow, dominates in the Radstädter Tauern and functions as a barrier in the west (Figs. 1, 9). (2) The deep Mur valley kept the populations separated and prevented gene-flow. (3) Woodland areas nowadays separate the southern parts of regions CE (population 20) and E (population 29). During cold periods, however, due to the downshift of vegetation belts, these areas were possibly populated. However, if contact was not hindered for geomorphological reasons, the two ancestral groups might have been intermixing. The hybrid populations would have been extirpated during warmer periods due to the upshift of vegetation belts and the reduction of alpine vegetation to the present extent (or even less due to temperatures higher than today; cf. Lang, 1993). Another possibility could be that a hybrid zone (Hewitt, 2001) was possibly established between E and CE, preventing the amalgam-

TABLE 4. Summary of analysis of molecular variance (AMOVA) performed with different groupings. Because of its geographic isolation, population 1 (Folgorida-Adamello) from D was regarded as a separate group. *N* = number of groups. Statistics include degrees of freedom (df), sum of squares (SSD), variance-component estimates (CV), and percentage of total variance (%total).

N	Grouping	Source of variation	df	SSD	CV	%total
5	[E]-[CE]-[CW]-[D]-[Adamello]	among regions	4	689.423	5.123	27.95
		among populations	28	791.419	3.998	21.81
		within populations	125	1151.367	9.211	50.24
4	[E]-[CE]-[CW]-[D-Adamello]	among regions	3	639.142	4.971	27.12
		among populations	29	841.699	4.151	22.64
		within populations	125	1151.367	9.211	50.24
3	[E]-[CE-CW]-[D-Adamello]	among regions	2	411.002	3.459	19.00
		among populations	30	1069.841	5.535	30.40
		within populations	125	1151.367	9.211	50.59
2	[E]-[CE-CW-D-Adamello]	among regions	1	287.702	3.303	17.73
		among populations	31	1193.140	6.119	32.84
		within populations	125	1151.367	9.211	49.43

ation of genotypes over larger distances as exemplified by Martinsen et al. (2001). During warmer periods this presumed Pleistocene hybrid zone would have been eliminated.

Genetic variation and depauperation—A stepwise and drastic genetic depauperation from east to west (as revealed by H_{Sh}, Fig. 2; f_R, Fig. 3; and $\%P_{pop}$, Table 1; see also Fig. 8) is the most striking feature in the investigated populations of *Saponaria pumila*. Similar patterns due to Pleistocene climatic changes have been observed in other plants (e.g., Broyles, 1998; Shapcott, 1998). Regarding isolated populations, it is expected that genetic drift leads to an accumulation of new mutations and sometimes also to a loss of genetic diversity (Hewitt, 1996). The intensity of this process depends strongly on effective population size and the duration of isolation. Whether this depauperation is really due to bottlenecking in small Pleistocene refugia, however, and not to recent long-distance dispersal, is not discernable with diversity parameters such as H_{Sh} or $\%P_{pop}$ alone. But there are clear differences in the genetic identity of populations dependent on the time of isolation, as ''old'' bottlenecked populations accumulate unique markers. In *S. pumila* both ''young'' as well as ''old'' depauperate populations occur and allow a clear differentiation.

Geographic grouping—Region **East** (**E**) exhibits a high level of internal diversity (Table 1, Figs. 2, 3) and also a high number of unique markers (Table 3), which characterize this refugium. Within region E three peripheral populations are genetically isolated as indicated by higher bootstrap values in the neighbor-joining tree and by separation in the PCoA (data not shown). These are the presently (see Fig. 1) geographically isolated populations 31 (Koralpe), 30 (Saualpe), and 33 (Gleinalpe). Population 33 shows the highest f_r value in the entire data set, but low H_{Sh}, making it a typical example of an ''old'' depauperate population. Isolation during warm periods restricting gene flow is a likely reason for the genetic distinctiveness of these populations.

Within **Center-East** (**CE**) the pattern is not clear. As a group it is significantly different from E as indicated by Mantel tests (Fig. 7). The moderate to high level of genetic diversity, especially in the more southern populations (Table 1, Figs. 2, 3), and the presence of nine unique fragments (Table 3) suggest a refugium in this area rather than a postglacial recolonization. As mentioned above, only a very small area in the east of CE (easternmost Gurktaler Alpen, around population 20, see Fig. 1) was situated outside the ice sheet, but south-exposed slopes and ridges of the central Gurktaler Alpen (surroundings of population 19), southeastern Hohe Tauern

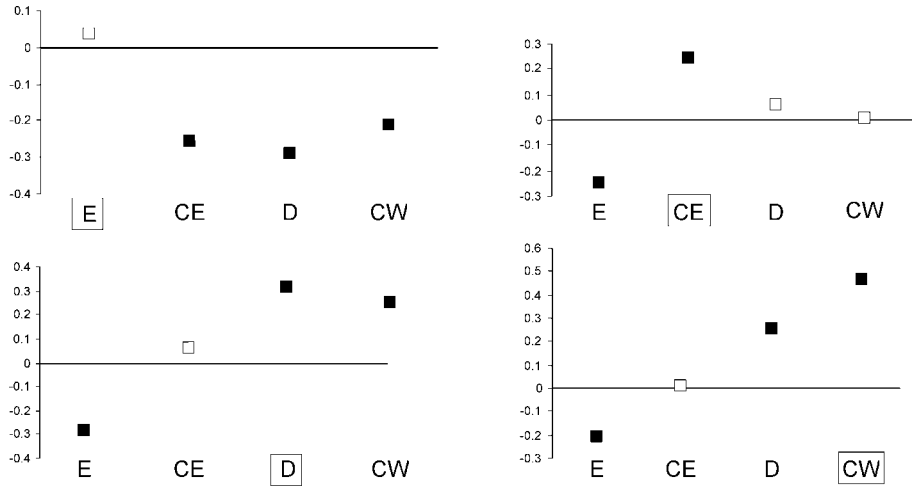

Fig. 7. Correlograms resulting from the Mantel permutation tests. Correlations of the four regions E, CE, CW, and D (following Table 1) with themselves and other regions. Filled squares indicate Bonferroni-corrected R_M values significantly different from zero at $P < 0.05$.

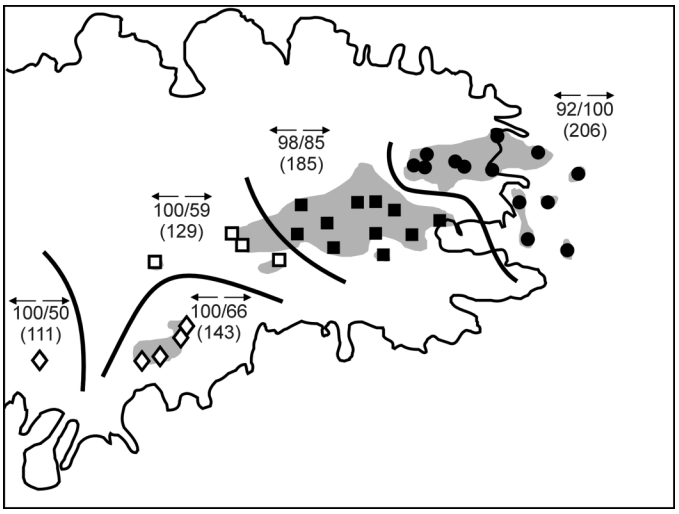

Fig. 8. Increase/decrease in the number of fragments in *Saponaria pumila* from east to west and vice versa. Because of its geographic isolation, population 1 (Folgorida-Adamello) is regarded as a separate unit. Left number in pair (←) = cumulative percentage of markers from east to west (E–CE–D–CW–Adamello); right number in pair (→) = cumulative percentage of markers from west to east (Adamello–CW–D–CE–E); number of markers (223 total) present in this region is given in parentheses.

(Reisseckgruppe, surroundings of population 15; Kreuzeckgruppe, surroundings of population 13), and Goldeck (population 14) were not glaciated. The high level of genetic diversity and divergence might indicate that *S. pumila* did not only survive in a small peripheral refugium but also on these peripheral nunataks.

The fact that all populations of **Center-West** (**CW**) are genetically depauperate, lack unique fragments (Table 1), *and* possess no or very few rare fragments is clear evidence for recent colonization via long-distance dispersal. This is also indicated by a positive correlation of Mantel R_M values of CW with CE and D (Fig. 7) in the Mantel tests. Even if UPGMA (Fig. 4) and PCoA (Fig. 5A) support CW, this group is only characterized by the shared absence of AFLP markers. The UPGMA shows that the genetic distances between populations 6, 7, and 8 are very low, even though the geographic distances are more than 50 km. If these isolated populations resulted from fragmentation of a formerly continuous distribution and of survival on nunataks during the last ice age, a higher level of genetic distinctiveness would be expected. Clear proof for recent or subrecent natural long-distance dispersal and establishment of viable populations as exemplified here is very rare (Ouborg, Piquot, and Van Groenendael, 1999; Cain, Milligan, and Strand, 2000), although the detection of processes involving long-distance dispersal are crucial in understanding the dynamics of populations and their distribution in space and time.

Even if Mantel tests (Fig. 7) do not support a separation of **Dolomites** (**D**) from CE and CW, it is very likely that these populations of *S. pumila* survived in a separate glacial refugium. Four unique fragments (Table 3), one present in nearly all investigated individuals, are confined to this region and the values of H_{Sh} (Fig. 2) and $\%P_{pop}$ (Table 1) in populations 2, 3, and 4 are moderately high compared to the highly depauperated populations from CW. Furthermore, the population most closely related to D is population 15 (Hocheck, CE; see Fig. 5A), which is geographically not the closest. Thus, colonization of D before the last glacial maximum appears more likely than postglacial spread. Glacial survival in situ in the southern Dolomites seems possible, as only the valleys were filled with glaciers and southern slopes remained unglaciated (Van Husen, 1987). Periglacial survival in the small siliceous region further south (Fig. 9A), or postglacial colonization would involve several long-distance dispersals to explain these genetic characteristics. The genetically extremely depauperate disjunct population in **Adamello** (population 1) originated from recent long-distance dispersal from the southern Dolomites and is thus a typical example of a ''young'' depauperate population. All fragments detected in this population are also present in the Dolomites.

Limits of migration—Why *S. pumila* did not migrate further westward is of particular interest. In contrast to the situation at the western border of E, there are no migration obstacles like limestone areas or deep valleys. There are also no obvious changes in mesoclimate in the adjacent westerly regions. Three possible explanations must be considered. (1) The dispersal ability of the species is so poor that the migration distance per year is very short. Even dispersal over several hundreds of meters is exceptional and, as a consequence, establishment of new viable populations over larger distances is extremely rare. Moreover, the westward migration is opposed by the (at least presently) prevailing winds from west/northwest or from the southwest/south. If this is the case, it is only a matter of time before larger areas will be colonized. (2) There is a vicariant species preventing the colonization of areas further west. There is no evidence for this. (3) Strong genetic depauperation accompanied by reduced population viability in the marginal populations is resulting in the inability to recolonize new areas. This hypothesis is opposed by the fact that genetically depauperated plant populations are abundant in northern Europe (e.g., Reinhammar, 1999) due to leading edge migration (Hewitt, 1996), where they recolonized large areas after the Pleistocene.

Hypothetical preglacial, glacial, and postglacial history of Saponaria pumila—We can only speculate about the early stages of the evolutionary history of *S. pumila*. There are no closely related taxa (Simmler, 1910), so the species may be ancient and perhaps has originated somewhere else. The history of the disjunction between Eastern Alps and the Southern Carpathians in Romania remains unclear. In the Alps it probably had been centered in the eastern Central Alps for a long time where it is still abundant. As this region was only locally glaciated during the whole Pleistocene (Voges, 1995), recurrent glacial survival was possible more or less in situ for a long period. Reacting to climatic fluctuations, *S. pumila* had only to migrate vertically up and down the same mountain slopes. As a result, we find these populations with high levels of diversity and identity. The importance of this area as a glacial refugium is also emphasized by the occurrence of some endemic and many relict taxa of arctic-alpine or Alpic-Carpathic distribution (Schneeweiss and Schönswetter, 1999). The divergence of E and CE occurred in this region probably several glacial cycles ago. Why the two regions could retain their genetic integrity at such a high level until today is not easy to see. Several possible explanations must be considered (see ***Geographic pattern and Pleistocene refugia***). Possibly before the Würm glaciation the southern Dolomites were colonized from CE via long-distance dispersal.

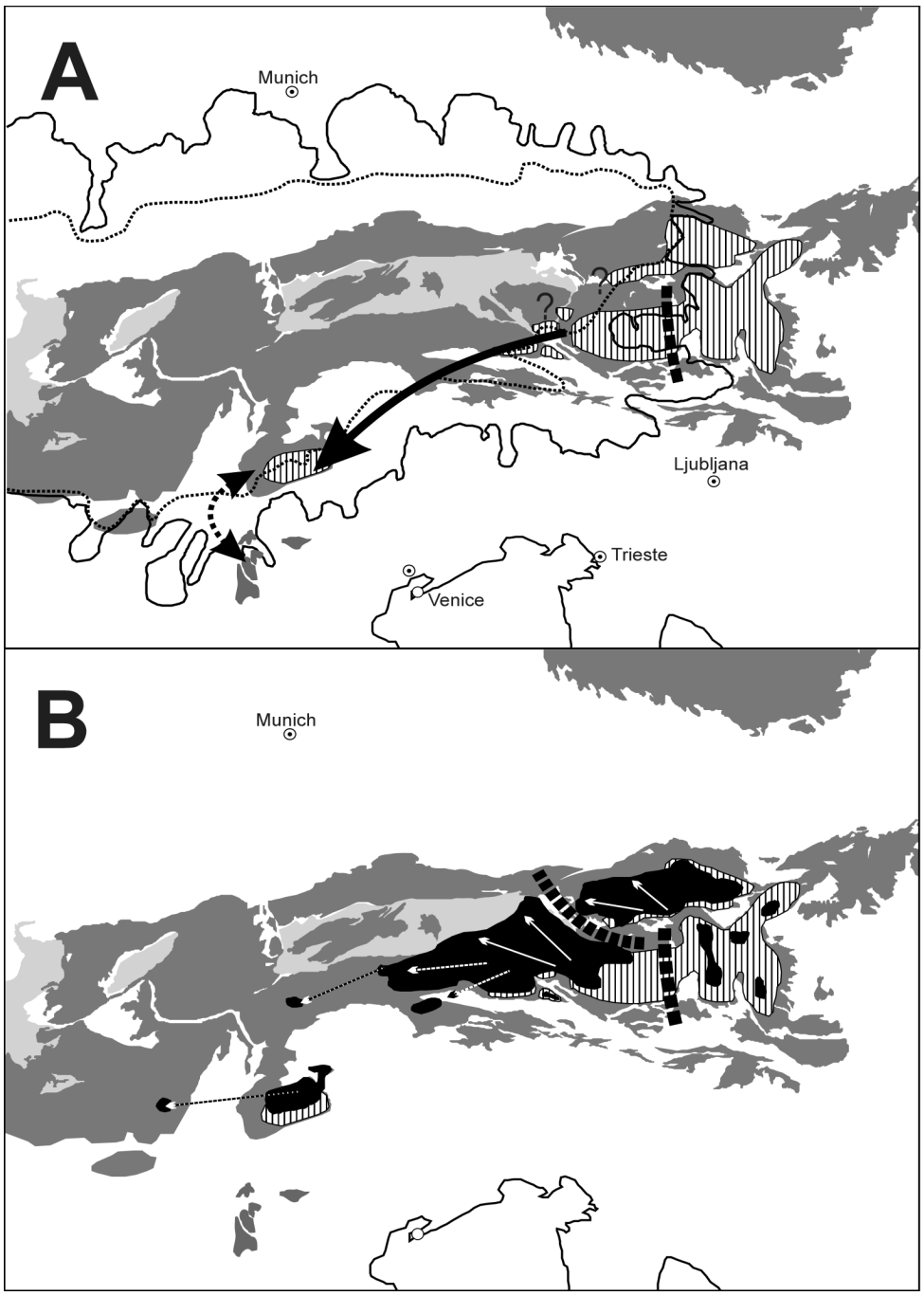

Fig. 9. Scenario of the biogeographic history of *Saponaria pumila* in the Eastern Alps. The maximum extent of the ice sheet (taken from Voges, 1995) is given as a black line. Regions above the estimated Würm snowline (Nagl, 1972; Van Husen, 1987) are within the dotted line. The siliceous regions of the Alps are given in dark gray, regions with predominating calcareous schists are given in light gray, and limestone regions are white. (A) Presumed distribution (hatched area) of *Saponaria pumila* during the last ice age (Würm). Areas with question marks indicate regions with siliceous bedrock where Pleistocene occurrence seems also possible. The arrow indicates the (pre-Würm?) dispersal event from CE to D, the dotted arrow indicates the alternative scenario of glacial survival south of D, and the bold dashed line indicates the possible contact area of E and CE. (B) Post-Pleistocene dispersals. White arrows indicate short-distance migration within mountain ranges, and dotted arrows indicate most recent long-distance dispersal between mountain ranges. The present distributional area of *Saponaria pumila* is given in black. The bold dashed lines indicate distributional barriers (see DISCUSSION).

The presumed situation during the coldest period of the last glaciation is outlined in Fig. 9A: *S. pumila* survived in the large eastern peripheral refugium (mainly E) and probably also on peripheral nunataks in the southeastern Hohe Tauern, western Gurktaler Alpen (CE), and also in the southern Dolomites (D). We favor the view that survival of alpine silicophilous plants on peripheral refugia was possible, and this is also supported by the presence of some endemics in the latter region (e.g., *Saxifraga depressa*, see Prosser, 2000). A refugium ''jumping'' between the Dolomites and small siliceous regions

at the southern border of the Alps, which do not exceed 1000 m asl, seems less likely as several long-distance dispersal events are necessary for this scenario (Fig. 9A, dotted arrow). Outside these regions, especially in the forelands of the Alps, the lack of favorable siliceous bedrock made survival impossible. After deglaciation, the remigration to the interior parts of the Alps began. The ''leading edge'' (Soltis et al., 1997) of the refugial populations recolonized areas adjacent to the west by dispersal only over short distances without significant loss of genetic diversity (Fig. 9B). Within E the migration was stopped by the limestone massif of Radstädter Tauern. In the easternmost Alps at the same time fragmentation and reduction of the distributional area started. Some isolated populations retained high levels of genetic diversity. It is very likely that extinction of populations on low mountains took place during the warmest periods. The westernmost part of the present distributional area was colonized via long-distance dispersal leading to severe bottlenecks and genetic depauperation (Fig. 9B). The few locations in Adamello originate from those in the Dolomites, while the westermost populations in the interior Alps originate from adjacent Hohe Tauern populations. These were the most recent events completing the puzzle of the phylogeography of *S. pumila* in the Alps.

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