RAPD Analysis Reveals Geographic Differentiations within *Allium schoenoprasum* L. (Alliaceae)

N. Friesen [1,2] and F. R. Blattner [1]

[1] Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany
[2] John Innes Centre, Norwich NR7 4UH, UK

Received: July 29, 1999; Accepted: February 1, 2000

Abstract: Random amplified polymorphic DNA (RAPD) analysis was used to study the phylogenetic relationships between species in *Allium* section *Schoenoprasum* and for the investigation of the intraspecific differentiation of *A. schoenoprasum.* RAPD analysis of 39 samples representing eight species of sect. *Schoenoprasum* and one sample of *A. atrosanguineum* (sect. *Annuloprasum*) resulted in 233 interpretable RAPD bands. The analysis clearly distinguishes the species of section *Schoenoprasum.* The arrangement of the accessions of *A. schoenoprasum* in all dendrograms mirrors the geographical distribution, with a clear differentiation between an Asian and European subgroup. Within the European group, Scandinavian material is clearly distinct from S and E European material. Informally described morphological types of *A. schoenoprasum* could not be confirmed by RAPD analysis but represent recurrent ecological adaptations. A combination of phenetic (UPGMA, neighbour-joining analysis), cladistic (parsimony analysis), and statistical (PCA) methods of data analysis resulted in clearer phylogenetic interpretations than each of the methods facilitates when used separately.

Key words: *Allium* sect. *Schoenoprasum*, Alliaceae, biogeography, ecology, evolution, RAPD.

Abbreviations:

RAPD:	random amplified polymorphic DNA
UPGMA:	unweighted pair group method using arithmetic averages
NJ:	neighbour-joining analysis
MP:	maximum parsimony
PCA:	principal coordinate analysis

Introduction

Allium L. section *Schoenoprasum* Dumort., like the other closely related sections *Cepa* Prokh. and *Annuloprason* Egor. which are characterized by fistulate leaves, belongs to subgenus *Rhiziridеum* (Koch) Wendelbo. In the last taxonomical revision of *Allium* sect. *Schoenoprasum* seven species and three subspecies were recognized (Friesen, 1996[10]). The most important species of this section is *A. schoenoprasum* L. (chives) a diploid, widespread in Eurasia and North America. *Allium schoenoprasum* s.l. also includes two tetraploid subspecies from Spain: ssp. *latiorifolium* (Sierra de Guadarrama) and ssp. *orosiae* (Hueska). The species is morphologically relatively diverse with an accordingly complicated nomenclatural history. Four morphological types were informally described (Stearn, 1978[33]; Friesen, 1996[10]), and *Allium buhseanum* Regel, from Iran and the Caucasus was also included as a synonym in *A. schoenoprasum* s.l. (Friesen, 1996[10]). Problems arise from the scattered geographical distribution of the four types throughout the area and from the quantitative characters differentiating them. Some examples might illustrate the situation.

In all parts of the area small plants (morphotype B) of *A. schoenoprasum* occur on limestone, which have been independently described as different variations but are morphologically very similar: var. *pumilum* Bunge (Altai, Siberia), var. *alvarense* Hylander (island Öland, Sweden), var. *jurmoënse* Eklung (Finland archipelago in the Gulf of Finland), and f. *kokinjae* Hay. (Balkan Peninsula). The robust variant which is distributed mainly in mountains of the entire area (type C) usually is named *A. sibiricum* L., *A. schoenoprasum* ssp. *sibiricum* (L.) Richter, or *A. schoenoprasum* var. *alpinum* DC. Type D, which have lengthwise ribbed leaves, can sometimes be found in Siberia and probably also in other regions, however, in herbarium material this character disappears. Sometimes type D constitutes homogenous populations, but usually it can be found together with typical *A. schoenoprasum* plants (types A and C). One of the more or less homogenous Siberian populations was described as an independent species, *A. udinicum,* by Antzupova (1989[1]).

Analyses of different molecular markers indicate the monophyly of sect. *Schoenoprasum.* The chloroplast genomes of all members of the section share a synapomorphic *Cla*I mutation in the *trn*T-*trn*D region which has not been found in any other taxon of *Allium* outside of section *Schoenoprasum* (Friesen et al., 1997 a[11]; Mes et al., 1997[22]). DNA sequences of the internal transcribed spacer region (ITS) of the nuclear ribosomal DNA (nrDNA) are also very similar and possess a 12 basepair deletion in the ITS-2, unique for sect. *Schoenoprasum* (Friesen et al., in preparation). The monophyly of *A. schoenoprasum* s.l., as well as the phylogenetic relationships of the subspecific taxa described, remains unclear. In order (1) to arrive at more precise hypotheses about the intraspecific differentiation of *A.*

Plant biol. 2 (2000) 297 – 305

ISSN 1435-8603

schoenoprasum, and (2) to decide whether variations *pumilum, alvarense,* and *jurmoënse* are natural entities or ecotypes with independent origins, we used RAPD analysis (Welsh and McClelland, 1990[38]; Williams et al., 1990[39]). RAPD analyses reveal even small genetic differences, since a large part of the nuclear genome will be scanned, as can be seen by mapping studies of segregating markers in a wide range of plant families (e.g., Rieseberg et al., 1993[28]; Bachmann and Hombergen, 1996[3]; Serquen et al., 1997[32]), and are successfully used for clarification of the phylogeographical questions (Gabrielson et al., 1997[17]; Friesen and Herrmann, 1998[13]; Purps and Kadereit, 1998[26]; Tollefsrud et al., 1998[35]; Friesen et al., 1999[15]). Another advantage of this method is that it is less expensive and can be performed more rapidly than most other methods (Morell et al., 1995[23]). However, RAPD techniques have some limitations, such as low reproducibility of some bands and the uncertain homology of co-migrating fragments in gel electrophoresis (Van der Zande and Bijlsma, 1994[36]; Harris, 1995[19]; Pillay and Kenny, 1995[25]; Rieseberg, 1996[27]). Most of the limitations of RAPD analysis can be overcome by carefully adjusting the reaction and detection conditions (Bachmann, 1997[2]; Colosi and Schaal, 1997[7]; Friesen and Klaas, 1998[14]).

Several reasons were considered in literature which make cladistic analysis inappropriate for RAPD data. Arguments against parsimony are founded on possible scoring of non-homologous bands, lack of genetic independence, the biased screening of only variable parts of the genome, or the lack of appropriate models of character evolution (Backeljau et al., 1995[14]). As most of the arguments also apply to morphological characters or DNA with unknown functional constraints ("noncoding" DNA, e.g., spacer and intron sequences) they could preclude the use of cladistics generally. Moreover, biased data might also influence other data analysis algorithms. We can only see one severe restriction for parsimony analysis of RAPD data. Cladistic theory and analysis relies on bi- or multifurcating lineages. It is inappropriate for the analysis of relationships within species or under inclusion of allopolyploids, where reticulate lineage relationships occur. RAPD studies are mostly used at taxonomic ranks where DNA sequences or RFLPs fail to detect differences between accessions or taxa (Bachmann, 1997[2]; Wolfe and Liston, 1998[40]). At this level gene flow between the organisms under study is generally possible, thus violating a major assumption of cladistic theory. Accordingly, distance-based, phenetic algorithms are normally used to analyze RAPD data. In our experience cladistic analyses could nevertheless be applied, in addition to phenetic analysis methods. In cases where data contain strong reticulate structure, parsimony will fail to find resolved trees (null hypothesis), whereas phenetic methods in all cases represent data in a tree-like way. On the other side, when trees or parts of trees are stable in parsimony analyses, this can be taken as a hint that the data (sub)set contains a reliable phylogenetic signal which is not swamped by gene flow (Roelofs and Bachmann, 1997[29]). This signal will be detected by most other methods of data analysis, too, but lacking the internal control against reticulate structures (Blattner and Friesen, in preparation). Combining cladistic and phenetic analysis methods thus allows more insights into data structure than excluding one method due to theoretical considerations. In our study of *Allium* sect. *Schoenoprasum* we show the advantages of the use of several analysis methods to understand the evolutionary history of closely related taxa.

Materials and Methods

A total of 38 accessions of eight species from *Allium* section *Schoenoprasum* and one accession of *A. atrosanguineum* of section *Annuloprason* from the living collection of the Department of Taxonomy of the IPK Gatersleben were investigated (Table **1**). The investigated accessions of *A. schoenoprasum* (29 accessions) more or less represent the entire geographical range of the species in Eurasia (Fig. **1**) and the informally described morphological types of Friesen (1996[10]).

DNA was isolated with the NucleoSpin Plant kit (Macherey-Nagel, Düren, Germany) according to the instructions of the manufacturer. Ten µl of the isolated DNA were dissolved in 150 µl of water and 4 µl (approximately 50 ng) of this DNA solution were used for each PCR amplification. The concentration of the extracted DNA was checked on an agarose gel.

Amplification was carried out using 11 arbitrary 10 bp primers (A04, A15, A16, AB04, AB11, AB18, AC02, G13, G19, D01 and D03) obtained from Operon Technologies, Alameda, CA. The amplification conditions were optimized according to Friesen et al. (1997 b[12]). One third of the reaction mixtures was separated on 1.5 % agarose gels in 0.5 × TBE buffer, followed by staining with ethidium bromide (Sambroock et al., 1989[31]). Clearly visible RAPD bands were scored manually for presence (1) or absence (0) from enlarged photographs of the gels. Differing band intensities were not taken into account to avoid errors introduced by competition among priming sites during the initial rounds of PCR (Bachmann, 1997[2]). Only bands reproducible in two independent amplification reactions were included in the data analyses. From the resulting binary data matrix distances and a character based analysis were conducted. Pairwise genetic distances were calculated using the Jaccard coefficient. Finally, phenograms were prepared based on UPGMA (unweighted pair group method using arithmetic averages) and neighbour-joining (NJ) cluster analyses (Saitou and Nei, 1987[30]) of the genetic distance matrix. The genetic distance matrix was also subjected to a principal coordinate analysis (PCA). From the distances, new independent axial coordinates are calculated which represent most of the variability of the original data. The taxa are then plotted as points in a three dimensional continuous space defined by the first three coordinates. These calculations were done in the NTSYS-PC program (Applied Biostatistic Inc. New York, 1993, version 1.8). Maximum parsimony analyses (MP) of the binary data matrix were done with PAUP* version 4d65 (a test version, kindly provided by D. Swofford) using the heuristic search option, MULPARS, ACCTRAN, and TBR branch swapping. Bootstrap analysis (Felsenstein, 1985[9]) was used to examine the statistical support of branches in the most parsimonious trees and neighbour-joining trees found. In addition to the analyses including all accessions, analyses with UPGMA, NJ, and MP were performed with all known polyploids (*A. altyncolicum* and *A. schoenoprasum* ssp. *latiorifolium*) excluded from the data matrix. Alternative tree topologies were evaluated with MacClade 3.05 (Maddison and Maddison, 1992[21]).

Table 1 The origin of the investigated accessions of *Allium* section *Schoenoprasum* and the outgroup *A. atrosanguineum* (Tax = accession numbers of the living collection of the Department of Taxonomy, Institute of Plant Genetics and Crop Plant Research). Morphological types A – D in *A. schoenoprasum* after Friesen (1996[10])

Taxon	Accession number and origin of plants
Section *Schoenoprasum* Dumort.	
A. altyncolicum Friesen	Tax 3157 (Russia, Altai), Tax 3159 (Russia, Altai)
A. karelinii Poljak.	Tax 2708 (Uzbekistan, Altai)
A. ledebourianum Schult. et Schult. fil	Tax 3173 (Russia, Altai), Tax 3641 (Mongolia, Mongolian Altai)
A. maximowiczii Regel	Tax 2772 (Russia, Burjatia, Romanovka)
A. maxim. var. *yesomonticola* (Hara) Shimuzu	Tax 6000 (Japan)
A. oliganthum Kar. et Kir.	Tax 3201 (Kasachstan, Tarbagatai)
A. schmitzii Coutinho	Tax 5226 (Portugal)
A. schoenoprasum L. sensu lato	
– Type A (*A. schoenoprasum* s. str.; *A. buhseanum* Regel)	Tax 0192 (Romania), Tax 0390 (Finland), Tax 0509 (Italy, Tretino), Tax 0702 (Ukraine, Carpatians), Tax 0854 (France, Corsica), Tax 0908 (Spain, Sierra Nevada), Tax 1215 (Hungary), Tax 1306 (Bot. Gard. Stockholm), Tax 3744 (Bulgaria, Rila Mts.), Tax 3897 (Iran, Mt. Barf Khone), Tax 5423 (Spain, Pyrenees, Port de la Bonaqua), Tax 5610 (Norway, Altafyord), Tax 5612 (Norway, Tromso)
– Type B (*A. gredense* Rivas Mateos; var. *alvarense* Hylander; var. *jurmoënse* Eklung; var. *pumilum* Bunge)	Tax 3369 (Sweden, Öland), Tax 3398 (Russia, Altai, Yuzhno-Chuisky range, Tara), Tax 5439 (Finland, Seili), Tax 5440 (Finland, Surmo)
– Type C (*A. foliosum* Clarion ex DC; var. *alpinum* DC; *A. sibiricum* L.; var. *sibiricum* (L.) Garcke; var. *laurentianum* Fernald)	Tax 0508 (Canada, Alberta, Rocky Mts.) Tax 1712 (Japan, Hokaido), Tax 1854 (Bulgaria, Vitosha range), Tax 3068 (Switzerland, Col du Sanetsch), Tax 3174 (Russia, Altai, Cholsun range), Tax 3446 (Russia, Chukotka), Tax 3460 (Switzerland, Bernese Alps), Tax 3892 (Russia, Altai, Shebalino), Tax 5411 (Russia, West Sajan)
– Type D (*A. udinicum* Antzupova)	Tax 3206 (Russia, Altai, Tyungur)
A. schoenoprasum ssp. *latiorifolium* (Pau) Rivas Martines et al.	Tax 5432 (Spain, Sierra de Guadarama, Penalara), 5434 (Spain, Sierra de Guadarama, Penalara)
Section *Annuloprason* Egorova	
A. atrosanguineum Kar. et Kir.	Tax 2907 (Kasachstan, Zailiysky Allatau)

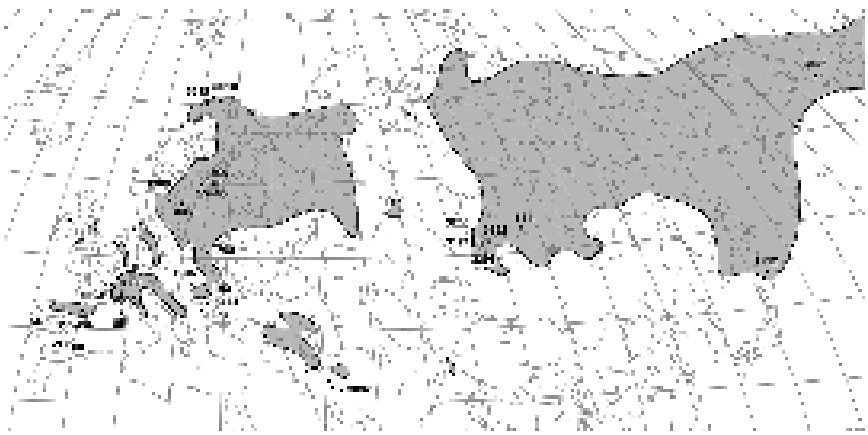

Fig. 1 Natural distribution of *Allium schoenoprasum* in Eurasia. Numbers indicate the origin of the investigated accessions.

Results

Eleven primers used to analyze 39 accessions of *Allium* sect. *Schoenoprasum* resulted in 233 unambiguously interpretable RAPD bands. Each primer produced between five and 18 bands per individual (Fig. **2**). In all analyses conducted *A. schoenoprasum* occurs as a monophyletic taxon, as do the other species of the section. The Iberian *A. schmitzii* is sister group to all species of section *Schoenoprasum*. Within *A. schoenoprasum*, the European accessions form a monophyletic unit. The morphologically similar intraspecific taxa of *A. schoenoprasum* (variations *pumilum, alvarense,* and *jurmoënse*) are not a natural entity but occur interspersed at different positions in the trees. The informally described morphotypes of *A. schoenoprasum* never form phylogenetical clustered subgroups.

The different algorithms used in the analyses resulted in some remarkable differences in the intraspecific groups obtained. In the UPGMA dendrogram (Fig. **3**) the Asian and the European clades occur as sister groups and the Canadian accession is

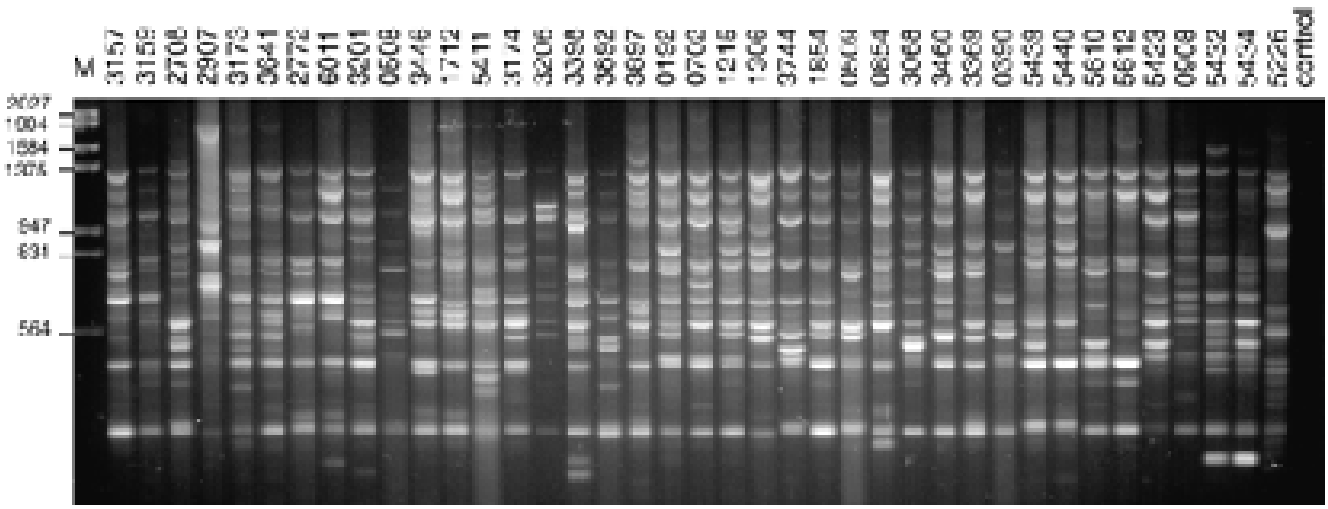

Fig. 2 Ethidium bromide stained agarose gel of an RAPD reaction with primer G19 of taxa from *Allium* sect. *Schoenoprasum* and *A. atrosanguineum*. Only prominent bands were scored for analysis. M = length standard. For origin of specimen see Table **1**.

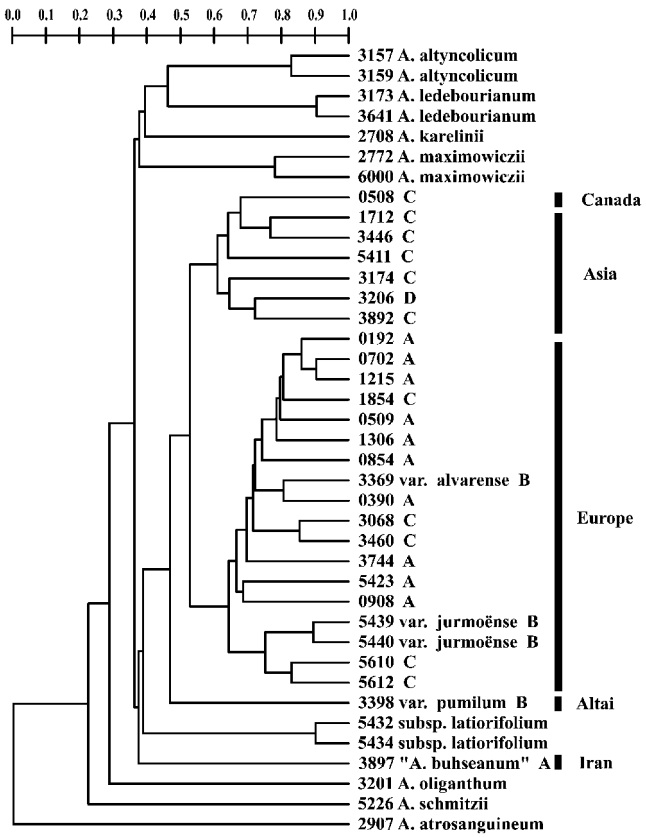

Fig. 3 UPGMA phenogram calculated from Jaccard distances of 38 accessions of seven species of *Allium* sect. *Schoenoprasum* and *A. atrosanguineum* based on 233 RAPD bands. For the accessions of *A. schoenoprasum,* no species name is given. A, B, C, and D depict the informally described morphological types within *A. schoenoprasum.*

part of the Asian clade. Within the European clade, four Scandinavian accessions are separated from the other European material. Three taxa of *A. schoenoprasum* (*"A. buhseanum"*, ssp. *latiorifolium,* and var. *pumilum*) appear at a position basal to the remaining accessions. The exclusion of polyploid taxa did not alter the topology of the phenogram of the other accessions. In the NJ analyses (Figs. **4A, B**) *"A. buhseanum"* is again sister group to the remaining accessions of *A. schoenoprasum* and the European accessions are a monophyletic unit within the other accessions. Tax 0508 from Canada appears at a sister group position of the European clade. The Altaian var. *pumilum* groups together with other accessions obtained from the Altai mountains, whereas the tetraploid ssp. *latiorifolium* is sister group to all other European accessions (Fig. **4A**). The exclusion of this taxon (Fig. **4B**) allows the recognition of three geographical groups within the European material (E Europe, C and S Europe, and N Europe) which are otherwise confused by two diploid accessions from Spain, grouping near the tetraploid (Fig. **4A**). The cladistic analysis of 136 parsimony informative RAPD characters (including polyploids) found 667 most parsimonious trees (444 steps, CI 0.520, RI 0.642). The consensus tree (Fig. **5A**) mostly resembles the tree found by the NJ analysis. The main difference concerns *"A. buhseanum"* which occurs at a somewhat strange position as sister group of *A. karelinii.* After exclusion of the polyploid taxa (114 parsimony informative characters) MP found 12 trees (394 steps, CI 0.556, RI 0.654). In the consensus tree (Fig. **5B**), which is better resolved than Fig. **5A**, *"A. buhseanum"* groups within the Asian accessions of *A. schoenoprasum.* In this group an Altaian accession (Tax 3206) occurs as sister group to all other *A. schoenoprasum.* Within the European material mostly C and S European accessions are basal and, as in the NJ analyses, the Scandinavian group (with bootstrap support) and E European group were found. In parsimony and neighbour-joining analyses an accession from N Italy (Tax 0509) appears as sister group of the Scandinavian material. The exclusion of *"A. buhseanum"* from the analyses did not alter the topology of the remaining taxa in the consensus trees (data not shown).

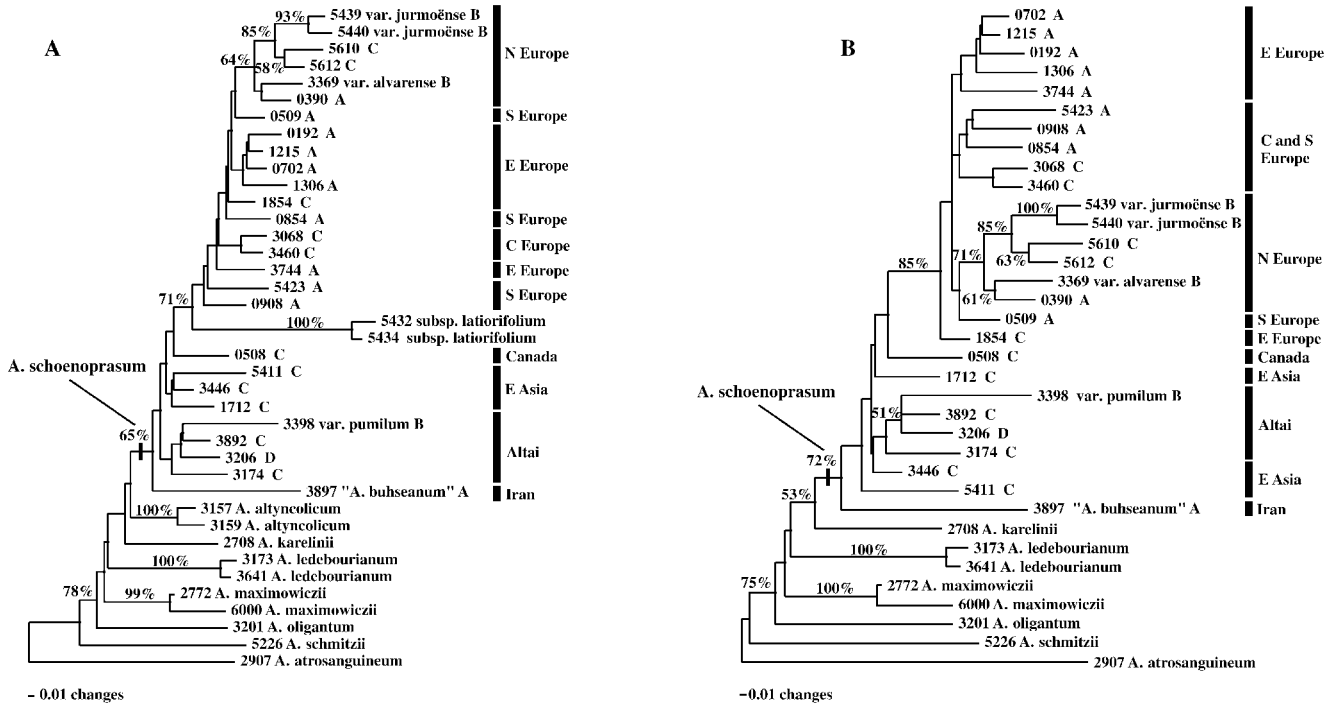

Fig. 4 Neighbour-joining phenogram calculated from the same distance matrix used for UPGMA analysis (Fig. **3**). Bootstrap values (500 resamples) above 50% are given along the branches. (**A**) Analysis including polyploid taxa (*A. altyncolicum* and *A. schoenoprasum* ssp. *latiorifolium*). (**B**) Analysis with known polyploids excluded.

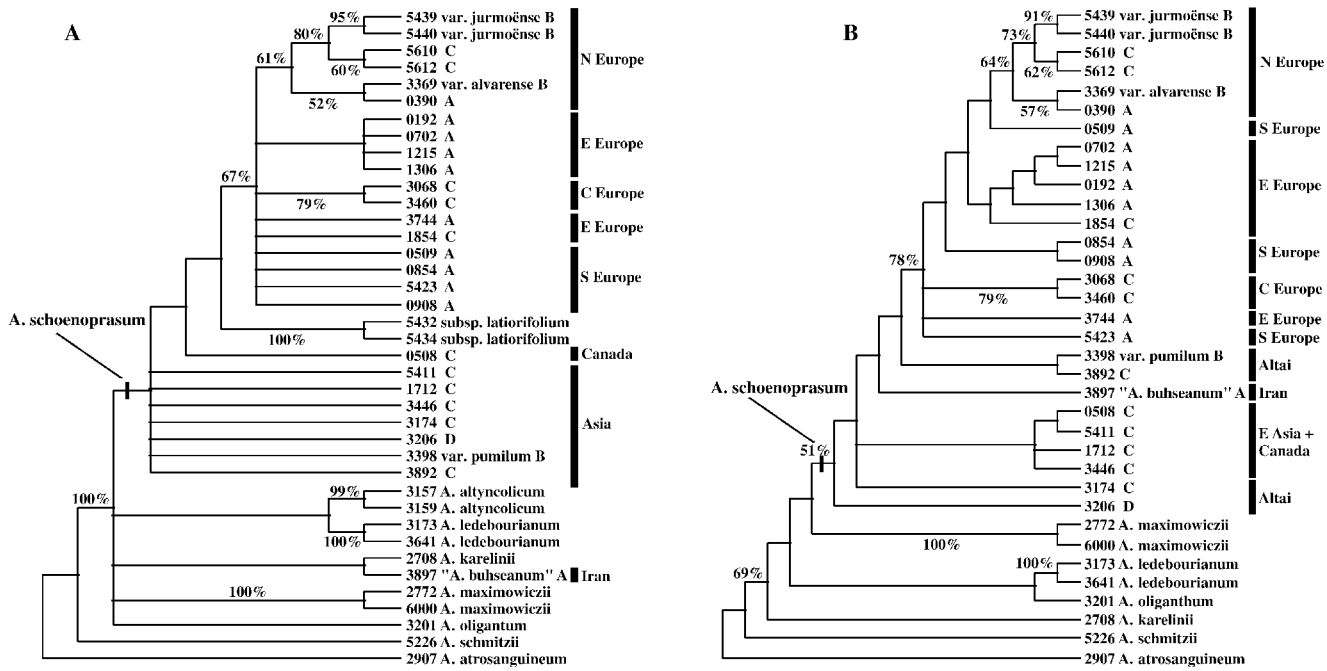

Fig. 5 Strict consensus trees of maximum parsimony analyses of 233 RAPD bands. (**A**) The analysis including polyploid taxa resulted in 667 most parsimonious trees (444 steps length, CI 0.520, RI 0.642). (**B**) After the exclusion of polyploids 12 trees (394 steps length, CI 0.556, RI 0.654) were found. For clades with bootstrap support above 50% (calculated from 500 resamples) the values are given along the branches.

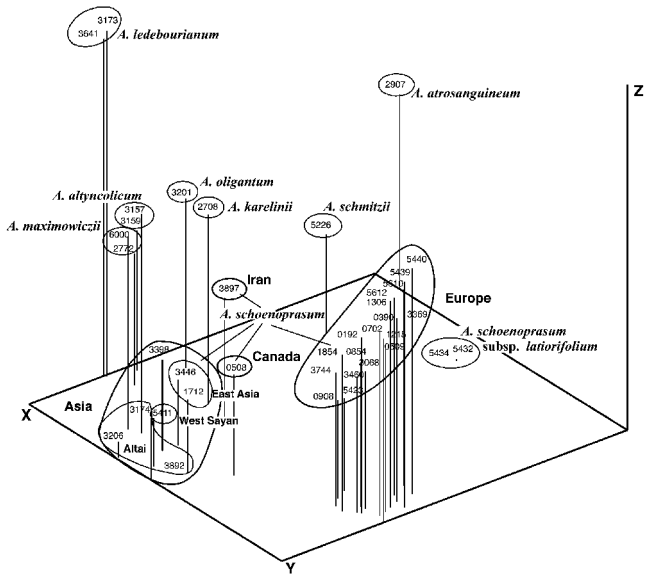

Fig. 6 Plot of the first three principal coordinates, calculated from the same RAPD distance matrix used in UPGMA and NJ analyses.

The principal coordinate analysis (Fig. **6**) clearly distinguishes the European and Asian accessions of *A. schoenoprasum.* In between these groups the Canadian and the Iranian *("A. buhseanum")* accessions occur. Interestingly, *"A. buhseanum"* is at a position which is intermediate between the two geographic groups of *A. schoenoprasum* and *A. karelinii,* that resembles its positions in the two MP analyses. The tetraploid *A. schoenoprasum* ssp. *latiorifolium* is quite distinct from the other accessions of the species, which is also shown by branch lengths in UPGMA and NJ analyses.

Discussion

The analyses of RAPD data of *Allium* sect. *Schoenoprasum* revealed all species of the section as monophyletic units. Within *A. schoenoprasum* the accessions are clearly geographically grouped (Asian vs. European origin) and the European material forms a monophyletic group. Within the European accessions, a Scandinavian group (with bootstrap support in the MP and NJ analyses) and an eastern European group (without bootstrap support) occur. The remaining accessions, mostly from central and southern Europe, do not form a clear group but are basal to the eastern and northern material. The informally described morphotypes A, C, and D (Friesen, 1996[10]) are distributed within all subgroups of *A. schoenoprasum,* thus lacking a common origin in each type. However, Asian type C occurs mostly in the basal part of *A. schoenoprasum.* Thus this type seems to be the initial form of *A. schoenoprasum* from which the other types repeatedly developed as morphological reactions on environmental conditions. The small morphotype B, which has been described under various names (var. *pumilum, alvarense,* and *jurmoënse*) and always occurs in limestone habitats, is also not a natural entity but seems to be a soil-dependent ecotype.

Different analysis methods used on the RAPD data revealed slightly different phylogenetic estimations. The most distinct tree was obtained by UPGMA analysis. This tree (Fig. **3**) differs mainly with respect to two features from NJ and MP analyses: (1) Asian and European accessions occur as sister groups instead of the European group being part of the Asian, and (2) *"A. buhseanum",* ssp. *latiorifolium,* and var. *pumilum* are basal to the remaining accessions of *A. schoenoprasum.* It is remarkable that these basal taxa all possess individual branches which are much longer than branches in their next relatives (Figs. **3** and **4A**). The RAPD data thus violate the requirement of ultrametric data (equal mutation rates in all lineages), necessary for UPGMA analysis (Swofford et al., 1996[34]) which seems to result in an effect comparable to long branch attraction (Felsenstein, 1978[8]) which is sometimes seen in MP analysis. The exclusion of polyploid taxa from the UPGMA analysis did not effect the topological positions of the remaining accessions (tree not shown).

In the NJ analyses (Fig. **4**) the accessions of *A. schoenoprasum* are clearly geographically ordered. The Altaian var. *pumilum,* though with a much longer branch, groups together with the other Altaian material, and Iberian ssp. *latiorifolium* is basal to the European accessions. *"Allium buhseanum"* from Iran is sister group to the remaining lines of *A. schoenoprasum.* The exclusion of tetraploid ssp. *latiorifolium* influences the groups found within the European sample. Whereas the geographically related C and S European accessions occur together with ssp. *latiorifolium* at the base of this clade its exclusion results in three distinct geographic groups from Scandinavia, C plus S Europe, and E Europe.

Cladistic analysis shows that MP is sensitive to reticulate data structures. The exclusion of two polyploid taxa resulted in about 98% reduction of the number of most parsimonious trees and a much better resolved strict consensus tree (Fig. **5**). Consistency index (0.520 vs. 0.556) and retention index (0.642 vs. 0.654) are only slightly influenced by the polyploids. Bootstrap support for the European group, as well as for *A. schoenoprasum,* is increased when polyploids are excluded. Within the European accessions, again the Scandinavian and the E European group occur. Unlike the NJ analysis, the exclusion of ssp. *latiorifolium* did not force C and S European material to group together. Instead, these accessions form a basal group within the European material. The position of *"A. buhseanum"* in the MP trees is distinct from NJ and UPGMA results. Including the polyploid taxa, it is sister group to *A. karelinii;* after their exclusion, it occurs at a position within the Asian accessions of *A. schoenoprasum.* Making *"A. buhseanum"* sister group to either *A. karelinii* or *A. schoenoprasum* (excluding polyploids) requires one additional step, whereas a sister group relationship to the other species in the study needs five to seven additional steps. Alternative positions within *A. schoenoprasum* need three (sister group to the E Asian clade) to 12 additional steps (sister group to the E European clade). This behaviour of *"A. buhseanum",* shifting between a position near or within Asian *A. schoenoprasum* and *A. karelinii,* is clearly visualized by the PCA graph, where *"A. buhseanum"* occurs at a position intermediate to *A. karelinii* and the groups of *A. schoenoprasum.* No clear conclusions can be drawn about the taxonomical status of *"A. buhseanum".* An ancient hybrid origin from ancestors of *A. schoenoprasum* and *A. karelinii* might well be possible, as a relatively recent introgression of an *A. karelinii* genotype in a southern population of *A. schoenoprasum.* Additional studies, including more accessions of *"A. buhseanum"* and comparisons of the karyotypes, might elucidate the origin and taxonomical

rank of this taxon, which shows some morphological differences to the rest of *A. schoenoprasum* (colour of the tepals is greyish-lilac, pedicels equaling the tepals, filaments of tepal length).

As in *"A. buhseanum",* where the position in cladistic and phenetic analyses is more easily interpretable with the help of PCA, this method again helps in understanding the position of ssp. *latiorifolium* in the other analyses. Apart from UPGMA, where it occurs at a basal position in *A. schoenoprasum,* the other analyses revealed it as sister group to the European clade. Such a geographically "correct" position would suggest that ssp. *latiorifolium* shares a common ancestor with all other European accessions, though some reservations because of the result of UPGMA might persist. The position of ssp. *latiorifolium* in the PCA graph (Fig. **6**) renders this interpretation unlikely. The tetraploid occurs at a position which is different from that of the other members of sect. *Schoenoprasum,* suggesting a hybrid origin of this taxon under inclusion of *A. schoenoprasum* and a recently occurring *Allium* species of sect. *Schoenoprasum* is very unlikely. *Allium schmitzii,* the only other member of sect. *Schoenoprasum* on the Iberian peninsula, can clearly be ruled out as second parent of ssp. *latiorifolium* by PCA. Recent results of a study of taxa from sect. *Cepa* including some accessions of sect. *Schoenoprasum* with microsatellite markers, reported alleles of sect. *Cepa* occurring in ssp. *latiorifolium* (Fischer and Bachmann, submitted). This makes a Spanish member of sect. *Cepa* the most likely second parent of ssp. *latiorifolium.*

The clear division between Asian and European accessions found in all analyses parallels the distribution of *A. schoenoprasum,* where eastern and western populations are divided by a 2000 km gap in the distribution area in W Siberia (Fig. **1**). In our analysis we had no accessions from the easternmost area of the European habitat. The inclusion of such accessions might eventually weaken the clear geographical distinction between European and Asian material, even when taking into account that recently only limited gene flow should occur. The sole accession from North America appears at a position between the Asian and European groups (Fig. **6**), slightly closer to the Asian accessions. From the position in the trees, no firm conclusion can be drawn about the direction of migration in *A. schoenoprasum* because the Canadian accession most often appears as sister group to the European clade. This position seems to suggest either a migration from Asia via North America to Europe or a colonization of both North America and Europe from descendants of E Asian populations. Both scenarios seem rather unlikely in the light of oceanic migration barriers and the restricted spreading ability of *Allium* seeds. Instead, the exclusively Eurasian distribution of sect. *Schoenoprasum,* with the exception of *A. schoenoprasum* which inhabits the entire temperate zone of the Northern Hemisphere, together with its position in the phylogenetic analyses, allows the conclusion that *A. schoenoprasum* originated in Eurasia and reached North America via E Asia and Beringia (Hanelt et al., 1992[18]; Nimis et al., 1998[24]). A larger sample of Asian and North American accessions of *A. schoenoprasum* might have resulted in a closer relationship of these groups in the analyses. The genetic similarity between Asian and Canadian accessions supports this second hypothesis.

The geographical differentiation found within European *A. schoenoprasum* revealed three groups. Whereas the Scandinavian accessions are supported by bootstrap analysis no such support was obtained for the groups of other regions. The lack of statistical support for the S and E European groups might be influenced by the sampling strategy or by the population history of the groups. The inclusion of additional accessions from W Russia as well as from the Caucasian region and Iran might have altered these groups. Alternatively, when gene flow between populations separated in different S and E European refugia during the last glaciation period (Bennett et al., 1991[5]) started again after the post-glacial expansion of their range, this could result in a blurred phylogeographic pattern too. Whereas no absolute conclusions about migration routes between Asia and Europe and between E and S Europe can be drawn, the clear differentiation between Scandinavia and the rest of Europe is in accordance with a phylogenetic pattern that one would expect for a recolonization of N Europe after the last glaciation. Bootstrap support of the northern clade, as well as the stable sister group relationship (Figs. **4, 5B**) of an accession from Trentino in N Italy (Tax 0509), a putative refuge during the ice ages (I. Stehlik, Zürich, pers. commun.), to the Scandinavian *A. schoenoprasum,* makes an interpretation of the phylogeographic pattern of recolonization of N Europe from S Europe possible. Accession Tax 1306, the only accession from a botanical garden (BG Stockholm) in the study, groups with E European material and seems not to be of Scandinavian origin.

In spite of a possible bias due to the limited sample of accessions, the general phylogenetic pattern in the trees is primarily geographical (bootstrap support for European group), a result in accordance with recent studies in other plant groups (Vellekoop et al., 1996[37]; Friesen and Herrmann, 1998[13]; Gabrielson and Brochmann, 1998[17]; Tollefsrud et al., 1998[35]). Ecology, although important for morphological differentiation (Blattner and Kadereit, 1999[6]), plays a minor role for the observable phylogenetic differentiation in *A. schoenoprasum.* Only in the Altaian var. *pumilum* (Tax 3398) are genetic differences more pronounced than in other groups, as shown by a longer branch in the NJ analyses (Fig. **4**). This might be the start of an ecologically-driven separation due to adaptations to differences in soil. In the European vars. *alvarense* and *jurmoënse* no larger genetic distances are observable which would suggest a shorter differentiation time available for Scandinavian accessions when colonization of Europe took place from Asia.

Since RAPDs screen a great number of loci in the genomes, thus detecting a wide range of minute genetic differences, they should be insensitive to biases introduced when analyzing phylogenetic relationships with a single gene or DNA region. Therefore, the method should provide a robust picture of the relative role of geography, ecology, and time on lineage differentiation (Bachmann, 1997[2]; Le Corre et al., 1997[20]). The use of a wide range of analysis methods (UPGMA, NJ, MP, and PCA) allowed more detailed insights in possible phylogenetic patterns in the data than using a single analysis method. Every method shows other properties of the data under study and only a combination of these methods allows general data interpretation. UPGMA, though the most frequently used method to analyze RAPD data, produces a tree which deviates strongly from the results of the other analysis methods. Thus, the sole use of UPGMA to analyze RAPDs is not recommended.

The use of MP to detect gene flow and concealed hybrids can help to find problematic groups in the analysis which, with phenetic methods, are not visible but still influence the results. PCA especially seems to be very helpful to obtain a completely different graphic representation of the data. Without PCA some of the tree topologies (e.g., *"A. buhseanum"* as sister group to *A. karelinii* in the MP analysis) appear unlikely, but when including the result of PCA, a biological explanation for such strange trees can be found.

Acknowledgements

We thank Drs Konrad Bachmann and Peter Hanelt for supporting the work and critical comments on the manuscript. The expert technical assistance of Mrs. Petra Oswald is gratefully acknowledged.

References

[1] Antzupova, T. P. (1989) New species of the genus *Allium* (Alliaceae) from Buryatia. Novosti Systematiki Vysshich rastenii, Leningrad 26, 38 – 39 (in Russian).
[2] Bachmann, K. (1997) Nuclear DNA markers in plant biosystematic research. Opera Botanica 132, 137 – 148.
[3] Bachmann, K. and Hombergen, E.-J. (1996) Mapping genes for phenotypic variation in *Microseris* (Lactuceae) with molecular markers. In Compositae: Biology and Utilization, Vol. 2 (Caligari, P. D. S. and Hind, D. J. N., eds.), London: Kew Gardens, pp. 23 – 43.
[4] Backeljau, T., De Bruyn, L., De Wolf, H., Jordaens, K., van Dongen, S., and Winnepenninckx, B. (1995) Random amplified polymorphic DNA (RAPD) and parsimony methods. Cladistics 11, 119 – 130.
[5] Bennett, K. D., Tzedakis, P. C., and Willis, K. J. (1991) Quartenary refugia of north European trees. Journal of Biogeography 18, 103 – 115.
[6] Blattner, F. R. and Kadereit, J. W. (1999) Morphological evolution and ecological diversification of the forest-dwelling poppies (Papaveraceae: Chelidonioideae) as deduced from a molecular phylogeny of the ITS region. Plant Systematics and Evolution 219, 181 – 197.
[7] Colosi, J. C. and Schaal, B. A. (1997) Wild proso millet *(Panicum miliaceum)* is genetically variable and distinct from crop varieties of proso millet. Weed Science 45, 509 – 518.
[8] Felsenstein, J. (1978) Cases in which parsimony and compatibility methods will be positively misleading. Systematic Zoology 27, 401 – 410.
[9] Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783 – 791.
[10] Friesen, N. (1996) A taxonomic and chorological revision of the genus *Allium* L. sect. *Schoenoprasum* Dumort. Candollea 51, 461 – 473.
[11] Friesen, N., Borisjuk, N., Klaas, M., Mes, T. H. M., and Hanelt, P. (1997 a) Allotetraploid origin of *Allium altyncolicum* (sect. *Schoenoprasum*) as investigated by karyological and molecular markers. Plant Systematics and Evolution 206, 317 – 335.
[12] Friesen, N., Fritsch, R. M., and Bachmann, K. (1997 b) Hybrid origin of some ornamental *Alliums* of subgenus *Melanocrommyum* verified with GISH and RAPD. Theoretical and Applied Genetics 95, 1229 – 1238.
[13] Friesen, N. and Herrmann, N. (1998) Taxonomy, chorology and evolution of *Allium lusitanicum* – the European *"A. senescens"*. Linzer Biologische Beiträge 30, 815 – 830.
[14] Friesen, N. and Klaas, M. (1998) Origin of some minor vegetatively propagated *Allium* crops studied with RAPD and GISH. Genetic Resources and Crop Evolution 45, 510 – 523.
[15] Friesen, N., Pollner, S., Bachmann, K., and Blattner, F. R. (1999) RAPDs and non-coding chloroplast DNA reveal a single origin of the cultivated *Allium fistulosum* from *A. altaicum*. American Journal of Botany 86, 554 – 562.
[16] Gabrielsen, T. M., Bachmann, K., Jakobsen, K. S., and Brochmann, C. (1997) Glacial survival does not matter: RAPD phylogeography of Nordic *Saxifraga oppositifolia*. Molecular Ecology 6, 831 – 842.
[17] Gabrielsen, T. M. and Brochmann, C. (1998) Sex after all: high levels of diversity detected in the Arctic clonal plant *Saxifraga cernua* using RAPD markers. Molecular Ecology 7, 1701 – 1708.
[18] Hanelt, P., Schultze-Motel, J., Fritsch, R., Kruse, J., Maaß, H. I., Ohle, H., and Pistrick, K. (1992) Infragenetic grouping of *Allium* – the Gaterslebenen approach. In The Genus *Allium* – Taxonomic Problems and Genetic Resources (Hanelt, P., Hammer, K., and Knüpffer, H., eds.), Gatersleben: IPK, pp. 107 – 123.
[19] Harris, S. A. (1995) Systematics and randomly amplified polymorphic DNA in the genus *Leucaea* (Leguminosae, Mimosoideae). Plant Systematics and Evolution 197, 195 – 208.
[20] Le Corre, V., Dumolin-Lapègue, S., and Kremer, A. (1997) Genetic variation at allozyme and RAPD loci in sessile oak *Quercus petraea* (Matt.) Liebl.: the role of history and geography. Molecular Ecology 6, 519 – 529.
[21] Maddison, W. P. and Maddison, D. R. (1992) MacClade: interactive analysis of phylogeny and character evolution, version 3.05. Sunderland: Sinauer.
[22] Mes, T. H. M., Friesen, N., Fritsch, R. M. Klaas, M., and Bachmann, K. (1997) Criteria for sampling in *Allium* based on chloroplast DNA PCR-RFLP's. Systematic Botany 22, 701 – 712.
[23] Morell, M. K., Peakall, R., Appels, R., Preston, L. R., and Lloyd, H. L. (1995) DNA profiling techniques for plant variety identification. Australian Journal of Experimental Agriculture 35, 807 – 819.
[24] Nimis, P. L., Malyshev, L. I., Bolognini, G., and Friesen, N. (1998) A multivariate phytogeographic analysis of plant diversity in the Putorana Plateau (N Siberia). Opera Botanica 136, 1 – 72.
[25] Pillay, M. and Kenny, S. T. (1995) Anomalies in direct pairwise comparisons of RAPD fragments for genetic analysis. BioTechniques 19, 694 – 698.
[26] Purps, D. M. L. and Kadereit, J. W. (1998) RAPD evidence for a sister group relationship of the presumed progenitor-derivate species pair *Senecio nebrodensis* and *S. viscosus* (Asteraceae). Plant Systematics and Evolution 211, 57 – 70.
[27] Rieseberg, L. H. (1996) Homology among RAPD fragments in interspecific comparisons. Molecular Ecology 5, 99 – 105.
[28] Rieseberg, L. H., Choi, H., Chan, R., and Spore, C. (1993) Genomic map of a diploid hybrid species. Heredity 70, 285 – 293.
[29] Roelofs, D. and Bachmann, K. (1997) Comparison of chloroplast and nuclear phylogeny in the autogamous annual *Microseris douglasii* (Asteraceae, Lactuceae). Plant Systematics and Evolution 204, 49 – 63.
[30] Saitou, N. and Nei, M. (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Journal of Molecular Evolution 4, 406 – 425.
[31] Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.
[32] Serquen, F. C., Bacher, J., and Straub, J. E. (1997) Mapping and QTL analysis of horticultural traits in a narrow cross in cucumber (*Cucumis sativus* L.) using random-amplified polymorphic DNA markers. Molecular Breeding 3, 257 – 268.
[33] Stearn, W. T. (1978) European species of *Allium* and allied genera of Alliaceae – a synonymic enumeration. Annales Musei Goulandris 4, 83 – 198.
[34] Swofford, D. L., Olsen, G. J., Waddell, P. J., and Hillis, D. M. (1996) Phylogenetic inference. In Molecular Systematics, 2nd ed. (Hillis, D. M., Moritz, C., and Mable, B. K., eds.), Sunderland: Sinauer, pp. 407 – 514.

[35] Tollefsrud, M. M., Bachmann, K., Jakobsen, K. S., and Brochmann, C. (1998) Glacial survival does not matter – II: RAPD phylogeography of Nordic *Saxifraga cespitosa*. Molecular Ecology 7, 1217 – 1232.
[36] van der Zande, L. and Bijlsma, R. (1994) Limitation of the RAPD technique in phylogeny reconstruction in *Drosophila*. Journal of Evolutionary Biology 8, 645 – 656.
[37] Vellekoop, P., Buntjer, J. B., Maas, J. W., and van Brederode, J. (1996) Can the spread of agriculture in Europe be followed by tracing the spread of the weed *Silene latifolia*. A RAPD study. Theoretical and Applied Genetics 92, 1085 – 1090.
[38] Welsh, J. and McClelland, M. (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Research 18, 7213 – 7218.
[39] Williams, J. G., Kubelik, A. R., Livak, K. J., Rafalski, J. A., and Tingey, S. V. (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18, 6531 – 6535.
[40] Wolfe, A. D. and Liston, A. (1998) Contributions of PCR-based methods to plant systematics and evolutionary biology. In Molecular Systematics of Plants II: DNA Sequencing (Soltis, D. E., Soltis, P. S., and Doyle, J. J., eds.), Dordrecht: Kluwer, pp. 43 – 86.

N. Friesen

John Innes Centre
Norwich NR7 4UH
UK

E-mail: nikolai.friesen@bbsrc.ac.uk

Section Editor: M. Hasebe