ORIGINAL ARTICLE

Amplified fragment length polymorphism (AFLP) suggests old and recent immigration into the Alps by the arctic-alpine annual *Comastoma tenellum* (Gentianaceae)

Peter Schönswetter[1]*, Andreas Tribsch[2] and Harald Niklfeld[1]

[1]*Department of Plant Chorology and Vegetation Science and* [2]*Department of Systematics and Evolution of Higher Plants, Institute of Botany, University of Vienna, Vienna, Austria*

ABSTRACT

Aim This study aims to elucidate the phylogeography of the arctic-alpine annual *Comastoma tenellum* (Rottb.) Toyok. (Gentianaceae) and to unravel the history of its immigration into the Alps.

Location Although samples from Alaska and Central Asia were also included, our study focusses on Europe, especially on the Alps.

Methods We applied amplified fragment length polymorphism (AFLP) fingerprinting on 37 populations (162 individuals) of *C. tenellum* and analysed the results phenetically.

Results As *C. tenellum* is mainly inbreeding, there is typically little to no intrapopulational genetic variation. Two populations from Alaska and Altai are strongly separated from all other accessions. The majority of the populations from the Alps group together with high bootstrap support. They fall into an unsupported Alps I group (northwards of Gran Paradiso) and a well-supported Alps II group (south-western Alps). The remaining European populations form a weakly-supported branch constituting accessions from the Carpathians, Scandinavia and two populations from the Eastern Alps.

Main conclusions *Comastoma tenellum* reached the Alps at least twice. The first immigration event resulted in a lineage that is clearly separated from the other European accessions. The immigration must have occurred well before the last glaciation because this lineage shows further phylogeographical structuring into two groups (Alps II in the south-western Alps and Alps I in the rest of the Alps). This pattern is presumably due to isolation in different glacial refugia. In addition to the old immigration event, the species reached the Alps in recent times either from Scandinavia or from the Carpathians via long-distance dispersal. These immigrations resulted in (at least) two populations that are spatially small and poor in individuals.

Keywords

Amplified fragment length polymorphism (AFLP), Pleistocene, *Comastoma tenellum*, Alps, arctic-alpines, phylogeography.

*Correspondence: Peter Schönswetter, Institute of Botany, University of Vienna, Rennweg 14, A-1030 Vienna, Austria.
E-mail: peter.schoenswetter@univie.ac.at
Present address: Peter Schönswetter, National Centre for Biosystematics (NCB), The Natural History Museums and Botanical Garden, University of Oslo, PO Box 1172, Blindern, N-0318 Oslo, Norway.

INTRODUCTION

Arctic-alpine plant taxa are disjunctly distributed in arctic latitudes and more southern mountain ranges (Hultén & Fries, 1986). Many of them occur in vast areas of the Northern hemisphere and are important elements of the vegetation cover. However, in striking contrast to the rapidly accumulating body of literature exploring phylogeographical patterns of arctic or alpine plants on a regional scale (e.g. within the central and southern European mountain ranges or Scandinavia; e.g. Gabrielsen *et al.*, 1997; Stehlik *et al.*, 2001, 2002a,b; Holderegger *et al.*, 2002; Kropf *et al.*, 2002, 2003; Schönswetter

et al., 2002, 2003a, 2004; Stehlik, 2002; Tribsch *et al.*, 2002; Brochmann *et al.*, 2003), there is still comparatively little knowledge of phylogeographical patterns on a wider geographical scale including both European alpine and arctic habitats.

Abbott *et al.* (2000) provided the first full area phylogeography of an arctic-alpine taxon, *Saxifraga oppositifolia* L. (Saxifragaceae). In the Arctic, the species first occurred in western Beringia. Subsequent migration eastwards and westwards led to the present circumpolar distribution. High levels of cpDNA variation, possibly indicative of glacial refugia in the Arctic, were found in north-western North America, Greenland and the Taymyr peninsula. Després *et al.* (2002) explored the phylogeography of *Trollius europaeus* L. in the Pyrenees, the Alps and Fennoscandia applying the amplified fragment length polymorphism (AFLP) technique. The authors showed that the species probably survived the last glaciation in more or less interconnected southern populations, represented nowadays by the relict populations found at high elevation in the Pyrenees, the Alps and the Carpathians. The high arctic populations originated from one single south-eastern lineage. Low genetic diversity in Fennoscandia was attributed to a past founder event during northward recolonization. In a further AFLP study, Schönswetter *et al.* (2003b) elucidated the phylogeography of *Ranunculus glacialis* L. subsp. *glacialis* including populations from the Alps, the Pyrenees, the Tatra and northern Europe. The taxon experienced old vicariance in the Alps and the Tatra. The Pyrenees were apparently more recently colonized. Populations in Northern Europe, by contrast, were similar to those from the Eastern Alps but genetically much less variable. It was thus concluded that *R. glacialis* colonized Northern Europe fairly recently, accompanied by a strong founder event.

Arctic-alpine distributions may have been shaped by processes acting on different time scales. They might be of recent origin, promoted by late-glacial or post-glacial migrations. Recent range expansion should lead to a lack of genetic differentiation among populations from different portions of the distribution area, due to the short time span being insufficient for creating substantial genetic divergence. In contrast, old range expansions accompanied by disruption of the distribution area should lead to recognizable genetic differentiation due to the lack of gene flow. It has been shown, e.g. for Alpine subendemic *Saponaria pumila* Janchen (Tribsch *et al.*, 2002) and arctic-alpine *R. glacialis* (Schönswetter *et al.*, 2003b) that processes on different time horizons can be differentiated.

In this study, we report phylogeographic data on *Comastoma tenellum* (Rottb.) Toyok. (syn. *Gentiana tenella* Rottb., *Gentianella tenella* [Rottb.] Börner; Gentianaceae) obtained by AFLP fingerprinting. Our sampling covers, at least punctually, large portions of the species' entire distribution. Firstly, we aim to explore the large-scale phylogeographical structure among populations from Alaska, the Altai, the Alps, the Carpathians and Scandinavia. Secondly, we aim to elucidate the phylogeographic pattern of our study taxon within the Alps. For the origin of the Alpine populations, several scenarios appear plausible and are surveyed in this paper. (a) The populations originate from a fairly recent immigration, thus they should not be genetically divergent from the source populations that can be readily traced with AFLPs (as shown by Schönswetter *et al.*, 2003b). (b) The populations originate from an old dispersal and/or vicariant event and are thus strongly differentiated from their source populations. At least with the applied technique, it will no longer be possible to trace the source populations. Furthermore, due to separation in Pleistocene refugia, the Alpine populations might exhibit a phylogeographical structure. Additionally, (c), the Alpine populations could have originated from a single or from multiple immigration events.

MATERIALS AND METHODS

The study species

Comastoma tenellum is a small (2–10 cm) diploid annual plant. It exhibits an arctic-alpine distribution and is found in (sub)arctic regions of Eurasia and North America as well as in more southern mountain ranges such as the Atlas, the Pyrenees, the Alps, the Carpathians, the Caucasus, the Central Asian mountain system and the southern Rocky Mountains (Hultén & Fries, 1986). In the Alps the species is widespread in the higher parts, growing on limestone and basic silicates from 1700 to 3100 m (Hegi, 1925). The flowers produce nectar (Hegi, 1925) and occasional visits by bumblebees were observed (P. Schönswetter and A. Tribsch, pers. obs.). However, autogamy seems to predominate at least during periods with unfavourable weather (Hegi, 1925) as the theca often already open in the flower buds (P. Schönswetter and A. Tribsch, pers. obs.). The seeds are lens-shaped, 0.7 mm long (Hegi, 1925) and lack morphological adaptations for long-distance dispersal.

Sampling

We sampled 31 populations of *C. tenellum* from the Alps (populations 1–31), two populations from Scandinavia (32–33), and one each from the Tatra (34), the southern Carpathians (35), the Altai (36) and Alaska (37), respectively (Fig. 1). As an outgroup, we included samples of *C. malyschevii* (Zuev) V.V. Zuev from Altai. Details of the sampling locations as well as the number of sampled individuals per population are given in Table 1. Voucher specimens for all populations are deposited in the herbarium of the Institute of Botany of the University of Vienna (WU).

DNA isolation and AFLP fingerprinting

Protocols for DNA isolation as well as for AFLP fingerprinting are described in Schönswetter *et al.* (2002) and Schönswetter *et al.* (2004), respectively. For the selective amplification, we used three primer combinations: *Eco*RI ACC (NED)-*Mse*I
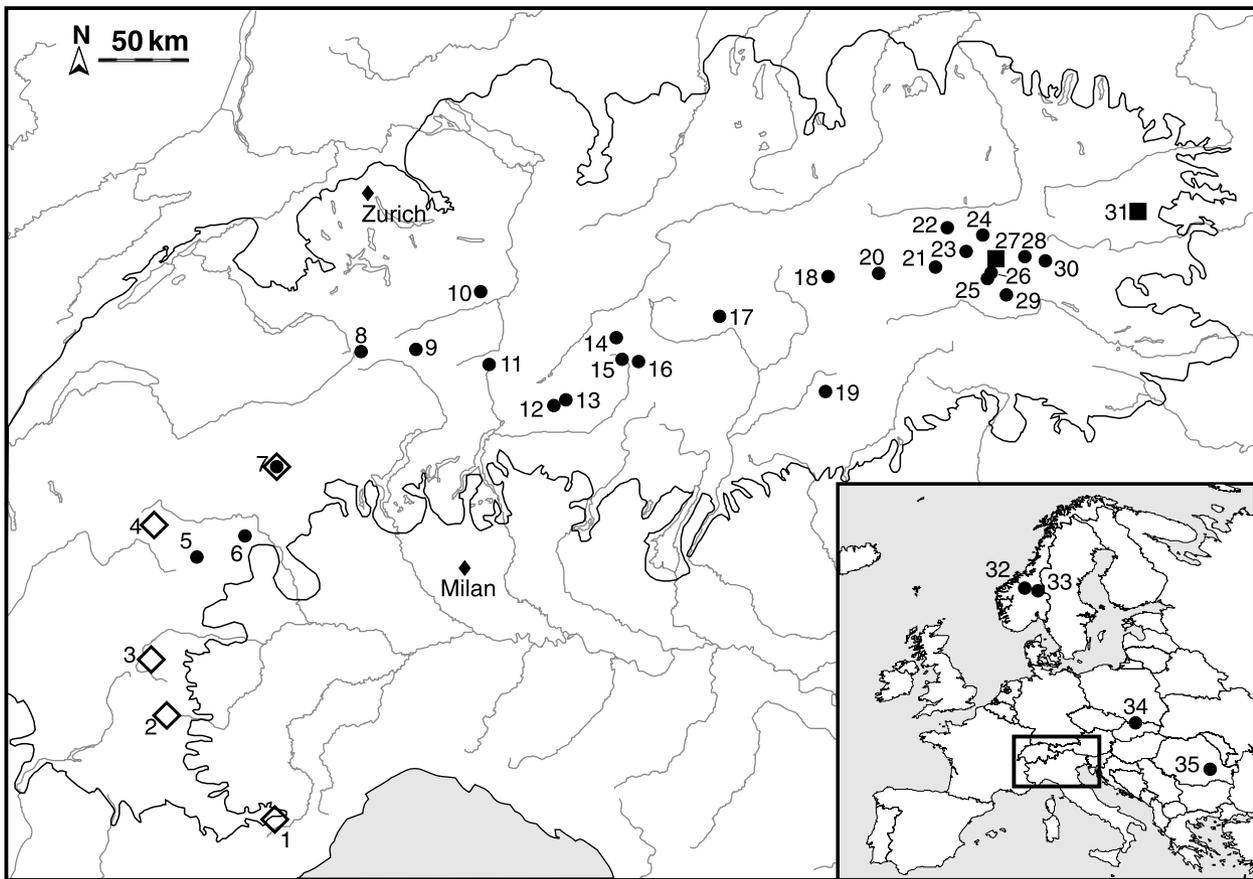
Figure 1 Sampled populations of *Comastoma tenellum* (Rottb.) Toyok. in the European Alps along with the maximum extent of the Pleistocene ice shield during the last glacial period (black line; modified from Jäckli, 1970; Van Husen, 1987; Voges, 1995). Rhombs, populations of Alps I; dots, Alps II; filled squares, Alps III. Population 7 has AFLP phenotypes of Alps I and Alps II. Bottom right insert gives the locations of populations 32–35 and indicates the position of the Alpine sampling area. Population numbers refer to Table 1. Populations 36 from Altai (Central Asia) and 37 from Alaska are not shown.

CAT, *Eco*RI ACA (6-Fam)-*Mse*I CAC, and *Eco*RI AGG (HEX)-*Mse*I CTG. The fluorescence-labelled selective amplification-products were separated on a 5% polyacrylamide gel with an internal size standard (GeneScan-500 [ROX], PE Applied Biosystems, Foster City, CA, USA) on an automated sequencer (ABI 377, PE Applied Biosystems). 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 (ver. 1.6.0©, Montana State University 1999; http://hordeum.msu.montana.edu/genographer/) for scoring of the fragments. The results of the scoring were exported as a presence/absence matrix and used for further analysis.

Data analysis

The number of AFLP fragments and the percentage of polymorphic fragments per population were calculated. We performed a neighbour-joining analysis with TREECON 1.3b (Van de Peer & De Wachter, 1997) based on Nei & Li's (1979) distance among all AFLP phenotypes. The tree was rooted with

C. malyschevii. Robustness of clusters was estimated using the bootstrap approach (Felsenstein, 1985) with 1000 replicates. Analyses of molecular variance (AMOVAS) were computed with ARLEQUIN 1.1 (Schneider *et al.*, 1997).

RESULTS

With the three primer combinations used, 130 unambiguously scoreable AFLP fragments were generated, 129 of which (99.2%) were polymorphic. Excluding the outgroup *C. malyschevii*, 122 fragments (93.8%) were polymorphic. The length of the fragments varied from 54 to 477 bp. We detected 102 different AFLP phenotypes in the 167 investigated individuals. Identical AFLP phenotypes were normally found only within populations; only the single investigated individual of population 30 shared its phenotype with three individuals from population 26. The number of AFLP-fragments per population varied from 38 in population 1 to 53 in population 17 (mean 45.66, SD = 3.92; Table 1). The percentage of polymorphic fragments ranged from zero in populations 5 and 24 to 38.00 in population 33 (mean 9.30, SD = 8.93; Table 1).

	Location	Country	Co-ordinates (E/N)	N	Frag.	%$_{poly}$
1	Bocchetta dell Aseo	I	7.78/44.13	3	38	7.89
2	Col Agnel	F/I	6.97/44.68	3	40	7.50
3	Monte Genevris	I	6.87/44.98	3	39	10.26
4	Col du Petit St. Bernard	F/I	6.87/45.67	5	40	5.00
5	Gran Paradiso	I	7.20/45.52	4	43	0.00
6	Champorcher	I	7.55/45.62	5	48	2.08
7	Gornergrat	CH	7.80/45.98	5	53	32.08
8	Furkapass	CH	8.40/46.57	5	43	6.98
9	Passo Lucomagno	CH	8.80/46.58	4	42	7.14
10	Cassonsgrat	CH	9.27/46.87	4	42	9.52
11	Splügenpass	CH/I	9.33/46.50	5	47	4.26
12	Pizzo Scalino	I	9.82/46.30	5	48	2.08
13	Bocchetta Forbici	I	9.90/46.32	5	47	2.13
14	Ofenpass	CH	10.27/46.63	5	49	2.04
15	Val Cancan	CH/I	10.32/46.53	5	49	4.08
16	Monte Scorluzzo	I	10.42/46.52	5	51	15.69
17	Pfossental	I	11.02/46.75	5	53	16.98
18	Tristenspitz	I	11.82/46.95	5	48	10.42
19	Cima Margerita	I	11.80/46.37	3	48	2.08
20	Totenkarspitze	A	12.18/46.97	5	47	2.13
21	Kalser Höhe	A	12.60/47.00	5	49	2.04
22	Kitzsteinhorn	A	12.68/47.20	5	47	10.64
23	Hochtor	A	12.83/47.08	5	46	13.04
24	Kogelkarkopf	A	12.94/47.16	3	40	0.00
25	Sadnig	A	12.98/46.93	5	45	8.89
26	Ochsentrieb	A	13.00/46.97	5	43	9.30
27	Wurtenkees	A	13.02/47.00	5	48	12.50
28	Striedenkopf	A	13.12/46.86	4	40	7.50
29	Ankogel	A	13.25/47.05	5	45	4.44
30	Wandspitze	A	13.53/47.02	1	43	–
31	Sölkpass	A	14.08/47.27	5	48	10.42
32	Hjerkinn	NO	9.55/62.25	5	45	2.22
33	Dalholen	NO	9.83/62.17	4	50	38.00
34	Kopa Magury	PL	20.00/49.22	1	43	–
35	Baba Mare	RO	25.47/45.41	5	43	11.63
36	Altai, Aktash	RU	87.73/50.32	5	44	31.82
37	Seward Peninsula	USA	−166.35/65.26	5	50	4.00
38	*C. malyschevii*: Altai, southern Chuya range	RU	88.21/49.66	5	50	18.00

Table 1 Numbering of populations, location, country*; co-ordinates; number of investigated individuals (*N*); number of observed AFLP-fragments (Frag.) per population; percentage of fragments which exhibit intrapopulational polymorphisms (%$_{poly}$) for all 37 investigated populations of *C. tenellum* and the outgroup taxon *C. malyschevii*

A, Austria; CH, Switzerland; F, France; I, Italy; NO, Norway; PL, Poland; RU, Russia; USA, United States of America.

The neighbour-joining analysis (Fig. 2) revealed the following groups: *Comastoma malyschevii* is strongly differentiated from all *C. tenellum* populations. Other strongly separated groups are the accessions from Alaska (population 37; 100% bootstrap support) and from Altai (population 36; 91% bootstrap support). The majority of the populations from the Alps group together with high bootstrap support (98%). This branch resolves into two groups, the unsupported Alps I comprising populations 5–26 and 28–30 with the exception of two individuals from population 7 and the well-supported (98% bootstrap support) Alps II with populations 1–4 plus three individuals from population 7. The remaining European populations form a weakly supported (55% bootstrap support)

branch. It comprises accessions from the Southern Carpathians (population 35; 91% bootstrap support), the Tatra (population 34), Scandinavia (populations 32, 33) and two populations from the eastern part of the Central Alps, (populations 27 and 31; 60% bootstrap support) referred to here as Alps III. Alps I and Alps II together have 19 private fragments, one of which is fixed. Of these, 14 are private to Alps I, two are exclusive to Alps II and three are found in both groups.

Non-hierarchical analyses of molecular variance (AMOVAs, Table 2) assigned 87% of the overall genetic variation to variation among the 37 populations. If only the populations from the Alps are taken into account, this value changes only slightly to 85%. Hierarchical AMOVAs of the Alpine
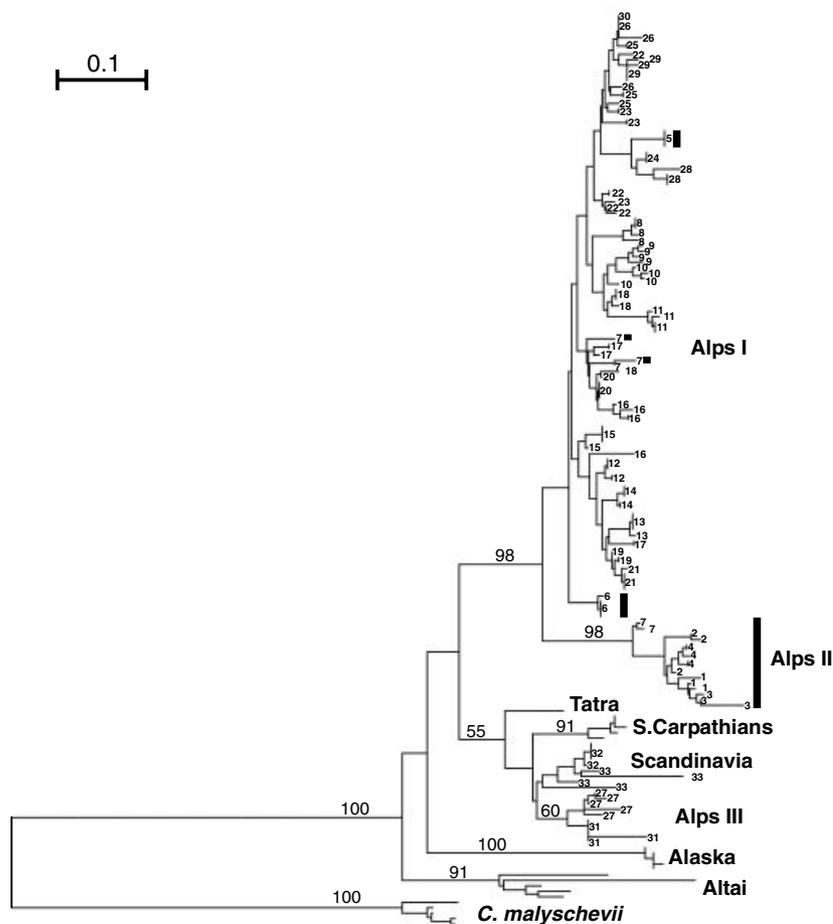
0.1

Figure 2 Neighbour-joining tree based on genetic distances of 162 individuals of *Comastoma tenellum* (Rottb.) Toyok. from the Alps, the Carpathians, Scandinavia, the Altai and Alaska rooted with five individuals from Central Asian *C. malyschevii* (Zuev) V.V. Zuev. Numbers above branches indicate bootstrap values higher than 50% (1000 replicates). To enforce legibility, they are given only for the major branches. For the Alpine and Scandinavian individuals, the population numbers are given. For individuals with identical AFLP profiles, the population number is given only once. In order to illustrate the mixing of Eastern and Western alpine individuals in Alps I, the individuals from populations 1 to 7 are marked by bold vertical lines.

Table 2 Analysis of molecular variance (AMOVA) of the 37 investigated populations of *Comastoma tenellum*

Source of variation	d.f.	Sum of squares	Variance components	% Total variance	F_{ST}**
Among populations 1–37	36	1164.82	7.21	87.24	0.87
Within populations	124	130.63	1.05	12.76	
Among Alpine populations (1–31)	30	720.44	5.23	84.95	0.85
Within Alpine populations	106	98.23	0.93	15.05	
Among Alps I*, Alps II*, Alps III	2	338.07	7.50	66.76	0.93
Among populations	27	370.87	2.95	26.24	
Within populations	102	80.23	0.79	7.00	
Among Alps I* and Alps II*	1	154.97	5.76	60.44	0.92
Among populations	26	362.37	3.02	31.71	
Within populations	94	70.23	0.75	7.85	

*Excluding population 7 that was split between Alps I (two individuals) and Alps II (three individuals).

**All *P*-values were <0.001.

populations attributed 67% of the overall variation to variation among Alps I, Alps II and Alps III, 26% to variation among the populations within the groups and only 7% to variation within populations. When disregarding Alps III, the among-groups component accounting for variation between Alps I and Alps II is 60% (Table 2).

DISCUSSION

Our AFLP data corroborate the hypothesis that *C. tenellum* is predominantly selfing (Hegi, 1925). Analyses of molecular variance (AMOVAs; Table 2) attribute a very low percentage of the overall variation to the within-population component and

the genetic diversity within populations (%$_{poly}$; Table 1) is low in most of the investigated populations. These results are in line with the observation that a high proportion of total genetic diversity residing among and not within populations is characteristic for annual, selfing species (Hamrick & Godt, 1996). Furthermore, similar AMOVA results were obtained in other studies on facultative or obligatory autogamous plants (e.g. Jiménez *et al.*, 2002; Holderegger *et al.*, 2003; Tremetsberger *et al.*, 2003). A few populations (populations 7, 33, 36), however, exhibit a comparatively high percentage of polymorphic fragments. The highest percentage of polymorphic fragments was found in population 7 resulting from the co-occurrence of individuals of two different lineages, Alps I and Alps II. We can not offer a sound explanation for the high level of variation in populations 33 and 36 from Scandinavia and Altai, respectively. High genetic variation can be expected in contact zones, as in population 7, but also in outbreeding populations. We have no information if *C. tenelleum* is selfing throughout its range. Alternatively, the low variation in most of the investigated populations could be also a result of genetic bottlenecks or a selection of autogamous individuals in the course of long-distance colonization ('Baker's Rule'; Baker, 1955; Pannel & Barrett, 1998). To distinguish between these hypotheses and to rule out secondary contact zones as causes for high genetic diversity in few of the investigated populations, a denser sampling from these regions including more individuals per population would be necessary.

Large-scale phylogeographical structure

The circumpolar, arctic-alpine *C. tenellum* did not obtain its wide extant distribution in post-glacial times. In contrast, old vicariance is suggested by the strong phylogeographical structure revealed by the neighbour-joining analysis (Fig. 2). The populations from Altai (Central Asia) and from Alaska are well separated from the European accessions. The resolution in the basal nodes of the tree, however, is weak and the lack of statistical support discourages conclusions about the relationships among Altaic, Alaskan and European populations.

The genus *Comastoma* has its centre of diversity and presumably its origin in Central Asia (Meusel *et al.*, 1978; Hagen & Kadereit, 2002). In spite of this, we found clear phylogeographical structure within Europe for *C. tenellum* suggesting that the species has been present there for a fairly long-time. The European populations form two major branches, one well-supported strictly Alpine group characterized by a relatively high number of private fragments and a second group with the accessions from Carpathians, Scandinavia and two populations from the eastern part of the Central Alps in Austria. This pattern partly contrasts with that found in other arctic-alpine plant taxa. In *S. oppositifolia* (Abbott *et al.*, 2000), only widespread cpDNA haplotypes were found in the Alps, indicating no long-term isolation of the Alpine populations from the arctic accessions. In *R. glacialis* (Schönswetter *et al.*, 2003b) the Carpathian (Tatra) population was strongly divergent from the Alpine ones, but northern European

(Scandinavian) populations were nested in an Eastern Alpine lineage. The latter close relationship of Alpine and Scandinavian populations is in contrast to the pattern observed in *C. tenellum*. Similar to the situation in our study taxon, a close relationship between Carpathian and Scandinavian populations was observed in *T. europaeus* (Després *et al.*, 2002).

Old and recent immigration into the Alps

Within the Alps, there is strong differentiation into the population groups Alps I, Alps II and Alps III (Fig. 2). Analyses of molecular variance (AMOVAs) indicate that two-thirds of the overall genetic variation of the Alpine populations is explained by variation among the three major population groups (Table 2). Alps I and Alps II were isolated from the other populations investigated for a long-time as they form a monophyletic group with high bootstrap support (Fig. 2) and are characterized by private fragments. Alps III, in contrast, is nested in a branch mainly formed by Carpathian and Scandinavian accessions. Hierarchical AMOVAs revealed that there is strong differentiation between Alps I and Alps II, as three-fifths of the overall genetic variation is explained by the differentiation of the two groups (Table 2). Furthermore, both groups have private fragments.

Alps I and Alps II cover unequally large portions of the Alpine arch (Fig. 1). Whereas populations of Alps I are widespread north- and eastwards of the Gran Paradiso group (Italy), Alps II inhabits only the south-western Alps. It can be assumed that isolation of populations in Pleistocene refugia, maybe at the periphery of the Alps or in the lowlands surrounding the Alps, led to the present phylogeographical structure. This implies that *C. tenellum* reached the Alps or at least Middle Europe well before the last glacial maximum. Alternatively, the species might have immigrated into the south-western Alps from the Pyrenees where it is very rare today (e.g. Bolòs *et al.*, 1990). A close relationship between Alpine and Pyrenean plant populations was already detected in previous phylogeographic studies (Kropf *et al.*, 2002, 2003; Schönswetter *et al.*, 2002). Individuals of Alps I and Alps II co-occur in contact zones, as exemplified by the assignment of the investigated individuals of population 7 from south-western Switzerland to Alps I and Alps II, respectively. A similar co-occurrence of individuals derived from divergent phylogeographical lineages was detected in the previously investigated *Erinus alpinus* L. in a population from eastern Switzerland (Stehlik *et al.*, 2002b). Alternatively, this co-occurrence might be caused by long-distance dispersal. The density of sampled populations in the Penninic Alps, however, is much too low for allowing conclusions.

Potential glacial refugia for Alps II were most probably situated in the south-western Alps, an area that suffered comparatively little from Pleistocene glaciations (Penck & Brückner, 1909) and thus is characterized by a high level of endemism (Pawłowski, 1970). Distinct phylogeographic groups in that region were already identified in other studies (Schönswetter *et al.*, 2002, 2004). Within the large population group

Alps I, there is hardly any structure. Populations from Western and Eastern Alps do not form groups, but are intermingled in the neighbour-joining tree (Fig. 2). The lack of a genetic structure suggests glacial survival in and post-glacial spread from only one Pleistocene refugium. As *C. tenellum* grows on limestone as well as on basic silicates, large areas at the southern and eastern periphery of the Alps might have acted as refugia. Alternatively, even glacial survival in the plains surrounding the Alps seems probable, as *C. tenellum* often descends to alluvial terraces in the Alps. In Central Asia, the species also occurs in steppe-like vegetation (A. Tribsch, pers. obs.). The results from *C. tenellum* contrast with those of other Alpine taxa so far investigated with similar sampling strategies, where strong phylogeographical patterns were detected that could be related to presumed glacial refugia at the periphery of the Alps and/or on nunataks (Schönswetter *et al.*, 2002, 2003a, 2004; Stehlik *et al.*, 2001, 2002a,b; Kropf *et al.*, 2002, 2003; Stehlik, 2002; Tribsch *et al.*, 2002; but see Holderegger *et al.*, 2002).

In spite of the dense sampling in the Eastern Alps, we found only two populations (populations 27 and 31) that belong to group Alps III. The neighbour-joining analysis demonstrated a close relationship with Carpathian and Scandinavian populations. Neither genetic distances nor the distribution of AFLP markers (not shown), however, allowed identification of the closest relatives of these two populations. Both are small in spatial extent and harbour only few individuals. This is especially true for the geographically-isolated population 31, by far the easternmost population of *C. tenellum* in the Alps. It was discovered only recently (Schönswetter & Tribsch, 2003) and is not much larger than a single square meter! The small extent of the two populations of Alps III obviously indicates recent, possibly even very recent immigration from source populations in Scandinavia or the Carpathians to the eastern Central Alps of Austria.

So, irrespective of the immigration pathways *C. tenellum* reached the Alps at least twice, first by a likely older colonization event resulting in the largely geographically vicariant lineages Alps I and Alps II, and, second, by a relatively recent (late glacial or post-glacial) long-distance dispersal event. The latter is remarkable for three reasons. (1) The seeds of *C. tenellum* lack morphological adaptations for dispersal by wind. Hence, bird-mediated seed dispersal appears a possible cause for the observed long-distance dispersal in *C. tenellum*. However, recent molecular studies have shown that long-distance dispersal is much more frequent than previously believed. There is, e.g. molecular evidence for presumably post-glacial trans-atlantic dispersal for 'short-distance dispersers' (reviewed in Abbott & Brochmann, 2003; Brochmann *et al.*, 2003). (2) As the dispersal event occurred after the last glacial maximum, there existed no stepping stones between the disjunct portions of the distribution area of *C. tenellum* in Europe. In contrast, continuous migration through the lowland or stepwise dispersal involving different mountain ranges that have no extant alpine vegetation belt, were certainly more straightforward during the cold stages of the Pleistocene. (3) The eastern Central Alps of Austria, especially the range of

the Niedere Tauern, where population 31 grows, are a centre of rare disjunct arctic-alpine and Alpic-Carpathian taxa in the Alps (Schneeweiss & Schönswetter, 1999). Recent immigration of *C. tenellum* from Scandinavia or the Carpathians might suggest that at least some of these disjunctions promoted by unknown dispersal agents might rather be results of recent dispersals than of old vicariance.

REFERENCES

Abbott, R.J. & Brochmann, C. (2003) History and evolution of the arctic flora: in the footsteps of Eric Hultén. *Molecular Ecology*, **11,** 299–313.

Abbott, R.J., Smith, L.C., Milne, R.I., Crawford, R.M.M., Wolff, K. & Balfour, J. (2000) Molecular analysis of plant migration and refugia in the Arctic. *Science*, **289,** 1343–1346.

Baker, H.G. (1955) Self-compatibility and establishment after 'long-distance' dispersal. *Evolution*, **9,** 347–348.

Bolòs, O., Vigo, J., Masalles, R.M. & Ninot, J.M. (1990) *Flora Manual dels Paisos Catalans*. Editorial Pòrtic S.A., Barcelona.

Brochmann, C., Gabrielsen, T.M., Nordal, I., Landvik, J.Y. & Elven, R. (2003) Glacial survival or *tabula rasa*? The history of North Atlantik biota revisited. *Taxon*, **52,** 417–450.

Després, L., Loriot, S. & Gaudeul, M. (2002) Geographic pattern of genetic variation in the European globeflower *Trollius europaeus* L. (Ranunculaceae) inferred from amplified fragment length polymorphism markers. *Molecular Ecology*, **11,** 2337–2347.

Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, **39,** 783–791.

Gabrielsen, T.M., Bachmann, K., Jakobsen, K.S. & Brochmann, C. (1997) Glacial survival does not matter: RAPD phylogeography of Nordic *Saxifraga oppositifolia*. *Molecular Ecology*, **6,** 831–842.

Hagen, K.B. & Kadereit, J.W. (2002) Phylogeny and flower evolution of the Swertiinae (Gentianaceae-Gentianeae): Homoplasy and the principle of variable proportions. *Systematic Botany*, **27,** 548–572.

Hamrick, J.L. & Godt, M.J.W. (1996) Effects of life history traits on genetic diversity in plant species. *Philosophical*

Transactions of the Royal Society in London, Series B, **351**, 1291–1298.

Hegi, G. (1925) *Illustrierte Flora von Mitteleuropa. Band V, Teil 3*. Parey, Berlin-Hamburg.

Holderegger, R., Stehlik, I. & Abbott, R.J. (2002) Molecular analysis of the Pleistocene history of *Saxifraga oppositifolia* in the Alps. *Molecular Ecology*, **11**, 1409–1418.

Holderegger, R., Stehlik, I., Lewis Smith, R.I. & Abbott, R.J. (2003) Populations of Antarctic Hairgrass (*Deschampsia antarctica*) show low genetic diversity. *Arctic, Antarctic, and Alpine Research*, **35**, 214–217.

Hultén, E. & Fries, M. (1986) *Atlas of North European vascular plants I*. Koeltz Scientific Books, Königstein.

Jäckli, H. (1970) Die Schweiz zur letzten Eiszeit (1:550 000). *Atlas der Schweiz* (ed. by Eidgenössiche Landestopographie). Eidgenössiche Landestopographie, Wabern-Bern.

Jiménez, J., Sánchez-Gómez, P., Güemes, J., Werner, O. & Rosselló, J.A. (2002) Genetic variability in a narrow endemic snapdragon (*Antirrhinum subbaeticum*, Scrophulariaceae) using RAPD markers. *Heredity*, **89**, 387–393.

Kropf, M., Kadereit, J. & Comes, H.P. (2002) Late Quaternary distributional stasis in the submediterranean mountain plant *Anthyllis montana* L. (Fabaceae) inferred from ITS sequences and amplified fragment length polymorphism markers. *Molecular Ecology*, **11**, 447–463.

Kropf, M., Kadereit, J. & Comes, H.P. (2003) Differential cycles of range contraction and expansion of European high mountain plants during the Late Quaternary: insights from *Pritzelago alpina* (L.) O. Kuntze (Brassicaceae). *Molecular Ecology*, **12**, 931–949.

Meusel, H., Jäger, E., Rauschert, S. & Weinert, E. (1978) *Vergleichende Chorologie der zentraleuropäischen Flora. Band II, Text*. G. Fischer, Jena.

Nei, M. & Li, W.H. (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the United States of America*, **76**, 5269–5273.

Pannel, J.R. & Barrett, S.C.H. (1998). Baker's Law revisited: reproductive assurance in a metapopulation. *Evolution*, **52**, 657–668.

Pawłowski, B. (1970) Remarques sur l'endémism dans la Flore des Alpes et des Carpates. *Vegetatio*, **21**, 181–243.

Penck, A. & Brückner, E. (1909) *Die Alpen im Eiszeitalter*. Tauchnitz, Leipzig.

Schneeweiss, G.M. & Schönswetter, P. (1999) Feinverbreitung, Ökologie und Gesellschaftsanschluß reliktischer Gefäßpflanzen der östlichen Niederen Tauern (Steiermark, Österreich). *Stapfia*, **61**, 1–242.

Schneider, S., Kueffer, J.M., Roessli, D. & Excoffier, L. (1997) *Arlequin 1.1: a software for population genetic analysis*. Genetics and Biometry Laboratory, University of Geneva, Geneva.

Schönswetter, P. & Tribsch, A. (2003) *(61) Comastoma tenellum. Floristische Neufunde (57–73)* (ed. by M.A. Fischer and H. Niklfeld), pp. 287–297. *Neilreichia*, **2–3**, 289–290.

Schönswetter, P., Tribsch, A., Barfuss, M. & Niklfeld, H. (2002) Several Pleistocene refugia detected in the high alpine plant *Phyteuma globulariifolium* Sternb. & Hoppe (Campanulaceae) in the European Alps. *Molecular Ecology*, **11**, 2637–2647.

Schönswetter, P., Tribsch, A., Schneeweiss, G.M. & Niklfeld, H. (2003a) Disjunctions in relict alpine plants: Phylogeography of *Androsace brevis* and *A. wulfeniana* (Primulaceae). *Botanical Journal of the Linnean Society*, **141**, 437–446.

Schönswetter, P., Paun, O., Tribsch, A. & Niklfeld, H. (2003b) Out of the Alps: Colonisation of the Arctic by East Alpine populations of *Ranunculus glacialis* (Ranunculaceae). *Molecular Ecology*, **12**, 3373–3381.

Schönswetter, P., Tribsch, A., Stehlik, I. & Niklfeld, H. (2004) Glacial history of high alpine *Ranunculus glacialis* (Ranunculaceae) in the European Alps in a comparative phylogeographical context. *Biological Journal of the Linnean Society*, **81**, 183–195.

Stehlik, I. (2002) Glacial history of the alpine *Rumex nivalis* (Polygonaceae): a comparison of common phylogeographic methods with nested clade analysis. *American Journal of Botany*, **89**, 2007–2016.

Stehlik, I., Schneller, J.J. & Bachmann, K. (2001) Resistance or emigration: Response of the high-alpine plant *Eritrichium nanum* (L.) Gaudin to the ice age within the Central Alps. *Molecular Ecology*, **10**, 357–370.

Stehlik, I., Blattner, F.R., Holderegger, R. & Bachmann, K. (2002a) Multiple glacial nunatak survival of the high alpine *Eritrichium nanum* (L.) Gaudin within the European Alps. *Molecular Ecology*, **11**, 2027–2036.

Stehlik, I., Schneller, J.J. & Bachmann, K. (2002b) Immigration and *in situ* glacial survival in the low-alpine *Erinus alpinus* (Scrophulariaceae). *Biological Journal of the Linnean Society*, **77**, 87–103.

Tremetsberger, K., Stuessy, T.F., Guo, Y.-P., Baeza, C.M., Weiss, H. & Samuel, R.M. (2003) Amplified Fragment Length Polymorphism (AFLP) variation within and among populations of *Hypochaeris acaulis* (Asteraceae) of Andean southern South America. *Taxon*, **52**, 237–245.

Tribsch, A., Schönswetter, P. & Stuessy, T.F. (2002) *Saponaria pumila* (Caryophyllaceae) and the ice-age in the Eastern Alps. *American Journal of Botany*, **89**, 2024–2033.

Van de Peer, Y. & De Wachter, R. (1997) Construction of evolutionary distance trees with TREECON for Windows: accounting for variation in nucleotide substitution rate among sites. *Computer Applications in Bioscience*, **13**, 227–230.

Van Husen, D. (1987) *Die Ostalpen in den Eiszeiten*. Geologische Bundesanstalt, Wien.

Voges, A. (1995) *International Quaternary Map of Europe, B10 Bern*. Bundesanstalt für Geowissenschaften und Rohstoffe/UNESCO, Hannover.

BIOSKETCHES

Peter Schönswetter did his PhD on the phylogeography of high alpine species in the Alps and now extends his work to arctic-alpine taxa.

Andreas Tribsch is working on the synthesis of distribution patterns of endemic taxa of the European Alps with phylogeographic data.

Harald Niklfeld's interests focus on comparative interpretation of distribution patterns of vascular plants in Central Europe.