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Amplified Fragment Length Polymorphism (AFLP) reveals no genetic divergence of the Eastern Alpine endemic *Oxytropis campestris* subsp. *tiroliensis* (Fabaceae) from widespread subsp. *campestris*

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Abstract. Applying Amplified Fragment Length Polymorphism, we explored genetic differences between widespread *Oxytropis campestris* subsp. *campestris* and *O. campestris* subsp. *tiroliensis*, a presumed glacial relict restricted to a small area along the main chain of the Eastern Alps. We could not find genetic differences between the two "taxa". Neither do the morphological characters given in the literature discriminate between them. Therefore *Oxytropis campestris* subsp. *tiroliensis* is unlikely a glacial relict that survived Pleistocene glaciations on nunataks, but rather a genetically insignificantly differentiated phenotype that arose in the course of postglacial recolonisation. There is no phylogeographical structure in *O. campestris* s.l. in the Alps most probably due to the fact that the taxon did not survive the cold stages of the Pleistocene in the interior of the Alps but immigrated to that region at a later date.

Key words: AFLP, Central Alps, *Oxytropis campestris*, endemism, migration, phylogeography, Pleistocene.

In the Eastern Alps, the vast majority of endemic vascular plants concentrate in areas that remained unglaciated during the Last Glacial Maximum (LGM) 18 ky ago (Pawlowski 1970, Tribsch and Schönswetter, 2003). Main centres of endemism are the northeastern, southeastern and southern Calcareous Alps for calcicolous plants (Merxmüller 1952, 1953, 1954; Pawlowski 1970) and the easternmost Central Alps and the Bergamasc Alps for silicicolous plants (Tribsch and Schönswetter, 2003).

Regardless of the overall good agreement of the patterns of endemism with Pleistocene refugia, some endemic taxa such as *Braya alpina* Sternb. & Hoppe (Brassicaceae), *Comastoma nanum* (Wulf.) Toyok. (Gentianaceae) and *Oxytropis campestris* (L.) DC. subsp. *tiroliensis* (Sieber ex Fritsch) Leins & Merxm. are confined to central parts of the Eastern Alps that were strongly glaciated during the LGM (Tribsch and Schönswetter, 2003). Their

restriction to this area has been interpreted as indicative for glacial survival on nunataks, i.e. mountain tops protruding from the Pleistocene ice shield (e.g. Brockmann-Jerosch and Brockmann-Jerosch 1926). Alternatively, such a distribution pattern might be explained by a preference for high alpine habitats that are much more abundant in central parts of the Alps than in peripheral ranges.

Phylogeographic studies on Alpine plants have given contradictory results with respect to glacial survival on nunataks. Stehlik et al. (2001, 2002a,b) and Stehlik (2002) found evidence for nunatak survival in central parts of the Alps. Schönswetter et al. (2002, 2003), Tribsch et al. (2002) and Tribsch and Schönswetter (2003) explained the detected phylogeographic patterns with survival in refugia at the southern and eastern periphery of the Alps including peripheral nunatak areas. All studies, however, focused on single taxa that are widespread in the Alps. In order to further test the still controversial nunatak hypothesis, we selected *O. campestris* subsp. *tiroliensis* as an endemic of central parts of the Eastern Alps that is completely absent from the presumed refugial areas. Here, we investigate its relationship to the presumed closest relative, subsp. *campestris*, including many populations of both subspecies covering the entire Alps.

Oxytropis campestris subsp. *tiroliensis* is restricted to the central Alps of eastern-most Switzerland, northern Italy and western to central Austria (Fig. 1). According to Leins and Merxmüller (1966), subsp. *tiroliensis* differs from the widespread yellowish flowering subsp. *campestris* in several floral characters, especially in the polychromatic bluish flower colour, a narrower standard and shorter calyx teeth. Due to the existence of transitional types to subsp. *campestris*, subsp. *tiroliensis* is recognised at the subspecific level (Leins and Merxmüller 1966, 1968; Pignatti 1982; Adler et al. 1994; but see Heß et al. 1972). According to Leins and Merxmüller (1966), in Europe the hexaploid *O. campestris* s.l. includes the type subspecies that is widespread in middle and southern European mountain ranges (Hultén and Fries 1986), subsp. *tiroliensis* and the northern Eurasian subsp. *sordida* (Willd.) Hartmann fil. The latter two have often been confused due to their similarly variable bluish flower colour (e.g. Ascherson and Graebner 1906–1910) but exhibit clear differences in the length of the calyx teeths and in the form of the pods (Leins and Merxmüller 1966).

The main goal of this study is to investigate the degree of genetic divergence between the Eastern Central Alpine endemic *O. campestris* subsp. *tiroliensis* and its widespread relative subsp. *campestris*. If the former is indeed a nunatak survivor that has differentiated in geographic isolation from subsp. *campestris*, we expect relatively high genetic divergence. Furthermore, we aim to elucidate the phylogeography of *O. campestris* s.l. in the Alps based on broad sampling of many populations, including also some populations from Pyrenees and Carpathians.

Materials and methods

Sampling. Leaf material was collected in the field and immediately stored in silica gel. We sampled 46 populations of *O. campestris* s.l. in the Alps. Based on the flower colour, 24 were identified as subsp. *campestris*, four were subsp. *tiroliensis*, three were intermediate and 15 were not in flower. In addition, we sampled two populations of subsp. *campestris* from the Pyrenees and one from the Tatra, as well as one population of subsp. *sordida* from north-western Siberia. The sample size varied from two to ten investigated individuals per population (mean = 3.88, SD = 1.49). Voucher specimens of all populations were deposited in the herbarium of the Institute of Botany of the University of Vienna (WU).

DNA isolation and AFLP fingerprinting. Total genomic DNA was extracted from comparable amounts of dried tissue following a CTAB-protocol (Doyle and Doyle 1987) with modifications as described in Schönswetter et al. (2002). The quality of the extracted DNA was checked on 1% TAE-agarose gels and quantified photometrically (UV 160A Spectrophotometer, Shimadzu). The AFLP procedure followed Vos et al. (1995) with modifications as in Schönswetter et al. (in press a).

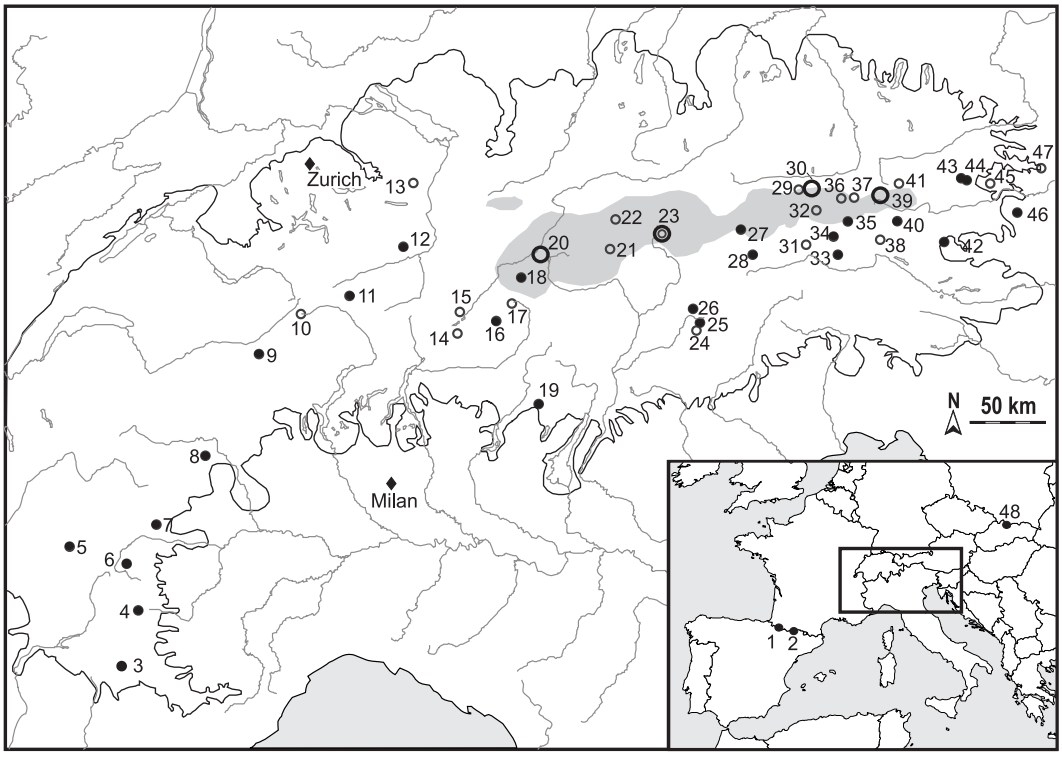

Fig. 1. Distribution of *Oxytropis campestris* subsp. *tiroliensis* in the Alps (shaded) and sampled populations of *O. campestris* subsp. *campestris* and *O. campestris* subsp. *tiroliensis* (numbered, see Table. 1). Population sordida-49 from northwestern Siberia is not shown. Dots, flowering populations of *O. campestris* subsp. *campestris*; small circles, not flowering or intermediate (populations tirol-32, tirol-36, tirol-41) populations of *O. campestris* s.l.; large circles, flowering populations of *O. campestris* subsp. *tiroliensis*. The maximum extent of the Pleistocene ice shield during the last glacial period (Würm) is illustrated with a black line (modified from Jäckli 1970, van Husen 1987 and Voges 1995)

After initial primer trials, three primer combinations with *Mse*I primers with four selective nucleotides were chosen: *Eco*RI AGC (NED)-*Mse*I CTGA, *Eco*RI AAG (HEX)-*Mse*I CTGA and *Eco*RI ACT (6-FAM)-*Mse*I CATA. The fluorescence-labelled selective amplification products were separated on a 5% polyacrylamide gel with an internal size standard (GeneScan-500 [ROX], PE Applied Biosystems) on an automated sequencer (ABI 377, Perkin Elmer). We repeated the analysis of some individuals in order to test the reproducibility of the AFLP technique. Raw data were collected and aligned with the internal size standard using ABI Prism GeneScan Analysis Software (PE Applied Biosystems). Subsequently, GeneScan files were imported into Genographer (version 1.1.6, Montana State University 1998; http://hordeum.msu.montana.edu/genographer/) for scoring as presence/absence data. Peaks of low intensity were included into the analysis when unambiguous scoring was possible.

The entire data set, **data set 1** (195 individuals) comprising all investigated individuals, was divided up for some of the analyses. **Data set 2** (177 individuals) contained all Alpine populations of *O. campestris* subspp. *campestris* and *tiroliensis*. For **data set 3** (104 individuals), intermediate or not flowering populations were excluded from data set 2. The affiliation of all populations to the respective data sets is given in Table 1.

Data analysis. The number of AFLP fragments per populations and the mean number of fragments per individual was estimated for all populations.

A Neighbour Joining (NJ) analysis of data set 1 based on Nei and Li's (1979) genetic distance was performed and bootstrapped (1000 permutations) using Treecon 1.3b (Van de Peer and De Wachter

Table 1. OTU, affiliation of the investigated population of *Oxytropis campestris* to the subspp. *campestris* (camp), *tiroliensis* (tirol) and *sordida* (sordida), respectively. For Alpine populations (locations 3 to 47) of *O. campestris* s.l. unequivocal assignability to subspp. *campestris* or *tiroliensis* based on flower colour is specified with an exclamation mark, question marks denote populations with intermediate flower colour. The remaining Alpine populations did not flower. Loc. nr., number designating sampling location; N, number of investigated individuals; location name, country (A = Austria; CH = Switzerland; E = Spain; F = France; I = Italy; PL = Poland; RU = Russia); coordinates; Morph., population used for morphometric analyses; $Frag_{Ind}$, mean number of AFLP-fragments per individual; $Frag_{Pop}$, number of AFLP-fragments per population; Data set, affiliation to one of the three data sets

OTU	Loc. nr.	N	Location name	Country	Coordinates (E/N)	Morph.	$Frag_{Ind}$	$Frag_{Pop}$	Data set		
camp	1	4	Col du Pourtalet	F/E			46.00	66	1	–	–
camp	2	5	Ordizeta	E	0.27/42.65		44.33	59	1	–	–
!camp	3	5	Col Restefond	F	6.83/44.33		48.33	84	1	2	3
!camp	4	3	Col Agnel	F/I	6.97/44.68		49.33	64	1	2	3
!camp	5	3	Col Galibier	F	6.38/45.07		49.00	68	1	2	3
!camp	6	–	Monte Genevris	I	6.87/44.98	x	–	–	–	–	–
!camp	7	3	Monte Palon	I	7.13/45.20		50.00	61	1	2	3
!camp	8	3	Champorcher	I	7.55/45.62		41.33	56	1	2	3
!camp	9	3	Simplon Pass	I/CH	8.02/46.23		47.33	59	1	2	3
camp	10	3	Nufenen Pass	CH	8.38/46.47		50.33	65	1	2	–
!camp	11	3	Passo Lucomagno	CH	8.80/46.58	x	46.00	63	1	2	3
!camp	12	3	Cassonsgrat	CH	9.27/46.87		46.67	64	1	2	3
camp	13	3	Säntis	CH	9.35/47.24		45.00	61	1	2	–
camp	14	3	Val Muretto	I	9.73/46.35		50.33	64	1	2	–
camp	15	3	Piz Julier	CH	9.75/46.48		45.00	64	1	2	–
!camp	16	3	Monte Vago	I	10.07/46.43		47.33	65	1	2	3
camp	17	5	Monte Torraccia	I	10.20/46.53		46.67	79	1	2	–
!camp	18	5	Alp Buffalora	CH	10.28/45.65		50.00	68	1	2	3
!camp	19	3	Passo Crocedomini	I	10.43/45.93		46.33	67	1	2	3
!tirol	20	5	Piz Lad	I/CH	10.47/46.83	x	47.33	73	1	2	3
camp	21	5	Gaisbergtal	A	11.05/46.85		46.67	78	1	2	–
camp	22	5	Schrankogel	A	11.10/47.03		40.67	60	1	2	–
camp	23	5	Rollspitz	I	11.50/46.95	x	45.67	77	1	2	–
!tirol	23	9	Rollspitz	I	11.50/46.95		47.67	75	1	2	3
camp	24	3	Cima Margerita	I	11.80/46.37		45.67	67	1	2	–
!camp	25	3	Passo Pordoi	I	11.82/46.42		48.67	75	1	2	3
!camp	26	3	Passo Sella	I	11.77/46.50		46.00	62	1	2	3
!camp	27	2	Totenkarspitz	A	12.18/46.97	x	45.50	55	1	2	3
!camp	28	3	Kalksteiner Jöchl	A	12.28/46.82		43.67	71	1	2	3
camp	29	3	Kitzsteinhorn	A	12.68/47.20		41.67	59	1	2	–
!tirol	30	5	Jägerscharte	A	12.76/47.20		47.00	73	1	2	3
!camp	31	3	Schleinitz	A	12.75/46.88		40.33	55	1	2	3
!camp	32	5	Hochtor	A	12.83/47.08		43.67	74	1	2	–
?tirol	32	5	Hochtor	A	12.83/47.08		44.33	67	1	2	–
camp	33	3	Roter Beil	A	13.02/46.82		44.00	61	1	2	–
!camp	34	3	Sadnig	A	12.98/46.93	x	36.00	45	1	2	3
!camp	35	3	Mallnitzer Tauern	A	13.10/47.02		46.67	70	1	2	3
?tirol	36	3	Türchlwand	A	13.05/47.15		44.00	59	1	2	–
camp	37	3	Gamskarkogel	A	13.16/47.16		45.00	67	1	2	–

Table 1 (continued)

camp	38	3	Hochkedl	A	13.38/46.91	45.67	66	1	2	–
!tirol	39	10	Weißeck	A	13.38/47.16	41.00	85	1	2	3
!camp	40	3	Wandspitze	A	13.53/47.02	47.00	66	1	2	3
?tirol	41	3	Gamsleitenspitz	A	13.55/47.24	41.33	60	1	2	–
!camp	42	5	Bretthöhe	A	13.93/46.90	46.00	83	1	2	3
!camp	43	3	Sölkpaß	A	14.08/47.27	40.67	70	1	2	3
!camp	44	3	Rettelkirchspitze	A	14.13/47.26	39.33	64	1	2	3
camp	45	4	Schießeck	A	14.33/47.24	46.33	73	1	2	–
!camp	46	4	Zirbitzkogel	A	14.57/47.07	46.33	78	1	2	3
camp	47	5	Lamprechtshöhe	A	14.77/47.33	46.00	66	1	2	–
camp	48	5	Wielka Turnia	PL	19.92/49.25	44.33	67	1	–	–
sordida	49	4	Yamburg	RU	74.63/69.72	49.00	73	1	–	–

1997) and plotted with MEGA ver. 2.1 (Kumar et al. 2001).

Principal Co-ordinate Analyses (PCoAs) based on inter-individual Jaccard similarities ($C_J = a/(a + b + c)$, where a is the number of fragments shared between two individuals and b and c are present in only one individual) were calculated and plotted with SPSS 10.0.7 (SPSS Inc. 1989–1999).

Analyses of Molecular Variance (AMOVAs) were calculated with ARLEQUIN 2.0 (Schneider et al. 1997). Mantel tests were applied in two different ways: (a) to compare the genetic matrix of Jaccard distances ($D = 1 - C_J$) between individuals of data set 2 to a matrix of geographical distances in kilometres, i.e. the "classical" use of a Mantel test; and (b) to test the genetic distance matrix of data set 3 against a model matrix where all pairwise comparisons between the population groups were coded. All Mantel R_M-values were calculated and Bonferroni-corrected using R-PACKAGE 4.0 (Casgrain and Legendre 1999). The probability of the test statistic was assessed with 1000 permutations.

Morphology. In order to re-evaluate the characters differentiating subspp. *campestris* and *tiroliensis* (i.e. a more narrow standard and shorter calyx teeth in the latter; Leins and Merxmüller 1966), we measured (1) the length and the maximal width of the blade of the standard and (2) the length of the median calyx tooth. One flower was investigated at full anthesis of 16 randomly selected individuals of subsp. *campestris* (populations camp-6, camp-11, camp-27, camp-34) and of 15 individuals of subsp. *tiroliensis* (populations tirol-20, tirol-23). Additionally, we measured one representative flower of a specimen of subsp. *tiroliensis* (Tirolia centr., Padaster bei Trins im Gschnitztal, leg. Kerner in Fl. Exs. Austro-Hungarica 13, herbarium WU) cited by Leins and Merxmüller (1966). T-tests were carried out using SPSS 10.0.7.

Results

With the three primer combinations used, 192 unambiguously scorable fragments have been generated of which 184 (95.8%) were polymorphic. The length of the fragments ranged from 58 to 489 bp. The mean number of fragments per individual, $Frag_{Ind}$, varied from 36.0 in camp-34 to 50.3 in pop. camp-10 (mean = 45.45, SD = 3.06; Table 1).The total number of fragments per population, $Frag_{Pop}$, ranged from 45 in camp-34 to 85 in tirol-39 (mean $Frag_{Pop} = 67.02$, SD = 8.04).

In the NJ analysis of data set 1 (Fig. 2) there was hardly any structure. Only one population (sordida-49) clustered with bootstrap support of > 50 (BS 78). None of subspp. *campestris* and *tiroliensis* formed distinct groups. In search for possible phylogeographical structure, we labelled the individuals according to the geological border of the Western and Eastern Alps (Hinterrhein – Splügenpaß – Lago di Como), an important borderline in previous phylogeographic studies, e.g. Schönswetter et al. (in press a, in press b). However, there is no geographical structure as well. In the PCoA of data set 2 there is no

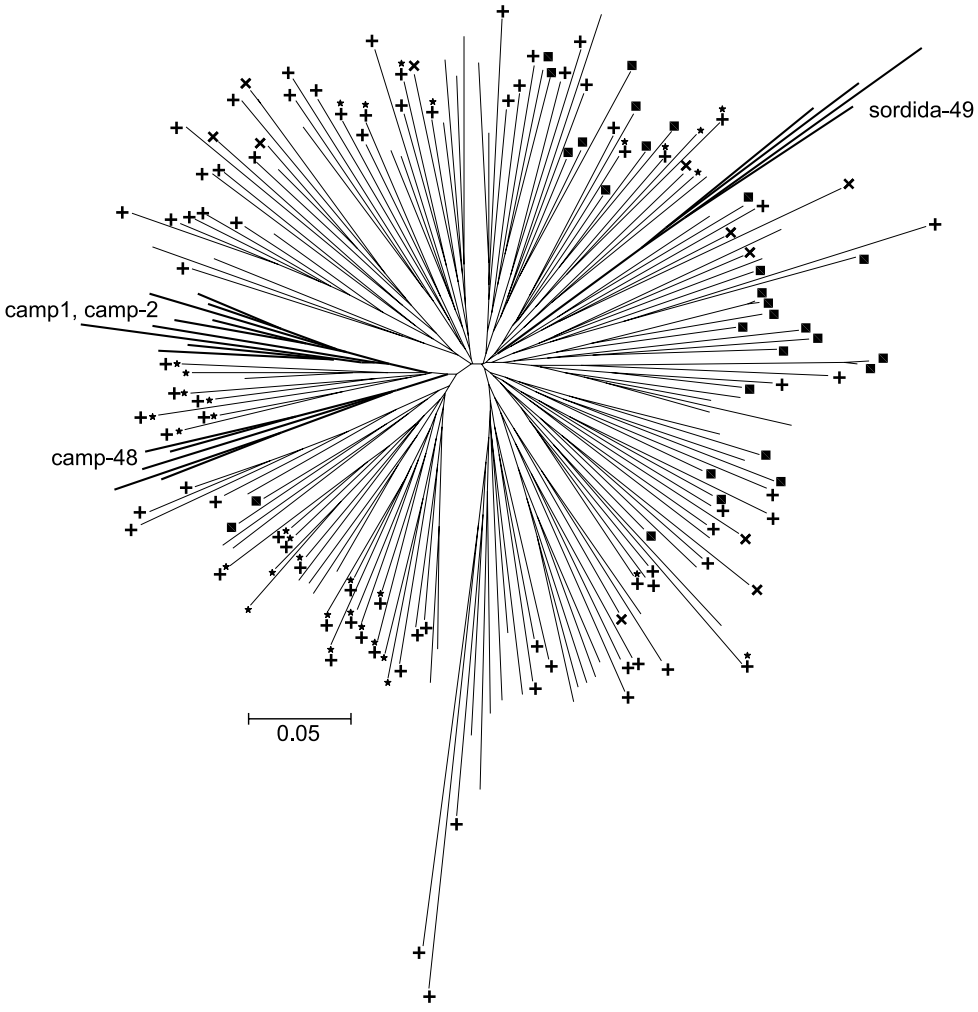

Fig. 2. Neighbour Joining analysis of the 195 investigated individuals of *O. campestris* (data set 1). The populations of subsp. *campestris* from the Pyrenees (camp-1, camp-2) and Tatra Mts. (camp-48) as well as the accession of subsp. *sordida* (sordida-49) were labelled. The latter had bootstrap support of 78%, all other groups had no support $> 50\%$. If flower colour was known, the Alpine populations were differentiated into subsp. *campestris* (crosses), subsp. *tiroliensis* (squares), and forms with intermediate flower colour (x). Non-flowering populations are not labelled. Additionally, all Western Alpine accessions are marked with asterisks

pattern reflecting genetic division between subsp. *campestris* and *tiroliensis* along any of the axes (Fig. 3ab).

In non-hierarchical AMOVAs of data set 2, 82% of the overall variation were attributed to variation within populations (Table 2). In a hierarchical AMOVA of data set 3, only 3% of the overall variation were explained by variation between *O. campestris* subspp. *campestris* and *tiroliensis*. The separation of Eastern and Western Alpine populations (see above) explained 4% of the overall variance in data set 2. Other geographic separations (not shown) did not yield higher explanation values.

The Mantel R_M value for the congruence of genetic and geographical distances in data set 2 was negative and non-significant ($R_M = -0.11$, $P = 0.31$). The second Mantel test comparing the genetic distance matrix of data set 3 and the model matrix of population groups resulted in insignificantly positive correlation of subsp. *campestris* with itself ($R_M = 0.040$,

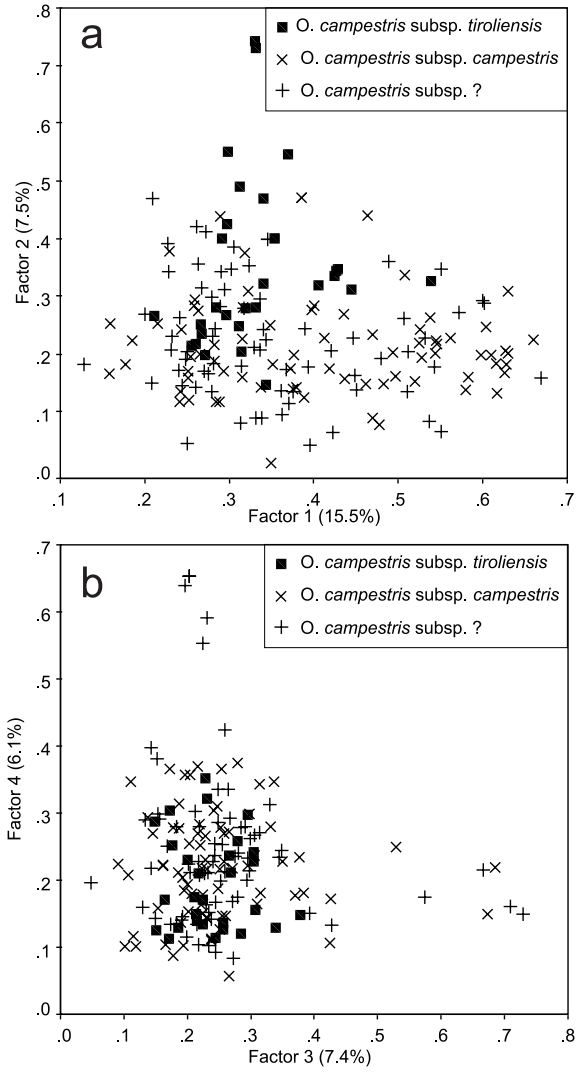

Fig. 3. Principal Co-ordinate Analysis (PCoA) of data set 2. Populations of *Oxytropis campestris* subspp. *campestris* and *tiroliensis* were differentiated according to their flower colour. Not flowering and intermediate individuals were labelled with +. (**a**) Axes 1 and 2; (**b**) axes 3 and 4

$P = 0.273$) and of subsp. *campestris* with subsp. *tiroliensis* ($R_M = 0.041$, $P = 0.175$). Correlating subsp. *tiroliensis* with itself yielded a significantly negative R_M value ($R_M = -0.155$, $P = 0.001$).

A summary of the results of the morphological measurements is given in Table 3. T-tests revealed no statistically significant differences for either the dimensions of the standard or the length of the calyx teeths (Table 3).

Discussion

No evidence for genetic divergence of *O. campestris* subsp. *tiroliensis* from subsp. *campestris*. We did not detect significant genetic differences between subspp. *tiroliensis* and *campestris*. (1) In the NJ analysis of data set 1 (Fig. 2) there was no separation of subspp. *tiroliensis* and *campestris* and both taxa were intermixed. (2) We found, accordingly, no separation in the PCoA of data set 2 (Fig. 3). (3) The Mantel test comparing the genetic distance matrix with pairwise comparisons of subsp. *tiroliensis* and *campestris* resulted in a significantly negative R_M value for subsp. *tiroliensis* tested against itself. This indicates that genetic distances do not at all correspond to taxon boundaries. (4) In a hierarchical AMOVA (Table 2) only 3% of the overall variation were explained by the differentiation of subspp. *tiroliensis* and *campestris*. A similar value was obtained for the *ad hoc* separation of Western and Eastern Alpine populations of both taxa (Table 2). The lack of divergence of subsp. *tiroliensis* is not only restricted to the genetic level, but also applies to morphological characters. A re-evaluation of the differential characters mentioned by Leins and Merxmüller (1966) revealed no differences neither in the shape of the standard nor in the length of the calyx teeth. Thus the insignificant genetic differentiation of *O. campestris* subsp. *tiroliensis* from subsp. *campestris* as well as the strongly overlapping morphological characters suggest that this taxon does not deserve taxonomic recognition.

The only character distinguishing subsp. *tiroliensis* from subsp. *campestris* is the bluish colour of the corolla. Flower colour, however, has been shown to be an unreliable indicator of genetic divergence in the Alaskan species groups of *O. campestris* and *O. arctica* (Jorgensen et al., 2003). In that study, no genetic basis for the taxonomic recognition of taxa based on either flower colour ("Russian

Table 2. Analysis of molecular variance (AMOVA) in Alpine populations of *Oxytropis campestris* subspp. *campestris* and *tiroliensis*

Source of variation	d.f.	Sum of squares	Variance components	% Total variance	F_{ST}[a]
Among Alpine populations of *O. campestris* s.l (data set 2)	47	964.32	2.50	18.35	0.18
Within populations	133	1479.67	11.13	81.65	
Eastern Alps vs. Western Alps (data set 2)	1	46.92	0.53	3.79	0.20
Among populations	43	847.48	2.22	15.77	
Within populations	126	1424.47	11.31	80.44	
Between Alpine populations of subspp. *campestris* and *tiroliensis* (data set 3)	1	44.02	0.45	3.22	0.17
Among populations	26	489.09	2.00	14.18	
Within populations	77	894.97	11.62	82.60	

[a] All P-values were < 0.001.

Table 3. Comparison of morphological characters of *Oxytropis campestris* subspp. *campestris* and *tiroliensis*

Character	Taxon	N	Mean	SD	*t*-test
Length of standard/ width of standard	*campestris*	16	1.35	0.18	$t = -0.525$,
	tiroliensis	16	1.38	0.15	$P = 0.604$
Calyx length in mm	*campestris*	16	1.71	0.40	$t = 1.423$,
	tiroliensis	16	1.57	0.21	$P = 0.227$

school'', e.g. Yurtsev 1999) or flower size (''American school'', e.g. Welsh 1991) was found. In contrast, the grouping of the accessions most probably reflects a vicariance pattern caused by a Pleistocene barrier formed by the Alaskan northern coastal ice shield. Generally, flower colour appears to be a quickly evolving character in *Oxytropis*. In a study of the extremely disjunct *O. deflexa*, Høland and Laane (1989) found that the geographically isolated Norwegian white to greyish-white flowering *O. deflexa* subsp. *norvegica* differs strongly from violet Asian subsp. *deflexa* and from western North American var. *foliolosa* in the composition of flower substances. However, because it is a comparatively thermophilic taxon restricted to an area that was covered by the Scandinavian ice shield, *O. deflexa* subsp. *norvegica* is believed to be less than 10 ky old.

Bluish flower colour in *O. campestris* is not restricted to the Alpine subsp. *tiroliensis*. It is shared with the northern Eurasian subsp. *sordida* that is differentiated from subsp. *tiroliensis* both morphologically (Leins and Merxmüller 1966) and genetically as indicated by the high bootstrap support (Fig. 2). Küpfer (1974) reported bluish populations of *O. campestris* from the Pyrenees as well. He explored possible introgression of co-occurring, violet flowering tetraploid *O. halleri* into populations of *O. campestris*. Based on the hexaploid chromosome number, the regular pollen and morphological characters (e.g. stipule morphology), however, he could exclude introgression.

The lack of significant genetic divergence of subsp. *tiroliensis* from subsp. *campestris* indicates that this taxon is no glacial relict that survived Pleistocene glaciations on nunataks in the central parts of the Alps. This finding agrees well with ecological characteristics that make nunatak survival of this taxon unlikely. Similar to the type subspecies, *Oxytropis campestris* subsp. *tiroliensis* is not a very hardy plant but rather a "thermophilic", xerophytic taxon that regularly descends to low altitudes (Polatschek 2000) in steppic habitats. In contrast, other presumed "nunatak taxa" (see, e.g. Brockmann-Jerosch and Brockmann-Jerosch 1926) restricted to the central Eastern Alps, such as *Braya alpina*, *Comastoma nanum*, *Taraxacum handelii* J. Murr and *T. reichenbachii* Huter occur exclusively in the high alpine to subnival vegetation belt (Tribsch and Schönswetter 2003).

Recent immigration of *O. campestris* s.l. into the Alps. Even on a large geographical scale, there is no obvious phylogeographic pattern in the investigated populations of *O. campestris*. The accession of subsp. *sordida* (sordida-49) from northwestern Siberia is most strongly differentiated (Fig. 2). The populations from Pyrenees (camp-1, camp-2) and Tatra (camp-48) form groups, but have no bootstrap support (Fig. 2). Accordingly, it is unlikely that Pyrenees, Alps and Tatra harbour gene pools that were isolated for a long time. In the Alps, neither NJ analysis of data set 1 (Fig. 2) nor PCoA of data set 2 (Fig. 3) revealed clear geographical grouping. As indicated by the Mantel test comparing geographic and genetic distances, there was no isolation by distance. Hierarchical AMOVA of data set 2 (Table 2) revealed that only 4% of the overall genetic variation were explained by differentiation between Eastern and Western Alpine populations.

The lack of a phylogeographical pattern in *O. campestris* is rather unexpected. It can hardly be explained by extensive recent pollen flow, as the species has specific habitat requirements (basic, but usually siliceous bedrock) and thus scattered occurrences in large parts of the Alps (P. Schönswetter and A. Tribsch, pers. obs.). In other studies on Alpine plants (Stehlik et al. 2001, 2002 a, 2002b; Schönswetter et al. 2002, 2003, in press a, in press b; Tribsch et al. 2002) strong phylogeographical patterns were detected caused by vicariance in glacial refugia. In the arctic-alpine coloniser *Saxifraga oppositifolia* L., however, Holderegger et al. (2002) found a phylogeographical structure similarly weak as in the present study.

Due to their very weak structure, our data thus suggest that *O. campestris* immigrated into the Alps after Pleistocene glaciations from unknown refugia. The taxon is well adapted to steppe-like habitats and often descends several hundred meters below timberline in dry meadows (Hegi 1925). Glacial survival and migration in lowland steppe and tundra vegetation (Frenzel et al. 1992) during cold stages of the ice-ages thus appear probable.

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