

The extreme disjunction between Beringia and Europe in *Ranunculus glacialis* s. l. (Ranunculaceae) does not coincide with the deepest genetic split – a story of the importance of temperate mountain ranges in arctic–alpine phylogeography

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Abstract

The arctic–alpine *Ranunculus glacialis* s. l. is distributed in high-mountain ranges of temperate Europe and in the North, where it displays an extreme disjunction between the North Atlantic Arctic and Beringia. Based on comprehensive sampling and employing plastid and nuclear marker systems, we (i) test whether the European/Beringian disjunction correlates with the main evolutionary diversification, (ii) reconstruct the phylogeographic history in the Arctic and in temperate mountains and (iii) assess the susceptibility of arctic and mountain populations to climate change. Both data sets revealed several well-defined lineages, mostly with a coherent geographic distribution. The deepest evolutionary split did not coincide with the European/Beringian disjunction but occurred within the Alps. The Beringian lineage and North Atlantic Arctic populations, which reached their current distribution via rapid postglacial colonization, show connections to two divergent pools of Central European populations. Thus, immigration into the Arctic probably occurred at least twice. The presence of a rare cpDNA lineage related to Beringia in the Carpathians supports the role of these mountains as a stepping stone between temperate Europe and the non-European Arctic, and as an important area of high-mountain biodiversity. The temperate and arctic ranges presented contrasting phylogeographic histories: a largely static distribution in the former and rapid latitudinal spread in the latter. The persistence of ancient lineages with a strictly regional distribution suggests that the ability of *R. glacialis* to survive repeated climatic changes within southern mountain ranges is greater than what recently was predicted for alpine plants from climatic envelope modelling.

Keywords: Arctic, climate change, disjunction, European Alpine System, migration, refugia

Received 23 March 2012; revision received 2 August 2012; accepted 6 August 2012

Introduction

Arctic–alpine species are disjunctly distributed in the high mountains of the Northern Hemisphere and the circumpolar Arctic (Löve & Löve 1974). Their geo-

graphic ranges vary considerably with respect to the set of temperate mountain ranges they occupy; within the Arctic they differ in their longitudinal extent and disjunction patterns (Hultén 1958). Organisms adapted to narrow ecological niches in high-altitude or high-latitude vegetation often have enormous circumpolar distributions. In the past, they underwent major range shifts in response to recurrent climatic and environmen-

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tal change, such as extension of the northern ice sheets during glacial periods (Hewitt 2004). Currently, range changes induced by ongoing climate warming are anticipated for the future (Thuiller *et al.* 2005; Alsos *et al.* 2009, 2012). Assumptions about the dispersal ability of arctic–alpine plants – and thus the tempo of their range shifts – have been modified recently. Earlier, the relative scarcity in the arctic flora of species with obvious adaptations for dispersal led to rejection of long-distance dispersal (LDD) as an important mechanism influencing the range dynamics of arctic–alpine plants (Hultén 1958; Dahl 1963). Disjunct arctic–alpine distributions were suggested to represent the remnants of previously wider distributions (Hultén 1937, 1958), assuming survival in regional glacial refugia (see also Abbott & Brochmann 2003) which were indeed demonstrated to have existed even in areas almost completely glaciated, such as Scandinavia (Parducci *et al.* 2012). Recent phylogeographic studies, however, have also emphasized the role of LDD by showing that dispersal is not a limiting factor in the habitat-tracking of arctic plants (e.g. Abbott & Brochmann 2003; Alsos *et al.* 2007).

As they experienced climatic fluctuations, cold-adapted species responded differently from temperate species (e.g. Hewitt 2004; Schmitt 2007; Stewart *et al.* 2010). While glacial periods increased habitat connectivity and thus facilitated gene exchange between isolated mountain ranges and between high-latitude areas, interglacials increased the isolation of high-mountain biomes as forests became established as climax vegetation in the temperate and boreal zones (Birks & Willis 2008). For a truer understanding of range formation and dynamics in arctic–alpine ecosystems, both continuously and disjunctly distributed species need to be investigated. Phylogeographic studies focusing on continuously distributed species (e.g. Abbott *et al.* 2000; Skrede *et al.* 2006; Alsos *et al.* 2009) have revealed several major intraspecific lineages linked to main refugia in North America (Beringia) and Eurasia, which reached their present wide distributions after large-scale colonization. Among species with disjunct distributions, one of the most intriguing patterns entails a disjunction between mainland Europe and the amph- or North Atlantic Arctic on one side and Beringia (the surroundings of the Bering Strait) on the other, without any links in the Asian or North American Arctic (Hultén 1958). This most spectacular and rare disjunction is found both in groups with taxonomic differentiation (e.g. *Ranunculus glacialis* s. l./Ranunculaceae, *Saxifraga rivularis*/Saxifragaceae, *S. stellaris* s.l.) and in those without it (e.g. *Luzula arcuata*/Juncaceae). The only case investigated in detail so far is that of *Saxifraga rivularis*, in which both vicariance between Beringia and the amph-Atlantic Arctic and postglacial LDD from the former to

the latter areas were found (Westergaard *et al.* 2010). Our grasp of the history of this pattern and its evolutionary role is hampered by the insufficiency of species studies. In addition, most arctic–alpine studies available so far have focused, sometimes exclusively, on either the alpine (temperate) or the arctic part of a species range (for rare exceptions see, e.g. Koch *et al.* 2006; Ehrlich *et al.* 2007; Winkler *et al.* 2012). Well-founded conclusions on range history and dynamics can only come from studies based on comprehensive sampling of both parts of the whole range.

An excellent system to investigate arctic–alpine range dynamics is *Ranunculus glacialis* s. l., an often abundant pioneer of siliceous scree, snow beds and rock crevices in the alpine and subnival belts of European mountains and moist arctic fellfields (Uotila 2001; Aeschmann *et al.* 2004). Its disjunct distribution range covers most high-mountain ranges of the European Alpine System (EAS: Sierra Nevada, Pyrenees, Alps, Carpathians and Scandinavian mountains) and also eastern Greenland, Iceland, Jan Mayen, the Faroe Islands, subarctic and arctic Scandinavia and southern Svalbard (Jalas & Suominen 1989); the second area comprises an amph-Beringian range in Alaska and easternmost Siberia (Chukchi Peninsula, Anadyr range; Hultén 1968). In the European Alps, *R. glacialis* is among the vascular plant species recorded at the altitudinal margin of higher plant life (Crawford 2008), and in the Arctic, it was considered a possible candidate for *in situ* nunatak survival during the Pleistocene glaciations (Birks 2008). Thus, it can serve as a platform for addressing problems of major disjunctions and of range dynamics within temperate mountains and within the Arctic.

Previous AFLP-based studies of *R. glacialis*, which included no material from the amph-Beringian region, revealed a clear phylogeographic structure with a major east–west split approximately in the middle of the Alps and two subordinate western and eastern splits (Schönswetter *et al.* 2004). Colonization of northern Europe from eastern Alpine source populations accompanied by a strong founder effect was inferred (Schönswetter *et al.* 2003). However, the restricted sampling outside the Alps a priori limited the large-scale conclusions, and the relationships among populations from the main ranges (Alps, Pyrenees, Carpathians) remained unresolved (Schönswetter *et al.* 2003). On the level of the genus, phylogenetic reconstructions of evolutionary relationships have indicated deep divergence within *R. glacialis* s. l., between the European *R. glacialis* and the Beringian *R. camissonis* (Paun *et al.* 2005; Hoffmann *et al.* 2010). Here, we reconstruct the phylogeographic history of the disjunctly distributed arctic–alpine *R. glacialis* s. l. in the spatial and relative temporal context, based on a comprehensive population

sampling covering all isolated parts of the species distribution. To overcome the limitations of previous AFLP-based studies (Schönswetter *et al.* 2003, 2004), we employ two sequence-based marker systems: noncoding plastid DNA regions and nuclear ribosomal internal transcribed spacers (ITS). We apply Bayesian inference methods to reconstruct the history of the lineages. (i) First, we test Hultén's (1958) classical hypothesis that the disjunction between Europe and Beringia corresponds to the most ancient split in the diversification of the species. Santisuk (1979) explicitly hypothesized that *R. glacialis* s. l. already had a pre-Quaternary arctic distribution which was disrupted during the glacial periods, resulting in speciation into two vicariant taxa which, consequently, constitute the most divergent lineages. (ii) Second, we endeavoured to reconstruct the spatiotemporal relationships among the populations from the disjunct European mountain ranges, which could not be resolved previously due to limitations imposed by the marker employed as well as by restricted sampling outside of the Alps (Schönswetter *et al.* 2003). (iii) Near-uniformity of populations from the ampho-Atlantic Arctic was previously suggested based on rapidly homogenizing, biparentally inherited AFLPs. Here, we test whether nonrecombining, uniparentally inherited plastid markers applied to a large sample support this hypothesis, or if traces of *in situ* nunatak survival can be detected as recently suggested for two other high arctic plant species (Westergaard *et al.* 2011). (iv) An understanding of the influence of past climate change on biogeographic history is critical to reliable predictions of future changes in species distributions and genetic diversity (Alsos *et al.* 2009). We therefore use the genetic data to draw a scenario of past range shifts and to suggest hypotheses about differences in the susceptibility of various parts of the species range to ongoing climatic change.

Material and methods

Study species

Ranunculus glacialis s. l. includes (i) *R. glacialis* L. subsp. *glacialis* occurring in the European Alpine System, the sub-arctic and arctic areas of Europe and eastern Greenland (Jalas & Suominen 1989), (ii) *R. glacialis* subsp. *alaskensis* Jurtzev, a recently described, narrowly distributed Beringian taxon endemic to the Seward Peninsula (Alaska; Yurtsev *et al.* 2012) and (iii) *R. camissonis* Schltdl. restricted to Beringia (Panarctic Flora; <http://nhm2.uio.no/paf/>), often treated as subspecies of *R. glacialis* in the past (e.g. Hultén 1968). *Ranunculus glacialis* subsp. *glacialis* is common in the Arctic, the Alps, the Tatra Mountains (Western Carpathians) and some parts of the Pyrenees, while it is

extremely rare in the Sierra Nevada and in the Romanian South-Eastern Carpathians, where it is considered critically endangered without any records for over a century (Negrean & Dihoru 2009); it was rediscovered there only in the course of this study (Ronikier 2010). In Beringia, *R. camissonis* is relatively widespread in several mountain ranges in Alaska and easternmost Siberia (Hultén 1968), while *R. glacialis* subsp. *alaskensis* is so far known only from four sites in the Kigluaik Mountains in Alaska. These two taxa differ in their habitats: *R. camissonis* grows on grassy turf and damp tundra on basic substrates, whereas *R. glacialis* subsp. *alaskensis* occurs in gravel, scree and snowbank environments mainly on acidic substrates (Yurtsev *et al.* 2012).

Ranunculus glacialis is a perennial herb adapted to low temperatures and unpredictable seasonal conditions of high mountains (Körner 2003). It is diploid with $2n = 16$ (e.g. Uotila 2001), predominantly outcrossing (Totland & Alatalo 2002; Wagner *et al.* 2010), and its ability of vegetative spread is very limited. The species produces single-seeded achenes that are relatively large and slightly winged, but devoid of traits facilitating LDD.

Sampling

Seventy-three populations were sampled across the entire distribution range of *R. glacialis* s. l. including all three taxa (Table 1). Choice of populations in the Alps was based on an earlier study to represent all previously detected AFLP groups (Schönswetter *et al.* 2004). Usually, three individuals per population were collected; five and seven populations were represented by two and single individuals, respectively, resulting in totally 200 samples. Leaf tissue collected in the field was dried and stored in silica gel. Voucher specimens are deposited in the herbaria of the Institute of Botany of the University of Vienna (WU), the Institute of Botany of the Polish Academy of Sciences (KRAM) or the Botanical Museum in Oslo (O).

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from 10 to 15 mg of dried leaf tissue using a Mixer Mill 300 (Retsch, Haan, Germany) and the DNeasy Plant Mini kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol. DNA quality and concentration were approximately estimated on 1% agarose gels stained with ethidium bromide.

Plastid DNA variation was tested in 13 noncoding plastid DNA regions on a subset of six samples using universal primers of Demesure *et al.* (1995) and Shaw *et al.* (2005, 2007). The *rpl32-trnL* (Shaw *et al.* 2007) and *rps12-rpl20* (Shaw *et al.* 2005) regions yielded highest variability and were selected for the study. The PCR

Table 1 Population number, taxonomic assignment, geographic origin, plastid DNA haplotypes and internal transcribed spacers (ITS) ribotypes of 73 sampled populations of *Ranunculus glacialis* s. l. Superscript numbers at cpDNA/ITS variants indicate their frequency in populations. ITS variants in brackets indicate intra-individual variation. Country codes: A, Austria; CH, Switzerland; DK, Denmark; E, Spain; F, France; I, Italy; IS, Iceland; N, Norway; PL, Poland; RO, Romania; RU, Russia; S, Sweden; SK, Slovakia; USA, United States of America

No	Taxon	Country	Region	Location	Latitude (°)	Longitude (°)	Plastid haplotype	ITS ribotype
1	<i>glacialis</i>	E	Sierra Nevada	Tajos de la Virgen	37.04 N	3.38 W	H13 ⁽³⁾	R1 ⁽³⁾
2	<i>glacialis</i>	E	Pyrenees	Picos del Infierno	42.79 N	0.25 W	H11 ⁽¹⁾	R18 ⁽¹⁾
3	<i>glacialis</i>	F	Pyrenees	Néouvielle, Pic Badet	42.79 N	0.12 E	H11 ⁽²⁾	R17 ⁽³⁾
4	<i>glacialis</i>	E	Pyrenees	Punta Suelza, Collado de Cao	42.65 N	0.27 E	H11 ⁽³⁾	R16 ⁽³⁾
5	<i>glacialis</i>	E	Pyrenees	Aigüestortes, Pic de la Mainera	42.52 N	1.03 E	H12 ⁽³⁾	R1 ⁽³⁾
6	<i>glacialis</i>	I	Alps	Alpi Liguri, Bocchino dell Aseo	44.15 N	7.78 E	H7 ⁽¹⁾ , H8 ⁽²⁾	R26 ⁽¹⁾ , [R26/35] ⁽²⁾
7	<i>glacialis</i>	F	Alps	Alpes Maritimes, Col de Restefond	44.34 N	6.85 E	H7 ⁽³⁾	[R25/36] ⁽²⁾ , [R26/35] ⁽¹⁾
8	<i>glacialis</i>	F	Alps	Alpes Cotiennes, Col de Vars	44.54 N	6.70 E	H7 ⁽³⁾	[R24/37] ⁽¹⁾ , R37 ⁽²⁾
9	<i>glacialis</i>	I	Alps	Alpi Cozie, Punta Cialancia	44.88 N	7.12 E	H8 ⁽³⁾	[R33/48] ⁽¹⁾ , R34 ⁽²⁾
10	<i>glacialis</i>	I	Alps	Alpi Cozie, Monte Genevris	45.00 N	6.88 E	H7 ⁽³⁾	R36 ⁽¹⁾ , [R36/41] ⁽¹⁾ , R41 ⁽¹⁾
11	<i>glacialis</i>	F	Alps	Alpes du Dauphiné, Col du Galibier	45.07 N	6.38 E	H7 ⁽³⁾	[R27/51] ⁽¹⁾ , R36 ⁽¹⁾ , [R36/41] ⁽¹⁾
12	<i>glacialis</i>	I	Alps	Alpi Graie, Gran Paradiso	45.53 N	7.20 E	H7 ⁽³⁾	[R27/44] ⁽²⁾ , [R38/42] ⁽¹⁾
13	<i>glacialis</i>	F	Alps	Alpes Grées, Petit St Bernard	45.67 N	6.87 E	H7 ⁽³⁾	[R27/45] ⁽¹⁾ , [R27/46] ⁽¹⁾ , [R29/47] ⁽¹⁾
14	<i>glacialis</i>	CH	Alps	Walliser Alpen, Grand St Bernard	45.87 N	7.17 E	H7 ⁽³⁾	[R38/43] ⁽¹⁾ , [R38/44] ⁽²⁾
15	<i>glacialis</i>	I	Alps	Alpi Pennine, Monte Nery	45.77 N	7.74 E	H5 ⁽³⁾	[R30/44] ⁽¹⁾ , [R38/44] ⁽²⁾
16	<i>glacialis</i>	CH	Alps	Lepontinische Alpen, Simplonpass	46.23 N	8.02 E	H5 ⁽³⁾	[R30/44] ⁽¹⁾ , [R38/44] ⁽¹⁾ , [R39/44] ⁽¹⁾
17	<i>glacialis</i>	CH	Alps	Uerner Alpen, Furkapass	46.57 N	8.40 E	H7 ⁽³⁾	[R27/44] ⁽²⁾ , [R39/44] ⁽¹⁾
18	<i>glacialis</i>	CH	Alps	Alpi Lepontine, Cima dell'Uomo	46.23 N	8.95 E	H7 ⁽³⁾	[R28/44] ⁽¹⁾ , [R28/51] ⁽¹⁾ , [R40/52] ⁽¹⁾
19	<i>glacialis</i>	I	Alps	Alpi Bergamasche, Monte Legnone	46.10 N	9.42 E	H7 ⁽¹⁾ , H9 ⁽¹⁾ , H10 ⁽¹⁾	R1 ⁽¹⁾ , [R1/19] ⁽¹⁾ , [R1/23] ⁽¹⁾
20	<i>glacialis</i>	CH	Alps	Lepontinische/ Rätische Alpen, Splügenpass	46.50 N	9.33 E	H7 ⁽³⁾	R1 ⁽³⁾
21	<i>glacialis</i>	CH	Alps	Glarner Alpen, Cassonsgrat	46.88 N	9.27 E	H7 ⁽³⁾	[R27/49] ⁽¹⁾ , [R31/49] ⁽¹⁾ , [R32/50] ⁽¹⁾
22	<i>glacialis</i>	I	Alps	Alpi Bergamasche, Pizzo di Coca	46.07 N	10.00 E	H7 ⁽³⁾	R1 ⁽¹⁾ , [R1/22] ⁽¹⁾ , R21 ⁽¹⁾
23	<i>glacialis</i>	I	Alps	Alpi di Livigno, Monte Brevia	46.48 N	10.05 E	H7 ⁽³⁾	R1 ⁽²⁾ , [R1/19] ⁽¹⁾
24	<i>glacialis</i>	A	Alps	Silvretta, Hohes Rad	46.88 N	10.10 E	H7 ⁽²⁾	R1 ⁽²⁾ , [R1/19] ⁽¹⁾
25	<i>glacialis</i>	I	Alps	Prealpi Bresciane e Gardesane, P. di Crocedomini	45.93 N	10.43 E	H7 ⁽³⁾	[R1/20] ⁽²⁾ , R20 ⁽¹⁾
26	<i>glacialis</i>	I	Alps	Sobretta/Ortles, Passo di Gávia	46.35 N	10.50 E	H6 ⁽¹⁾ , H7 ⁽¹⁾	R1 ⁽³⁾

Table 1 Continued

No	Taxon	Country	Region	Location	Latitude (°)	Longitude (°)	Plastid haplotype	ITS ribotype
27	<i>glacialis</i>	A	Alps	Ötztaler Alpen, Pfossental	46.75 N	11.02 E	H7 ⁽²⁾	R1 ⁽³⁾
28	<i>glacialis</i>	A	Alps	Tuxer Alpen, Glungezer	47.20 N	11.52 E	H7 ⁽³⁾	R1 ⁽¹⁾ , [R1/3] ⁽¹⁾ , R19 ⁽¹⁾
29	<i>glacialis</i>	I	Alps	Dolomiti, Monte Ziolera	46.17 N	11.45 E	H1 ⁽²⁾	[R1/3] ⁽¹⁾ , R3 ⁽¹⁾
30	<i>glacialis</i>	I	Alps	Dolomiti, Passo Pordoi	46.42 N	11.82 E	H1 ⁽¹⁾ , H2 ⁽¹⁾	R1 ⁽¹⁾ , [R1/3] ⁽¹⁾ , [R2/3] ⁽¹⁾
31	<i>glacialis</i>	I	Alps	Sarntaler Alpen, Schrotthorn	46.73 N	11.55 E	H1 ⁽³⁾	R1 ⁽²⁾ , [R1/2] ⁽¹⁾
32	<i>glacialis</i>	I	Alps	Zillertaler Alpen, Tristenspizze	46.96 N	11.82 E	H1 ⁽³⁾	[R1/3] ⁽¹⁾ , [R1/9] ⁽¹⁾ , [R1/10] ⁽¹⁾
33	<i>glacialis</i>	I	Alps	Alpi Carniche, Monte Peralba	46.63 N	12.72 E	H1 ⁽²⁾	R3 ⁽²⁾ , R5 ⁽¹⁾
34	<i>glacialis</i>	A	Alps	Hohe Tauern, Totenkarspizze	46.98 N	12.19 E	H1 ⁽³⁾	[R1/2] ⁽¹⁾ , [R1/11] ⁽¹⁾ , R3 ⁽¹⁾
35	<i>glacialis</i>	A	Alps	Hohe Tauern, Grossvenediger	47.12 N	12.30 E	H1 ⁽³⁾	R1 ⁽¹⁾ , [R1/4] ⁽²⁾
36	<i>glacialis</i>	A	Alps	Hohe Tauern, Schleinitz	46.90 N	12.75 E	H1 ⁽³⁾	[R1/3] ⁽²⁾ , R3 ⁽¹⁾
37	<i>glacialis</i>	A	Alps	Hohe Tauern, Ankogel	47.05 N	13.25 E	H1 ⁽³⁾	[R1/3] ⁽³⁾
38	<i>glacialis</i>	A	Alps	Hohe Tauern, Wandspizze	47.02 N	13.53 E	H1 ⁽³⁾	[R1/3] ⁽¹⁾ , R3 ⁽²⁾
39	<i>glacialis</i>	A	Alps	Gurktaler Alpen, Eisenhut	46.95 N	13.90 E	H1 ⁽¹⁾	[R1/3] ⁽¹⁾ , R3 ⁽²⁾
40	<i>glacialis</i>	A	Alps	Wölzer Tauern, Aarfeldspitz	47.27 N	14.09 E	H1 ⁽²⁾	R1 ⁽¹⁾ , [R1/3] ⁽¹⁾
41	<i>glacialis</i>	A	Alps	Steiermark, Seckauer Zinken	47.34 N	14.75 E	H1 ⁽³⁾	R1 ⁽¹⁾ , [R1/3] ⁽²⁾
42	<i>glacialis</i>	SK	Carpathians	Vysoké Tatry, Prielom (Rohatka)	49.18 N	20.14 E	H3 ⁽³⁾	R1 ⁽¹⁾ , [R1/3] ⁽²⁾
43	<i>glacialis</i>	SK	Carpathians	Vysoké Tatry, Hrubý vrch	49.17 N	20.03 E	H3 ⁽³⁾	R1 ⁽²⁾ , [R1/3] ⁽¹⁾
44	<i>glacialis</i>	PL	Carpathians	Wysokie Tatry, Krzyżne	49.23 N	20.05 E	H3 ⁽³⁾	R1 ⁽²⁾ , [R1/3] ⁽¹⁾
45	<i>glacialis</i>	SK	Carpathians	Západné Tatry, Tri Kopy	49.20 N	19.73 E	H15 ⁽³⁾	R1 ⁽¹⁾ , [R6/7] ⁽¹⁾ , R8 ⁽¹⁾
46	<i>glacialis</i>	RO	Carpathians	Munții Rodnei, Vf. Ineu	47.53 N	24.88 E	H14 ⁽³⁾	R1 ⁽³⁾
47	<i>glacialis</i>	RO	Carpathians	Munții Făgărașului, Vf. Gălbenele	45.61 N	24.76 E	H3 ⁽³⁾	R1 ⁽³⁾
48	<i>glacialis</i>	N	Scandinavia	Hordaland, Finse	60.61 N	7.53 E	H4 ⁽²⁾	R1 ⁽²⁾
49	<i>glacialis</i>	N	Scandinavia	Jotunheimen, Galdhøpiggen	61.63 N	8.33 E	H4 ⁽³⁾	R1 ⁽³⁾
50	<i>glacialis</i>	N	Scandinavia	Dovrefjell, Knudshøa	62.28 N	9.50 E	H4 ⁽³⁾	R1 ⁽³⁾
51	<i>glacialis</i>	N	Scandinavia	Sør-Trøndelag, Leirtjønnkollen	62.44 N	9.73 E	H4 ⁽³⁾	R1 ⁽³⁾
52	<i>glacialis</i>	N	Scandinavia	Trollheimen, Gjevillvasskamban	62.75 N	9.23 E	H4 ⁽¹⁾	R1 ⁽¹⁾
53	<i>glacialis</i>	S	Scandinavia	Sarek, Tjievvra	66.90 N	17.92 E	H4 ⁽³⁾	R1 ⁽³⁾
54	<i>glacialis</i>	S	Scandinavia	Sarek, Tsahtsa	67.18 N	17.26 E	H4 ⁽¹⁾	R1 ⁽¹⁾
55	<i>glacialis</i>	N	Scandinavia	Troms, Målselv	68.77 N	18.27 E	H4 ⁽²⁾	R1 ⁽²⁾
56	<i>glacialis</i>	N	Scandinavia	Troms, Mt. Lávkaslubbu	69.25 N	20.47 E	H4 ⁽³⁾	R1 ⁽³⁾
57	<i>glacialis</i>	N	Scandinavia	Troms, Krokaldalen	69.66 N	19.11 E	H4 ⁽²⁾	R1 ⁽²⁾

Table 1 Continued

No	Taxon	Country	Region	Location	Latitude (°)	Longitude (°)	Plastid haplotype	ITS ribotype
58	<i>glacialis</i>	N	Scandinavia	Finnmark/ Troms, Storvik	70.15 N	22.43 E	H4 ⁽¹⁾	R1 ⁽¹⁾
59	<i>glacialis</i>	N	Scandinavia	Finnmark/Troms	–	–	H4 ⁽¹⁾	R1 ⁽¹⁾
60	<i>glacialis</i>	IS	Iceland	Akureyri, Mt. Sulur	65.62 N	18.20 W	H4 ⁽³⁾	R1 ⁽³⁾
61	<i>glacialis</i>	IS	Iceland	Nordwestfjorde, Holmavik	65.74 N	22.12 W	H4 ⁽³⁾	R1 ⁽³⁾
62	<i>glacialis</i>	N	Jan Mayen	Jan Mayen, Blinddalen	70.98 N	8.57 W	H4 ⁽³⁾	R1 ⁽³⁾
63	<i>glacialis</i>	N	Svalbard	Sørkapp, Sørneset	76.57 N	16.54 E	H4 ⁽³⁾	R1 ⁽³⁾
64	<i>glacialis</i>	N	Svalbard	Wedel Jarlsberg Land, Nottinghambukta	77.07 N	15.16 E	H4 ⁽³⁾	R1 ⁽³⁾
65	<i>glacialis</i>	DK	Greenland	Kap Dalton S of Scoresbysund	69.49 N	24.10 W	H4 ⁽²⁾	R1 ⁽³⁾
66	<i>glacialis</i>	DK	Greenland	Bontekoe	73.12 N	21.40 W	H4 ⁽³⁾	R1 ⁽³⁾
67	<i>glacialis</i>	DK	Greenland	Daneborg, E of Zackenbergl	74.50 N	18.82 W	H4 ⁽³⁾	R1 ⁽³⁾
68	<i>camissonis</i>	USA	Alaska	Brooks range, Endicott Mts., NNE Mt. Inualurak	68.24 N	152.19 W	H16 ⁽²⁾	R15 ⁽²⁾
69	<i>camissonis</i>	USA	Alaska	Brooks range, Endicott Mts., NNE Mt. Inualurak	68.24 N	152.20 W	H16 ⁽³⁾	R15 ⁽³⁾
70	<i>camissonis</i>	USA	Alaska	Seward Peninsula, Minnie Creek	65.34 N	163.46 W	H16 ⁽¹⁾	[R12/13] ⁽¹⁾
71	<i>alaskensis</i>	USA	Alaska	Seward Peninsula, Crete Creek	64.88 N	166.13 W	H19 ⁽¹⁾	[R12/13] ⁽¹⁾
72	<i>alaskensis</i>	USA	Alaska	Seward Peninsula, Crete Creek	64.88 N	166.12 W	H17 ⁽¹⁾ , H18 ⁽²⁾	R14 ⁽³⁾
73	<i>camissonis</i>	RU	Chukotka	Laurentiya Bay	65.65 N	171.23 W	H19 ⁽³⁾	R12 ⁽³⁾

mix (total volume 25 µL) contained 1× PCR AmpliTaq Buffer (Applied Biosystems, Foster City, CA, USA), 2.5 mM Mg²⁺, 0.1 µM of each primer, 0.15 mM of each dNTP (Roche Diagnostics, Basel, Switzerland), 0.8% bovine serum albumin (BSA, New England Biolabs, Ipswich, MA, USA), 1 U AmpliTaq polymerase (Applied Biosystems), and 1 µL template DNA. PCR was performed with the following cycling conditions: initial denaturation for 5 min at 80 °C followed by 35 cycles of 1 min at 94 °C, 1 min at 50 °C, ramp of 0.3 °C/s to 65 °C and 4 min at 65 °C.

The Internal Transcribed Spacer region of the nuclear ribosomal DNA (containing ITS1, 5.8S and ITS2), previously used in phylogenetic reconstructions of *Ranunculus* (Hörandl *et al.* 2005; Paun *et al.* 2005; Hoffmann *et al.* 2010), was selected as nuclear marker. ITS PCR volumes of 12.5 µL contained 1× PCR AmpliTaq Buffer (Applied Biosystems), 2.5 mM Mg²⁺, 0.11 mM of each dNTP (Roche Diagnostics), 0.2 µM of each primer (ITS1A and ITS4; Fuertes Aguilar *et al.* 1999; White *et al.* 1990), 1 µg BSA, 1 U AmpliTaq DNA Polymerase (Applied Biosystems) and 0.5 µL DNA template. A touchdown cycling profile was used, starting with 5 min at 94 °C, followed by 35 cycles of 30 s at 94 °C,

30 s at 56 °C (with decrease in 0.4 °C per cycle and a constant temperature of 48 °C from the 15th cycle onwards) and 1 min at 72 °C, and a final extension step of 10 min at 72 °C. All PCRs were performed in a GeneAmp 9700 thermal cycler (Applied Biosystems).

Prior to sequencing, PCR products were purified using High Pure PCR Product Purification kit (Roche Diagnostics). Sequencing was performed using BigDye Terminator 3.1 (Applied Biosystems) with 5× sequencing buffer, according to the manufacturer's manual. Two strands were sequenced in all samples using the same primers as in the PCR. Cycle sequencing products were purified by EDTA/ethanol precipitation, resuspended in 12 µL formamide and separated on an ABI 3130 Genetic Analyser equipped with 36 cm capillaries and POP-7 polymer (Applied Biosystems). Sequences were analysed with DNA Sequencing Analysis Software version 5.1 (Applied Biosystems).

Data analysis

Sequences were assembled, edited and manually aligned using BIOEDIT 5.0.9. (Hall 1999). Plastid DNA sequences were concatenated, assuming linked inheri-

tance. As inversions introduce substitutional changes which actually are due to a structural mutation (Kelchner & Wendel 1996), the single inversion was reverted prior to analyses. In the case of ITS, intraindividual polymorphism was observed in numerous individuals. These amplicons were not cloned as in all cases the variation was found to be either clearly limited to groups of ribotypes within one compact geographical region (Western Alps) or to concern closely related ribotypes within populations. Additionally, initial visual inspection suggested that the majority of ambiguous sites could be explained as the result of co-occurrence of an unambiguous ribotype present in other individuals and a second one, which may or may not be present in unambiguous form in other individuals. Therefore, we used the software PHASE 2.1 (Stephens *et al.* 2001) with default settings in conjunction with the webtool SEQ-PHASE (Flot 2010) to reconstruct ribotypes.

Relationships between plastid haplotypes and ribotypes were analysed using TCS 1.21 (Clement *et al.* 2000), which constructs networks by implementing the statistical parsimony algorithm described by Templeton *et al.* (1992). In the plastid data set, indels larger than 1 bp as well as the single inversion were reduced to single-base pair changes. A poly-A stretch was excluded from the analysis as mononucleotide repeat characters are prone to homoplasy at large geographic and temporal scales (Ingvarsson *et al.* 2003). The analyses were run with default parsimony connection limit of 95% and treating gaps as fifth character state.

Phylogenetic analysis of both plastid and nuclear data sets was conducted using the approach implemented in BEAST 1.5.3–1.6.2 (Drummond & Rambaut 2007), as this allows taking into account the genealogical uncertainty due to the stochastic nature of the coalescence process. The best-fit substitution models were determined using the Akaike Information Criterion (AIC) as implemented in jMODELTEST 0.1 (Posada 2008). For both data sets, the best-fit substitution models had low Akaike weights (<0.2) and the set of models with a cumulative Akaike weight >0.95 included models with 5–10 (median 8) and 4–11 parameters (median 6) for the plastid and the nuclear data set, respectively. Therefore, we finally used the medium complex HKY+Gamma model with five parameters, subsuming the proportion of invariable sites in the gamma distribution modelled with six categories. As the less parameterized strict clock model was not significantly different from a relaxed clock model (data not shown), rate evolution was modelled in a strict clock framework. Due to the lack of external calibrations, we used strong priors on the substitution rates. For the plastid data set, substitution rate was modelled with a lognormal distribution with a mean of 7.5×10^{-3} substitutions per site per million years and a

standard deviation of 0.6, resulting in a modal value around 4×10^{-3} substitutions per site per million years in agreement with previously published rates (Yamane *et al.* 2003; Smith *et al.* 2008). For the nuclear data set, substitution rate was modelled with a truncated normal distribution with mean and standard deviation of 4.5×10^{-3} and 2×10^{-3} substitutions per site per million years, thus encompassing the range of previously reported ITS substitution rates for herbaceous plants (Kay *et al.* 2006). After initial analyses, the root was constrained to a maximum age of 10 million years. As these calibrations are derived from phylogenetically distant groups and result in conservative, yet too coarse temporal resolution (Appendix S1 and S2, Supporting information), we do not discuss absolute ages, but only use relative ages instead. We used the Bayesian skyline plot (Drummond *et al.* 2005) as the most general demographic model with the number of coalescent time intervals $m = 3$. Stationarity of the Markov chain, which was run for 3×10^7 (plastid data set) or 6×10^7 generations (nuclear data set), respectively, with sampling every 1000th generation, was determined using TRACER 1.4 (available from <http://tree.bio.ed.ac.uk/software/tracer/>). The first 10% of sampled generations was discarded as burn-in, after which all effective sample size (ESS) values were >100. A second run was conducted to confirm convergence of the Markov chain on the stationary distribution. All parameter estimates were based on these two runs combined (54 000 and 108 000 sampling points, respectively).

To assess the spatial distribution through time, we used the discrete geospatial model implemented in BEAST (Lemey *et al.* 2009) to obtain the probability distribution of the geographic locations of each node in the tree. Briefly, this model assigns each sequence to a fixed location in space (we used the four distinct European mountain regions [Sierra Nevada, Pyrenees, Alps and Carpathians] and the two disjunct Arctic areas [North Atlantic and amph-Beringian]) and estimates rates of diffusion between them using a continuous-time Markov chain model. Conditionally on the unobserved location at the root of the tree, which derives from a uniform distribution over all sampled locations, dispersal proceeds independently along each branch in the tree and ultimately gives rise to the observed locations at the tips. To achieve statistical efficiency, dispersal rates are allowed to be zero with some probability (determined by a truncated Poisson distribution) in the framework of Bayesian stochastic search variable selection (BSSVS). Significance of nonzero diffusion rates was assessed via a Bayes factor test as described by Lemey *et al.* (2009). Initial analyses using different prior means for the Poisson distribution (0.1, 0.693, 1, 2) indicated only minor quantitative differences (data not

shown) as reported also in other groups (Escobar García *et al.* 2012), and consequently, only the results with the default prior mean of 0.693 are shown. The possible influence of sampling asymmetry (many populations from the Alps and the European Arctic, fewer from elsewhere) was assessed by rerunning the analysis with a reduced data set containing maximally ten populations per region.

Results

Plastid DNA variation and phylogeographic structure

The effective number of samples was reduced in a few populations due to PCR amplification failure or insufficient sequence quality; the final sequence data set included 185 successfully analysed samples. The alignment was 1696 bp long (878 and 818 bp for *rpl32-trnL* and *rps12-rps20*, respectively) and included 35 polymorphic sites: 24 nucleotide substitutions, six single-base pair insertions/deletions (indels), three longer indels, one 3-bp inversion and a poly-A stretch with varying length. Nineteen haplotypes were identified in total (Fig. 1). Most populations (93%) were characterized by single haplotypes. Within-population variation, which only

concerned closely related haplotypes, was detected in three populations from the Western Alps (populations 6, 21, 26) and populations 32 and 72 from the Eastern Alps and from Alaska, respectively (Fig. 2; Table 1).

The statistical parsimony network revealed several geographically coherent lineages (Fig. 1). Eleven mutations separated haplotypes H1–H4, restricted to the Eastern Alps (H1–H2), the Carpathians (H3) and the North Atlantic region (H4), from the remaining haplotypes. The central part of the network was occupied by six closely related haplotypes (H5–H10) distributed in the Western Alps. A further lineage (H11–H13) occurred in the Pyrenees and the Sierra Nevada. Finally, H16–H19 from Beringia grouped with the two rare European haplotypes H14 and H15 from the Carpathians. Two alternative connections displayed in the network did not find support in the BEAST analysis.

The BEAST analysis corroborated recognition of several usually well-supported terminal lineages (Fig. 1; the full, annotated maximum clade credibility tree file is available in Appendix S1, Supporting information), partly comprising only single haplotypes. The distribution of those haplotypes is shown in Fig. 2. One main clade contained haplotypes from central and northern Europe (H1–H4; Eastern Alps, Carpathians, North

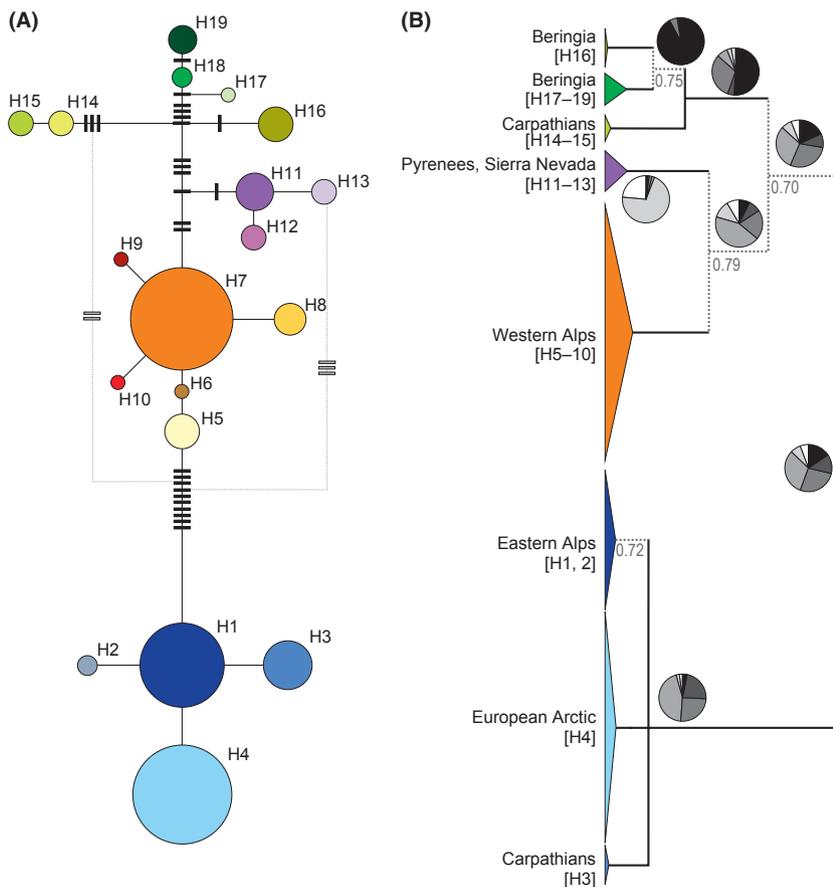


Fig. 1 Structuring of plastid DNA diversity encountered in *Ranunculus glacialis* s. l. (A) Statistical parsimony haplotype network. Haplotypes are colour-coded; their numbering corresponds to Table 1. Bars indicate haplotypes not detected in the data set. Dashed lines indicate alternative connections that are not supported by the BEAST analysis shown in B. (B) Simplified majority rule consensus tree from strict clock Bayesian analysis with the software BEAST, node heights corresponding to median relative ages. Numbers below branches indicate Bayesian posterior probabilities. Geographic distribution and pertaining haplotypes are given for each lineage. Ancestral area probabilities are indicated as pie charts at internal nodes and at nodes subtending geographically heterogeneous terminal clades (areas in clock-wise arrangement and distinguished by a black to white colour gradient: Beringia, European Arctic, Carpathians, Alps, Pyrenees, Sierra Nevada). Lineages receiving posterior probabilities (PP) ≥ 0.95 are shown with continuous lines; those with lower PP are illustrated with hatched lines.

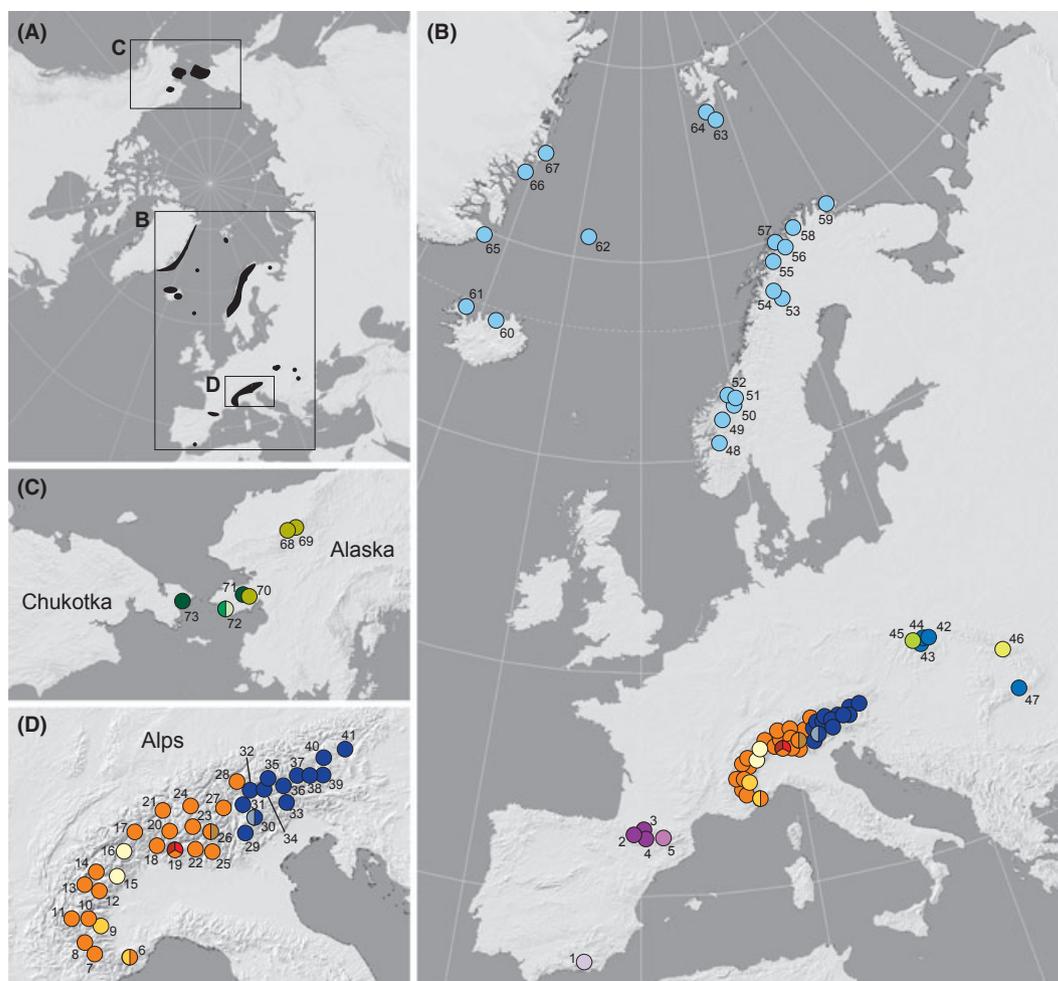


Fig. 2 Geographic distribution of plastid DNA variation encountered in 73 populations of *Ranunculus glacialis* s. l. Population numbers refer to Table 1; colour-coding of haplotypes corresponds to the statistical parsimony network presented in Fig. 1A. (A) Distribution of *R. glacialis* s. l.; frames indicate regions for which detailed maps are presented; (B–D) distribution of plastid haplotypes in Europe, Beringia and the European Alps, respectively.

Atlantic Arctic). The second, only poorly supported main clade included haplotypes from the western part of the Alps (H5–H10), the Pyrenees and the Sierra Nevada (H11–H13), the Carpathians (H14, H15) and Beringia (H16–H19). Among those, only the Beringian and the Carpathian populations formed a separate, highly supported lineage (Fig. 1).

ITS variation and phylogeographic structure

Internal transcribed spacer sequences were obtained from all samples studied and 52 ribotypes were identified, most of which were infrequent. Only four ribotypes exceeded a frequency of 5%, with R1 being the most common (Fig. 3). It had the widest distribution spanning the entire European Alpine System and the North Atlantic area, with a complete absence only from the Western Alps and from Beringia (Fig. 4,

Table 1). In 38 populations, intraindividual variation was detected. With the notable exception of most western Alpine populations, where two (populations 12–18, 21) or even three (populations 9, 11) regional ribotype lineages were present, intraindividual variation mostly involved closely related ribotypes (Table 1). The statistical parsimony network revealed several ribotype groups, four of which were also recognized by the BEAST analysis with a posterior probability >0.95 (Fig. 3). The group with the largest geographic extent, spanning the entire distribution area of *R. glacialis* subsp. *glacialis*, comprised the most frequent ribotype R1 and its derivatives such as the relatively abundant R3 occurring in the easternmost Alps and several Carpathian populations. In addition, this group contained two geographically well-defined lineages, the first comprising populations from the Pyrenees (R16–R18) and the second those from Beringia (R12–R15). Populations

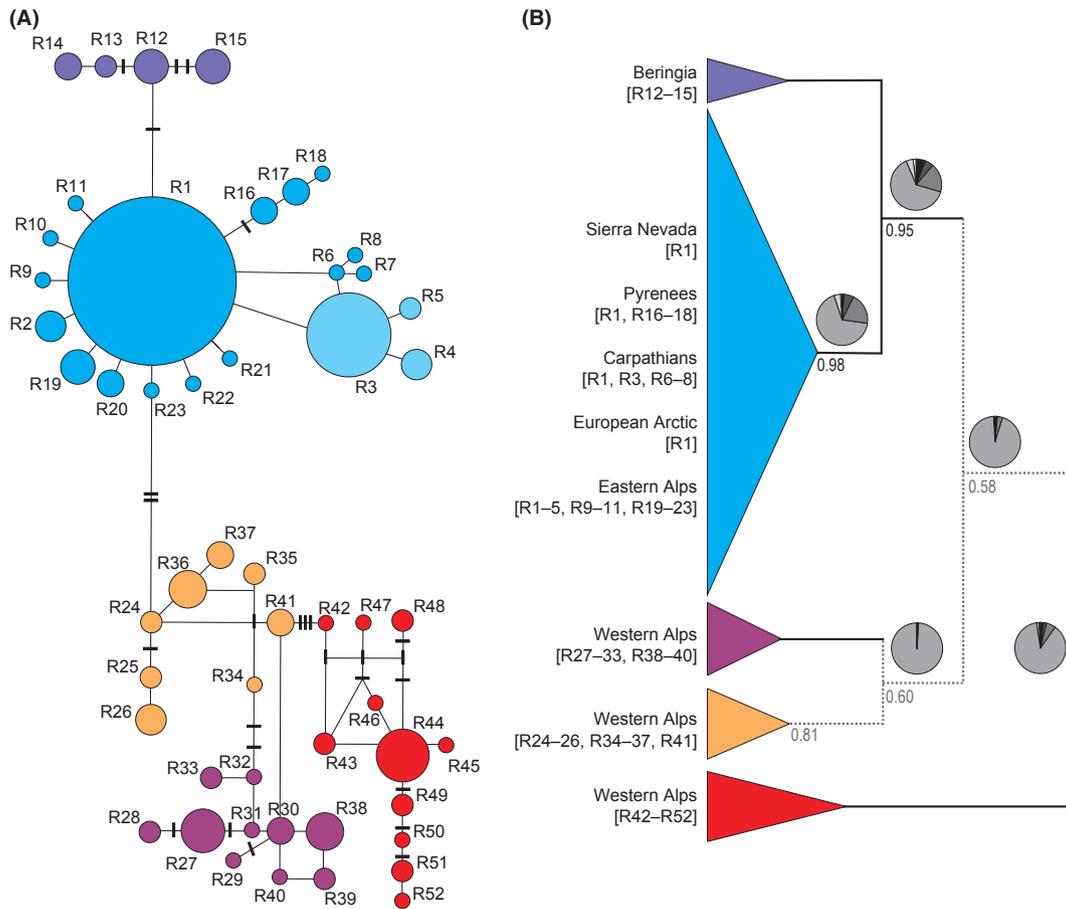


Fig. 3 Structuring of internal transcribed spacers (ITS) diversity encountered in *Ranunculus glacialis* s. l. (A) Statistical parsimony haplotype network. Ribotypes are colour-coded; their numbering corresponds to Table 1. Due to the large number of low-frequency ribotypes, colours are assigned to the lineages identified by *BEAST* rather than to single ribotypes. Ribotypes R3–R5, whose western distribution limit corresponds with the main split in the plastid data set (see text), are additionally shown in faint blue. Bars indicate ribotypes not detected in the data set. (B) Simplified majority rule consensus tree from strict clock Bayesian analysis with the software *BEAST*, node heights corresponding to median relative ages. Numbers below branches indicate Bayesian posterior probabilities. Geographic distribution and pertaining ribotypes are given for each lineage. Ancestral area probabilities are indicated as pie charts at internal nodes and at nodes subtending geographically heterogeneous terminal clades (areas in clock-wise arrangement and distinguished by a black to white colour gradient: Beringia, European Arctic, Carpathians, Alps, Pyrenees, Sierra Nevada). Lineages receiving posterior probabilities (PP) ≥ 0.95 are shown with continuous lines; those with lower PP are illustrated with hatched lines.

from the Western Alps hosted two to three lineages constituted by numerous closely related ribotypes of low frequency. Two groups (R27–R32, R38–R40 and R42–R52) were strictly co-occurring and absent from the southwesternmost Alps, where the third group (R24–R26, R34–R37, R41) dominated. The high diversity of southern European populations strongly contrasted with the uniformity in the ampho-Atlantic Arctic area (Fig. 4).

The *BEAST* reconstruction was only partially resolved (Fig. 3; the full, annotated maximum clade credibility tree file is available in Appendix S2, Supporting information). Ribotypes from the Western Alps fell into three lineages. All other populations clustered in one

clade, with two strongly supported subclades with Beringian and European distribution, respectively.

Range connectivity and ancestral areas of lineages

The analysis of range connectivity among discrete geographic regions based on plastid DNA (Fig. 5) established links (Bayes Factors in parentheses) between (i) the Pyrenees and the Sierra Nevada (24.8), (ii) the Carpathians and Beringia (5.02), (iii) the Alps and the Carpathians (4.91) and (iv) the Alps and the North Atlantic Arctic (2.99). In the ITS data set, geographic connections were supported only between the Alps and the Carpathians (BF = 9.54) and between the Carpathians and the

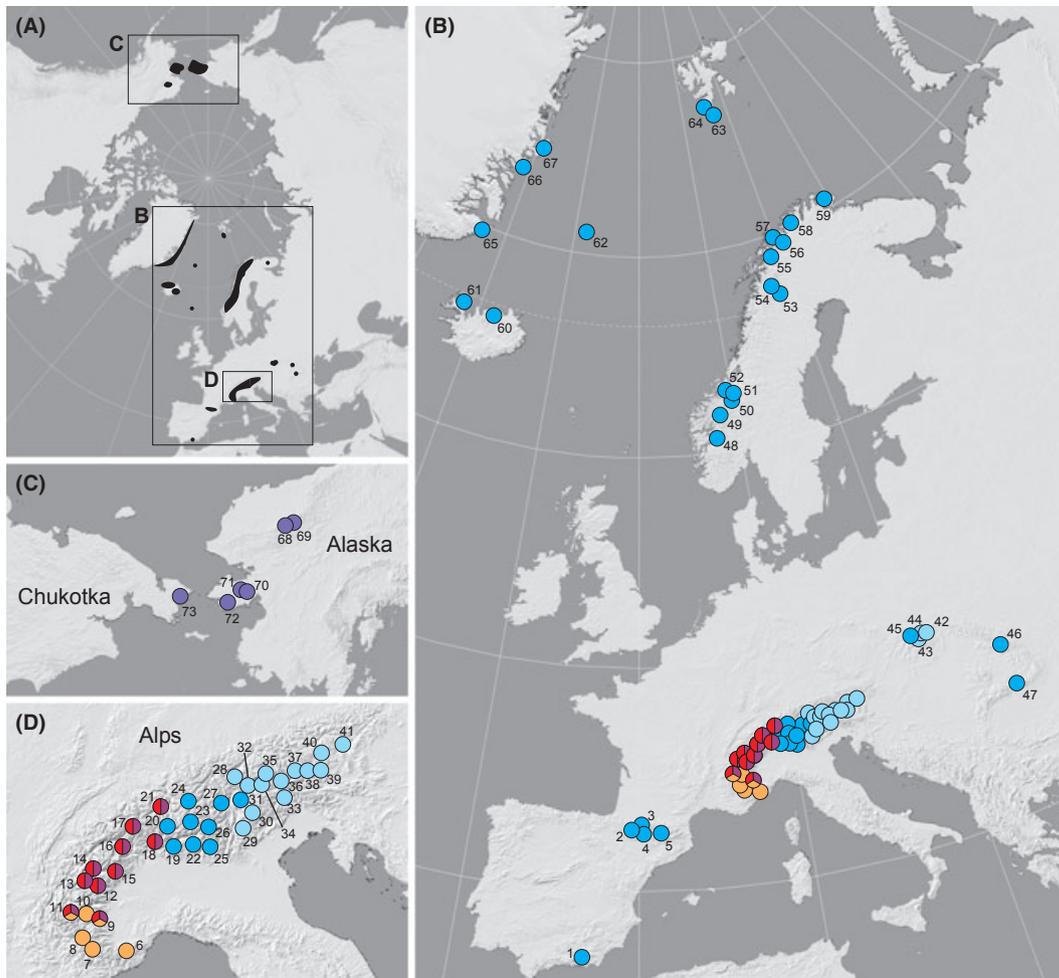


Fig. 4 Geographic distribution of internal transcribed spacers variation encountered in 73 populations of *Ranunculus glacialis* s. l. Population numbers refer to Table 1; colour-coding of ribotypes corresponds to the statistical parsimony network presented in Fig. 3. (A) Distribution of *R. glacialis* s. l.; frames indicate regions for which detailed maps are presented; (B–D) Distribution of ribotypes in Europe, Beringia and the European Alps, respectively.

North Atlantic Arctic (5.16). For both data sets, the set of supported geographic links remained unchanged when using reduced data sets with maximally ten populations per region (data not shown).

Ancestral location probabilities are indicated in Figs 1 and 3. For the plastid data set, there was a positive correlation between the geographic heterogeneity of a clade and the ambiguity in the reconstruction of its ancestral area, such that the set of geographic areas until their cumulative probability exceeded 0.8 included all areas present at the tips. At the root node, this set included all regions except the Sierra Nevada and the Pyrenees, with the Alps and the Carpathians having the highest probabilities (0.32 and 0.27, respectively). In the reconstructions based on the ITS data set, ambiguities were much less pronounced. For all nodes except that subtending an exclusively Beringian clade, the Alps

had the highest probability as ancestral area (0.64–0.99), even in geographically heterogeneous clades. Whereas for the plastid data set ancestral area probabilities remained unaffected by the number of populations per region, for the ITS data probabilities became more evenly distributed among regions in a data set containing maximally ten populations per region, although in most cases the Alps remained the best supported region (0.36–0.99; data not shown).

Discussion

Many arctic–alpine species display fragmented distribution ranges. One of the plants exhibiting an extreme disjunction between Europe and Beringia is *Ranunculus glacialis* s. l. Discovery of a plastid lineage found in two Carpathian populations as the closest relative of the



Fig. 5 Range connectivity inferred using a discrete phylogeographic model in *BEAST*. Connections supported by Bayes Factors $BF \geq 3$ from the plastid and the internal transcribed spacers data are shown by solid and broken lines, respectively. Line widths are proportional to the squared root of the respective Bayes factors.

Beringian haplotypes and nesting of the Beringian nuclear ribosomal ITS lineage within European lineages reject the hypothesis that the divergence between the European and Beringian populations constitutes the oldest evolutionary split within *R. glacialis* s. l. A southern European origin, most probably in the Alps, followed by a series of range expansions is suggested for all ITS lineages and several major plastid lineages (Figs 1 and 3). In the same line, the most ancient diversification seen in plastid and ITS sequences occurred within the European Alpine System (excluding the Scandinavian Mountains), a scenario that is also supported by the distribution of the closest relatives (Pawłowski 1929; Paun *et al.* 2005; Hörandl & Emadzade 2011). Formation of the European/Beringian disjunction was thus not the primary evolutionary process but occurred at a later stage of range formation.

Strong differentiation among mostly allopatric lineages in the European Alpine System

Plastid DNA sequences uncovered several distinct lineages with mostly sharply circumscribed distributions (Figs 1 and 2) suggesting stepwise range expansions rather than multiple long-distance dispersals within the

EAS. The main phylogeographic break is not correlated with a disjunction among mountain ranges, but is located within the Alps. Two ancient, strictly allopatric, east–west vicariant lineages abut along the Etsch/Adige valley and the Brenner Pass, an area long recognized as one of the major phylogeographic boundaries in the Alps (e.g. Kerner 1871; Merxmüller 1952–1954). As previously observed in AFLP-based studies on Alpine plants (e.g. Schönswetter & Tribsch 2005; Schönswetter *et al.* 2005; Alvarez *et al.* 2009; Thiel-Egenter *et al.* 2011) and animals (e.g. Pauls *et al.* 2006; Haubrich & Schmitt 2007), the genetic break does not coincide with a distribution gap. The set of three Western Alpine ITS lineages with partly overlapping distributions on the one hand and the widespread ITS lineage occurring in the Eastern Alps on the other hand are also strictly allopatric, but their boundary is further west than that revealed by plastid DNA; it is situated between Lake Constance and Lake Como, corroborating a phylogeographic break identified in a previous AFLP study (Schönswetter *et al.* 2004). This intraspecific borderline supports a classical biogeographic boundary between the Eastern and Western Alps (Aeschmann *et al.* 2011), which has often emerged in biogeographic (Pampanini 1903; Pawłowski 1970) or vegetation ecological (Ozenda 1985) contexts. It showed little congruence, however, with break zones of species or allele distributions in a recent multispecies study (Thiel-Egenter *et al.* 2011). The shift between the two splits seen in plastid vs. nuclear data may indicate differences in gene flow mediated by pollen and seeds (Ennos 1994). The break in the plastid data coincides, however, with the distributional separation of ITS ribotypes R3 and its derivatives R4 and R5 from the others (Fig. 4), corroborating the Brenner Pass as an important phylogeographic contact zone in the Eastern Alps. The patterns reflected by sequence divergence support earlier phylogeographic observations inferred from rapidly evolving AFLP markers (e.g. Schönswetter *et al.* 2002, 2003) but also provide a deeper temporal perspective for underlying biogeographic mechanisms, certainly going far beyond the time frame of the last glaciation and altogether demonstrating high range persistence and stability of refugia over time.

Populations from the western Alps showed strong internal differentiation and high diversity with six specific, though closely related, plastid haplotypes and 29 ribotypes falling into three lineages. This diversity may be a vestige of a complex glacial history involving several local refugia, which were amply present due to the weak glaciations of the siliceous inner margin of the Alps during the Last Glacial Maximum (Voges 1995). The alternative hypothesis is ancient hybridization, as the most divergent group of ribotypes (R42–R52) is exclusively present as intraindividual variation; under

this scenario, the high number of low-frequency ribotypes in this area would result from various stages of concerted evolution (Álvarez & Wendel 2003). Inclusion of available sequences of the closest relatives of *R. glacialis*, that is, *R. aconitifolius*, *R. kuepferi*, *R. plataniifolius* and *R. seguieri* (Paun *et al.* 2005; Hörandl & Emadzade 2011), in a NeighborNet analysis conducted using SplitsTree4 (Huson & Bryant 2006), however, did not indicate any traces of hybridization involving *R. glacialis* (data not shown).

Most Iberian populations host unique plastid haplotypes and ribotypes (Figs 1 and 3) in accordance with the deep AFLP divergence revealed for the single hitherto investigated Pyrenean population (Schönswetter *et al.* 2004). Long-term isolation and lack of gene exchange between the Pyrenees and the Western Alps contrasts with relatively recent colonization of the Pyrenees in *Phyteuma globulariifolium* or *Carex curvula* (Schönswetter *et al.* 2002; Puşcaş *et al.* 2008) and probable repeated dispersal between these areas in *Pulsatilla vernalis* (Ronikier *et al.* 2008b; but see Lihová *et al.* 2009 for a contrasting example). An exception to the occurrence of exclusively Iberian sequence variants is the common and widespread ribotype R1 present in the Sierra Nevada and the eastern Pyrenees, where it gave rise to a stepwise westward range expansion (R16–R18). The Iberian plastid lineage (H11–13) is distantly related to that in the Western Alps, albeit without strong support (Fig. 1). The terminal position of the plastid haplotype from the Sierra Nevada (H13) in the statistical parsimony network (Fig. 1) suggests that the southwestern edge of the species' distribution was colonized from the Pyrenees, a scenario highly supported by the Bayesian reconstruction of migration events (Fig. 5) and shown also by other mountain taxa of this area (Kropf *et al.* 2006).

Most Carpathian populations were genetically tightly linked to those from the Eastern Alps (Figs 2 and 4). The hypothesis of a relatively recent establishment of *R. glacialis* in the Carpathians is challenged, however, by the discovery of a rare and strongly divergent plastid lineage constituted by haplotypes H14 and H15, the next relatives of the Beringian haplotypes. This supports the importance of the Carpathians as a stepping stone between temperate Europe and the Arctic (Després *et al.* 2002; Albach *et al.* 2006). The ITS data from the same populations, however, did not mirror the Carpathian–Beringian connection as they harboured ribotypes falling into a widespread lineage distributed from the Sierra Nevada to the Carpathians. This discordance is likely due to homogenization of ITS repeats via concerted evolution (Álvarez & Wendel 2003) following hybridization with immigrants from the Alps. Occurrence of divergent groups in the Carpathians, and

particularly in the Tatra Mts., supports that this mountain range has been a meeting ground of various lineages through time (Ronikier *et al.* 2008b; Ronikier 2011; Winkler *et al.* 2012). Overall, persistence of a rare plastid lineage in the Tatra Mountains as well as in the localized and isolated population 46 in the Romanian Eastern Carpathians supports the importance of the Carpathians as a region important for intraspecific evolution of alpine plants (Ronikier *et al.* 2008a) and a refugium of European mountain biota (e.g. Mardulyn *et al.* 2009), thus also as priority area of conservation efforts.

Independent history of populations from the North Atlantic and the amphi-Beringian area

The arctic distribution of *R. glacialis* s. l. comprises two disjunct areas, one encompassing the North Atlantic Arctic from eastern Greenland to Scandinavia and Svalbard, the other spanning Beringia from easternmost Siberia to Alaska. Plastid and nuclear data sets unravelled distinct Beringian lineages, whose divergence, however, took place well after the initial diversification of the main lineages (Figs 1 and 3). Whereas in several arctic–alpine groups, Beringia was found to be an important source area for (re-)establishment of current circumpolar ranges (Abbott *et al.* 2000; Eidesen *et al.* 2007), in *R. glacialis* North Atlantic Arctic populations are closely related to those from the EAS. Plastid data clearly suggest that populations from both areas have independent ties to populations in the EAS without any signs of inner-Arctic gene flow (Fig. 2), supporting a repeated colonization of the Arctic and emphasizing the importance of temperate mountain ranges in arctic–alpine phylogeography. This scenario is in agreement with the ITS data (Fig. 3), although due to the wide distribution of ribotype R1 also in the North Atlantic Arctic inner-arctic gene flow cannot be excluded based on ITS data alone. Connections between the amphi-Beringian Arctic and the EAS have been found before in *Androsace* (Primulaceae, Schneeweiss *et al.* 2004), but, similarly as in *R. glacialis*, the precise mechanisms (long-distance dispersal or massive extirpations of intervening northern populations) remain unknown. Incongruent results were obtained with respect to the directionality of connections between the Beringian populations and those from the EAS. In the plastid data, after an initial colonization of Beringia from the EAS (Carpathians and/or Alps), a Beringian origin of the Carpathian haplotypes H15–H16, implying back-colonization from Beringia into the EAS, received more support than a Carpathian origin (even though the probability for the latter was not negligible either; Fig. 1). In contrast, the ITS data strongly supported a European (Alpine) origin and unidirectional coloniza-

tion of Beringia. These differences in inference of the ancestral area probably are the result of different evolutionary dynamics of the used markers (homogenization of ITS, resilience against genetic swamping of plastid markers; Álvarez & Wendel 2003; Currat *et al.* 2008). The same processes together with shifts in the local geographic distribution of plastid and nuclear lineages in the Alps due to differences in gene flow mediated by pollen and seeds may also account for the incongruence concerning the relationship of the Beringian to the Western Alpine lineage (Fig. 1 versus Fig. 3). Additional data will be necessary both to further test the directionality of the migration between Beringia and the EAS and to clarify the relationships of the Beringian to the Alpine lineages.

The distribution area of *R. glacialis* s. l. on both sides of the Bering Strait overlaps with an important Pleistocene refugium for arctic biota (Hultén 1937) whose relevance was confirmed by multidisciplinary evidence (Abbott & Brochmann 2003; Hewitt 2004; DeChaine 2008). Whereas large parts of Beringia remained unglaciated during the Last Glacial Maximum (Hultén 1937; DeChaine 2008), older glaciations were probably more extensive (Heiser & Roush 2001) and may have led to local isolation and diversification after primary colonization. The extent of morphological and ecological diversification led to the recognition of two Beringian taxa, *R. camissonis* and *R. glacialis* subsp. *alaskensis*. Whereas the distinctness of the Beringian lineage was apparent (Figs 2 and 4; Table 1), our data do not support a – morphologically plausible – closer relationship of subsp. *alaskensis* with the mostly European subsp. *glacialis* to the exclusion of *R. camissonis* as suggested by Yurtsev *et al.* (2012). No clear genetic differentiation between populations of *R. camissonis* and *R. glacialis* subsp. *alaskensis* was found (Figs 2 and 4; Table 1). For instance, population 73 of *R. camissonis* shared haplotype H19 with *R. glacialis* subsp. *alaskensis*, and populations 70 and 71 pertaining to both taxa exhibited the same ribotypes. More highly resolving population genetic data applied to a dedicated sampling coupled with morphological and ecological surveys of populations to track potential hybridization are needed to test for potential divergence of the two Beringian taxa.

Range expansion to the North Atlantic area took place more recently. This rejects the hypothesis of long separation of northern and southern European populations inferred from cultivation experiments and morphological observations (Böcher 1972), but corroborates an earlier AFLP study suggesting postglacial colonization from the Eastern Alps (Schönswetter *et al.* 2003). No sequence variation was found, although we included populations from the entire North Atlantic Arctic range of *R. glacialis*. The exclusively found plas-

tid haplotype H4 belongs to the lineage comprising the Eastern Alps and most of the Carpathian populations; it differs by a single mutational step from the Eastern Alpine haplotype H1. The presence of a distinct haplotype (Fig. 2) discourages postglacial migration directly from the Alps but rather suggests spread from populations of Alpine origin that persisted in a northern refugium south of the Scandinavian ice sheet. The time frame for the northward range expansion is set by the end of the Last Glacial Maximum and the retreat of the Scandinavian ice sheet starting 15 000 years ago (Svendsen *et al.* 2004). Macrofossil records of *R. glacialis* from Younger Dryas sediments (c. 12 000 years ago) from western Norway (Birks & Birks 2008) illustrate the velocity of this colonization. Even though *R. glacialis* belongs to the hardest candidates for *in situ* glacial survival on nunataks (Birks 2008), we did not find any support for such a scenario.

Contrasting range dynamics in the European Alpine System and the North Atlantic area in response to climate change

The contrasting phylogeographic patterns in the EAS and in the North Atlantic area indicate significantly different responses of *R. glacialis* towards a changing environment. Within the EAS, *R. glacialis* underwent a complex history of allopatric differentiation probably driven by isolation in refugia. This suggests a largely static distribution with climate-induced altitudinal migration, but mostly absent long-distance dispersal. This distributional stasis in the EAS stands in marked contrast to the rapid, latitudinal spread over very large distances in the North Atlantic area. A similar pattern with genetically diverse southern populations and a genetically uniform northern range was discovered based on AFLPs in another arctic–alpine species, *Arabis alpina* (Ehrich *et al.* 2007). Postglacial expansion to Scandinavia from northern refugia by uniform genetic lineages was also found in *Pulsatilla vernalis* (Ronikier *et al.* 2008b). Thus, frequent large-scale dispersal seems to be a common feature in arctic plant species and tracking habitats in the vast, open Arctic is efficient even in species lacking adaptations for long-distance dispersal (see also Alsos *et al.* 2007).

Species distribution models predict strong changes in plant distributions driven by the ongoing global warming, and arctic–alpine species face the highest risk of extirpation in southern parts of their ranges (Thuiller *et al.* 2005; Alsos *et al.* 2009, 2012; Espindola *et al.* 2012). This implies that for arctic–alpine species that originated and diversified in the European mountains such processes may cause an eradication of the most divergent lineages and thus significantly reduce

the species' evolutionary potential. In *R. glacialis*, this concerns especially the Beringian–European lineage represented in Europe by small and vulnerable Carpathian populations (Ronikier 2010). On the other hand, the genetic distinctness of populations from temperate mountain ranges supports the importance of low-latitude areas for the persistence of biodiversity during the Quaternary climatic oscillations (Hampe & Petit 2005). The stable persistence of regional lineages, which probably diverged several climatic cycles ago, may suggest a good ability to survive repeated range contractions within mountain ranges due to the broad spectrum of conditions maintained by microhabitat diversity in the mountain landscape, which may, as recently suggested (Scherrer & Körner 2011), buffer to some extent the direct influence of climate warming.

Acknowledgements

We thank Richard Abbott and three anonymous reviewers for insightful comments which helped to improve the manuscript. We are indebted to Inger Greve Alsos, Torbjørn Borgen, Gilles Corriol, Božo Frajman, David Murray, Carolyn Parker, Anna Ronikier, Inger Skrede, Heidi Solstad and Andreas Tribsch for company in the field or collecting samples. Collecting permits were granted by Tatrzanski Park Narodowy, Poland (no. Bot-203), Ministerstvo Životneho Prostredia, Slovakia (no. 1762/565/04-5.1) and Academia Română, Comisia pentru ocrotirea monumentelor naturii, Romania (no. 291); authorities granting permits for Alpine populations are listed in Schönswetter *et al.* (2004). We are grateful to Juan Lorite from the Parque Nacional de Sierra Nevada for providing plant material. The work was financially supported by grant no N303 013 32/0656 from the Polish Ministry of Science and Higher Education and partly through statutory funds of the Institute of Botany, Polish Academy of Sciences, to M.R.

References

- Abbott RJ, Brochmann C (2003) History and evolution of the arctic flora: in the footsteps of Eric Hultén. *Molecular Ecology*, **12**, 299–313.
- Abbott RJ, Smith LC, Milne RI, Crawford RMM, Wolff K, Balfour J (2000) Molecular analysis of plant migration and refugia in the Arctic. *Science*, **289**, 1343–1346.
- Aeschimann D, Lauber K, Moser DM, Theurillat JP (2004) Flora alpina. 1. Haupt, Bern.
- Aeschimann D, Rasolofo N, Theurillat JP (2011) Analyse de la flore des Alpes. 1: historique et biodiversité. *Candollea*, **66**, 27–55.
- Albach D, Schönswetter P, Tribsch A (2006) Comparative phylogeography of the *Veronica alpina* complex in Europe and North America. *Molecular Ecology*, **15**, 3269–3286.
- Alsos IG, Eidesen PB, Ehrich D *et al.* (2007) Frequent long-distance plant colonization in the changing Arctic. *Science*, **316**, 1606–1609.
- Alsos IG, Alm T, Normand S, Brochmann C (2009) Past and future range shifts and loss of diversity in dwarf willow (*Salix herbacea* L.) inferred from genetics, fossils and modeling. *Global Ecology and Biogeography*, **18**, 223–239.
- Alsos IG, Ehrich D, Thuiller W *et al.* (2012) Genetic consequences of climate change for northern plants. *Proceedings of the Royal Society Biological Sciences*, **279**, 2042–2051.
- Álvarez I, Wendel JF (2003) Ribosomal ITS sequences and plant phylogenetic inference. *Molecular Phylogenetics and Evolution*, **29**, 417–434.
- Alvarez N, Thiel-Egenter C, Tribsch A *et al.* (2009) History or ecology? Substrate type as a major driver of spatial genetic structure in Alpine plants. *Ecology Letters*, **12**, 632–640.
- Birks HH (2008) The Late-Quaternary history of arctic and alpine plants. *Plant Ecology and Diversity*, **1**, 135–146.
- Birks HJB, Birks HH (2008) Biological responses to rapid climate change at the Younger Dryas–Holocene transition at Kråkenes, western Norway. *The Holocene*, **18**, 19–30.
- Birks HJB, Willis KJ (2008) Alpines, trees and refugia in Europe. *Plant Ecology and Diversity*, **1**, 147–160.
- Böcher TW (1972) Evolutionary problems in the Arctic flora. In: *Taxonomy, Phytogeography and Evolution* (ed Valentine DH), pp. 101–113. Academic Press, London, New York.
- Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9**, 1657–1660.
- Crawford RMM (2008) Plants at the Margin. Ecological Limits and Climate Change. Cambridge University Press, Cambridge.
- Curat M, Ruedi M, Petit RJ, Excoffier L (2008) The hidden side of invasions: massive introgression by local genes. *Evolution*, **62**, 1908–1920.
- Dahl E (1963) Plant migrations across the North Atlantic Ocean and their importance for the paleogeography of the region. In: *North Atlantic Biota and their History* (eds Löve Á, Löve D), pp. 173–188. Pergamon Press, Oxford.
- DeChaine E (2008) A bridge or a barrier? Beringia's influence on the distribution and diversity of tundra plants. *Plant Ecology and Diversity*, **1**, 197–207.
- Demesure B, Sodzi N, Petit RJ (1995) A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. *Molecular Ecology*, **4**, 129–134.
- Després L, Lorient 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.
- Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, 214.
- Drummond AJ, Rambaut A, Shapiro B, Pybus OG (2005) Bayesian coalescent inference of past population dynamics from molecular sequences. *Molecular Biology and Evolution*, **22**, 1185–1192.
- Ehrich D, Gaudeul M, Assefa A *et al.* (2007) Genetic consequences of Pleistocene range shifts: contrast between the Arctic, the Alps and the East African mountains. *Molecular Ecology*, **16**, 2542–2559.
- Eidesen PB, Carlsen T, Molau U, Brochmann C (2007) Repeatedly out of Beringia: *Cassiope tetragona* embraces the Arctic. *Journal of Biogeography*, **34**, 1559–1574.
- Ennos RA (1994) Estimating the relative rates of pollen and seed migration among plant populations. *Heredity*, **72**, 250–259.

- Escobar García P, Winkler M, Flatscher R *et al.* (2012) Extensive range persistence in peripheral and interior refugia characterizes Pleistocene range dynamics in a widespread Alpine plant species (*Senecio carniolicus*, Asteraceae). *Molecular Ecology*, **21**, 1255–1270.
- Espíndola A, Pellissier L, Hordijk W, Maiorano L, Guisan A, Alvarez N (2012) Predicting present and future intra-specific genetic structure through niche hindcasting across 24 millennia. *Ecology Letters*, **15**, 649–657.
- Flot JF (2010) SeqPHASE: a web tool for interconverting PHASE input/output files and FASTA sequence alignments. *Molecular Ecology Resources*, **10**, 162–166.
- Fuertes Aguilar J, Roselló JA, Nieto Feliner G (1999) Molecular evidence for the compilospecies model of reticulate evolution in *Armeria* (Plumbaginaceae). *Systematic Biology*, **48**, 735–754.
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**, 95–98.
- Hampe A, Petit RM (2005) Conserving biodiversity under climate change: the rear edge matters. *Ecology Letters*, **8**, 461–467.
- Haubrich K, Schmitt T (2007) Cryptic differentiation in alpine-endemic, high-altitude butterflies reveals down-slope glacial refugia. *Molecular Ecology*, **16**, 3643–3658.
- Heiser PA, Roush JJ (2001) Pleistocene glaciations in Chukotka, Russia: moraine mapping using satellite synthetic aperture radar (SAR) imagery. *Quaternary Science Reviews*, **20**, 393–404.
- Hewitt GM (2004) The structure of biodiversity – insights from molecular phylogeography. *Frontiers in Zoology*, **1**, 4.
- Hoffmann MH, von Hagen KB, Hörandl E, Röser M, Tkach NV (2010) Sources of the arctic flora: origins of arctic species in *Ranunculus* and related genera. *International Journal of Plant Sciences*, **171**, 90–106.
- Hörandl E, Emadzade K (2011) The evolution and biogeography of alpine species in *Ranunculus* (Ranunculaceae): a global comparison. *Taxon*, **60**, 415–426.
- Hörandl E, Paun O, Johansson JT *et al.* (2005) Phylogenetic relationships and evolutionary traits in *Ranunculus* s.l. (Ranunculaceae) inferred from ITS sequence analysis. *Molecular Phylogenetics and Evolution*, **36**, 305–327.
- Hultén E (1937) Outline of the History of Arctic and Boreal Biota during the Quaternary Period. J Cramer, New York.
- Hultén E (1958) The ampho-Atlantic plants and their phytogeographical connections. *Kungliga Svenska Vetenskapsakademiens Handlingar, Fjärde Serien*, **7**, 1–340.
- Hultén E (1968) *Flora of Alaska and Neighboring Territories*. Stanford University Press, Stanford, California.
- Huson DH, Bryant D (2006) Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution*, **23**, 254–267.
- Ingvarsson PK, Ribstein S, Taylor DR (2003) Molecular evolution of insertions and deletion in the chloroplast genome of *Silene*. *Molecular Biology and Evolution*, **20**, 1737–1740.
- Jalas J, Suominen J (eds.) (1989) *Atlas Florae Europaeae*. Distribution of Vascular Plants in Europe. 8. Nymphaeaceae to Ranunculaceae. The Committee for Mapping the Flora of Europe & Societas Biologica Fennica Vanamo, Helsinki.
- Kay K, Whittall JB, Hodges SA (2006) A survey of nuclear ribosomal internal transcribed spacer substitution rates across angiosperms: an approximate molecular clock with life history effects. *BMC Evolutionary Biology*, **6**, 36.
- Kelchner SA, Wendel JF (1996) Hairpins create minute inversions in non-coding regions of chloroplast DNA. *Current Genetics*, **30**, 259–262.
- Kerner A (1871) Die natürlichen Floren im Gelände der deutschen Alpen. In: *Die deutschen Alpen für Einheimische und Fremde geschildert*, 2nd edn (ed. Schaubach A), pp. 126–189. Frommann, Jena.
- Koch M, Kiefer C, Ehrich D, Vogel J, Brochmann C, Mummenhoff K (2006) Three times out of Asia Minor: the phylogeography of *Arabis alpina* L. (Brassicaceae). *Molecular Ecology*, **15**, 825–839.
- Körner C (2003) *Alpine Plant Life*, 2nd edn. Springer, Heidelberg.
- Kropf M, Comes HP, Kadereit JW (2006) Long-distance dispersal vs vicariance: the origin and genetic diversity of alpine plants in the Spanish Sierra Nevada. *New Phytologist*, **172**, 169–184.
- Lemey P, Rambaut A, Drummond AJ, Suchard MA (2009) Bayesian phylogeography finds its roots. *PLoS Computational Biology*, **5**, e1000520.
- Lihová J, Carlsen T, Brochmann C, Marhold K (2009) Contrasting phylogeographies inferred for the two alpine sister species *Cardamine resedifolia* and *C. alpina* (Brassicaceae). *Journal of Biogeography*, **36**, 104–120.
- Löve A, Löve D (1974) Origin and evolution of the arctic and alpine floras. In: *Arctic and Alpine Environments* (eds Ives JD, Barry RG), pp. 571–603. Methuen, London.
- Mardulyn P, Mikhailov YE, Pasteels JM (2009) Testing phylogeographic hypotheses in a Euro-Siberian cold-adapted leaf beetle with coalescent simulations. *Evolution*, **63**, 2717–2729.
- Merxmüller H (1952) Untersuchungen zur Sipplgliederung und Arealbildung in den Alpen. I. *Jahrbuch des Vereins zum Schutze der Alpenpflanzen und -tiere*, **17**, 96–133.
- Merxmüller H (1953) Untersuchungen zur Sipplgliederung und Arealbildung in den Alpen. II. *Jahrbuch des Vereins zum Schutze der Alpenpflanzen und -tiere*, **18**, 138–158.
- Merxmüller H (1954) Untersuchungen zur Sipplgliederung und Arealbildung in den Alpen. III. *Jahrbuch des Vereins zum Schutze der Alpenpflanzen und -tiere*, **19**, 97–139.
- Negrean G, Dihoru G (2009) *Ranunculus glacialis* L. In: *Cartea Roşie a plantelor vasculare din România* (eds Dihoru G, Negrean G), pp. 443–444. Editura Academiei Române, Bucureşti.
- Ozenda P (1985) La végétation de la chaîne alpine dans l'espace montagnard Européen. Masson, Paris.
- Pampanini R (1903) Essai sur la géographie botanique des Alpes et en particulier des Alpes sud-orientales. *Mémoires de la Société Fribourgeoise des Sciences Naturelles, Serie Géologie et Géographie*, **3**, 1–215.
- Parducci L, Jørgensen T, Tollefsrud MM *et al.* (2012) Glacial survival of boreal trees in Northern Scandinavia. *Science*, **335**, 1083–1086.
- Pauls SU, Lumbsch HT, Haase P (2006) Phylogeography of the montane caddisfly *Drusus discolor*: evidence for multiple refugia and periglacial survival. *Molecular Ecology*, **15**, 2153–2169.
- Paun O, Lehnbech C, Johansson JT, Lockhart P, Hörandl E (2005) Phylogenetic relationships and biogeography of *Ranunculus* and allied genera (Ranunculaceae) in the Mediterranean region and in the European Alpine System. *Taxon*, **54**, 911–930.
- Pawlowski B (1929) Die geographischen Elemente und die Herkunft der Flora der subnivalen Vegetationsstufe im Tatra-Gebirge. *Bulletin de l'Académie Polonaise des Sciences et des Lettres*.

- Classe des Sciences Mathématiques et Naturelles, Sér. B: Sciences Naturelles, 1929, 161–202.
- Purowski B (1970) Remarques sur l'endémisme dans la flore des Alpes et des Carpates. *Vegetatio*, **21**, 181–243.
- Posada D (2008) jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution*, **25**, 1253–1256.
- Puşças M, Choler P, Tribsch A *et al.* (2008) Post-glacial history of the dominant alpine sedge *Carex curvula* in the European Alpine System inferred from nuclear and chloroplast markers. *Molecular Ecology*, **17**, 2417–2429.
- Ronikier M (2010) Distribution of the arctic-alpine *Ranunculus glacialis* (Ranunculaceae) in the Carpathians with a new locality in the Făgăraş mountains (Romania). *Polish Botanical Journal*, **55**, 199–207.
- Ronikier M (2011) Biogeography of high-mountain plants in the Carpathians: an emerging phylogeographical perspective. *Taxon*, **60**, 373–389.
- Ronikier M, Cieślak E, Korbecka G (2008a) High genetic differentiation in the alpine plant *Campanula alpina* Jacq. (Campanulaceae): evidence for glacial survival in several Carpathian regions and long-term isolation between the Carpathians and the Alps. *Molecular Ecology*, **17**, 1763–1775.
- Ronikier M, Costa A, Fuertes Aguilar J, Nieto Feliner G, Küpfer P, Mirek Z (2008b) Phylogeography of *Pulsatilla vernalis* (L.) Mill. (Ranunculaceae): chloroplast DNA reveals two evolutionary lineages across central Europe and Scandinavia. *Journal of Biogeography*, **35**, 1650–1664.
- Santisuk T (1979) A palynological study of the tribe Ranunculaceae (Ranunculaceae). *Opera Botanica*, **48**, 1–74.
- Scherrer D, Körner C (2011) Topographically controlled thermal-habitat differentiation buffers alpine plant diversity against climate warming. *Journal of Biogeography*, **38**, 406–416.
- Schmitt T (2007) Molecular biogeography of Europe: Pleistocene cycles and postglacial trends. *Frontiers in Zoology*, **4**, 11.
- Schneeweiss GM, Schönswetter P, Kelso S, Niklfeld H (2004) Complex biogeographic patterns in *Androsace* (Primulaceae) and related genera: evidence from phylogenetic analyses of nuclear ITS and plastid trnL-F sequences. *Systematic Biology*, **53**, 856–876.
- Schönswetter P, Tribsch A (2005) Vicariance and dispersal in the alpine perennial *Bupleurum stellatum* L. (Apiaceae). *Taxon*, **54**, 725–732.
- 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, Paun O, Tribsch A, Niklfeld H (2003) Out of the Alps: colonization of Northern Europe by East Alpine populations of the Glacier Buttercup *Ranunculus glacialis* L. (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.
- Schönswetter P, Stehlik I, Holderegger R, Tribsch A (2005) Molecular evidence for glacial refugia of mountain plants in the European Alps. *Molecular Ecology*, **14**, 3547–3555.
- Shaw J, Lickey EB, Beck JT *et al.* (2005) The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany*, **92**, 142–166.
- Shaw J, Lickey EB, Schilling EE, Small RL (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in Angiosperms: the tortoise and the hare III. *American Journal of Botany*, **94**, 275–288.
- Skrede I, Eidesen PB, Piñeiro Portela R, Brochmann C (2006) Refugia, differentiation and postglacial migration in arctic-alpine Eurasia, exemplified by the mountain avens (*Dryas octopetala* L.). *Molecular Ecology*, **15**, 1827–1840.
- Smith CI, Pellmyr O, Althoff DM, Balcázar-Lara M, Leebens-Mack J, Segraves KA (2008) Pattern and timing of diversification in *Yucca* (Agavaceae): specialized pollination does not escalate rates of diversification. *Proceedings of the Royal Society B Biological Sciences*, **275**, 249–258.
- Stephens M, Smith NJ, Donnelly P (2001) A new statistical method for haplotype reconstruction from population data. *American Journal of Human Genetics*, **68**, 978–989.
- Stewart JR, Lister AM, Barnes I, Dalén L (2010) Refugia revisited: individualistic responses of species in space and time. *Proceedings of the Royal Society Biological Sciences*, **277**, 661–671.
- Svendsen JI, Alexanderson H, Astakhov VI *et al.* (2004) Late quaternary ice sheet history of northern Eurasia. *Quaternary Science Reviews*, **23**, 1229–1271.
- Templeton AR, Crandall KA, Sing CF (1992) A cladistic analysis of the phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics*, **132**, 619–633.
- Thiel-Egenter C, Alvarez N, Holderegger R *et al.* (2011) Break zones in the distributions of alleles and species in alpine plants. *Journal of Biogeography*, **38**, 772–782.
- Thuiller W, Lavorel S, Araujo MB, Sykes MT, Prentice IC (2005) Climate change threats to plant diversity in Europe. *Proceedings of the National Academy of Sciences of the USA*, **102**, 8245–8250.
- Totland Ø, Alatalo JM (2002) Effects of temperature and date of snowmelt on growth, reproduction and flowering phenology in the arctic/alpine herb, *Ranunculus glacialis*. *Oecologia*, **133**, 168–175.
- Uotila P (2001) Ranunculaceae. In: Jonsell B (ed) *Flora Nordica*, **2**, 227–335.
- Voges A (1995) International Quaternary Map of Europe, B10 Bern. Bundesanstalt für Geowissenschaften und Rohstoffe/UNESCO, Hannover.
- Wagner J, Steinacher G, Ladinig U (2010) *Ranunculus glacialis* L.: successful reproduction at the altitudinal limits of higher plant life. *Protoplasma*, **243**, 117–128.
- Westergaard KB, Jørgensen MH, Gabrielsen TM, Alsos IG, Brochmann C (2010) The extreme Beringian/Atlantic disjunction in *Saxifraga rivularis* (Saxifragaceae) has formed at least twice. *Journal of Biogeography*, **37**, 1262–1276.
- Westergaard KB, Alsos IG, Popp M, Engelskjøn T, Flatberg KI, Brochmann C (2011) Glacial survival may matter after all: nunatak signatures in the rare European populations of two west-arctic species. *Molecular Ecology*, **20**, 376–393.
- White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: A Guide to Methods and Applications* (eds Innis MA, Gelfand DH, Sninsky JJ, White TJ), pp. 315–322. Academic Press, New York.
- Winkler M, Tribsch A, Schneeweiss GM *et al.* (2012) Tales of the unexpected: phylogeography of the arctic-alpine model

plant *Saxifraga oppositifolia* (Saxifragaceae) revisited. *Molecular Ecology*, **21**, 4618–4630.

Yamane K, Yasui Y, Ohnishi O (2003) Intraspecific cpDNA variations of diploid and tetraploid perennial buckwheat, *Fagopyrum cymosum* (Polygonaceae). *American Journal of Botany*, **90**, 339–346.

Yurtsev BA, Murray DF, Elven R (2012) *Ranunculus glacialis* subsp. *alaskensis* subsp. nov. (Ranunculaceae), a Beringian race of an otherwise Atlantic species. *Journal of the Botanical Research Institute of Texas*, **6**, 17–24.

M.R. and P.S. designed the study and collected most samples. M.R. performed the research. M.R., G.M.S. and P.S. analysed the data. M.R. wrote the draft and all authors revised the manuscript. M.R. is interested in the biogeography and evolution of alpine plants and fungi in the European mountains, with a focus on the Carpathians. G.M.S. is interested in different aspects of plant evolution, including genome evolution of parasitic plants, phylogeography, polyploid evolution and speciation. P.S. is interested in polyploid evolution and in spatiotemporal diversification of European alpine plants.

Data accessibility

DNA sequences (GenBank Accessions): JX105046–JX105241, JX118260–JX118638.

Sequence alignments: DRYAD data base entry doi:10.5061/dryad.7b87b. Full, annotated maximum clade credibility tree files from BEAST analyses are available as On-line supplementary material (Appendix S1 and S2, Supporting information).

Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 The annotated maximum clade credibility tree (including information such as clade support, absolute node ages, spatial distribution through time) from strict clock Bayesian analysis with the software BEAST based on plastid DNA sequence data from all studied populations.

Appendix S2 The annotated maximum clade credibility tree (including information such as clade support, absolute node ages, spatial distribution through time) from strict clock Bayesian analysis with the software BEAST based on ITS sequence data from all studied populations.

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