Phylogeography of *Swertia perennis* in Europe based on cpDNA markers

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Background. *Swertia perennis* (Gentianaceae) is a perennial diploid and clonal plant species that is discontinuously distributed in peat bogs in the mountains of Europe, Asia and North America as well as in the lowlands of Europe. The current geographical dispersion of *S. perennis* is probably the result of quaternary climatic changes that have played an important role in determining the distribution of *Swertia* and other plant and animal species.

Methods. In this study we used molecular techniques and combined data from chloroplast DNA markers (*trn*LF region and *trn*H-*psb*A spacer) to elucidate the phylogeography of *S. perennis* in Europe. Plants were collected from 28 populations in different locations in the lowlands and mountainous areas of Europe (e.g. the Carpathians, Sudetes, Bohemian Forest and Alps). cDNA was analysed to detect the genetic relationship between specimens from different locations.

Results. A total of 20 haplotypes were identified across the dataset. They were characterised by a high level of genetic variability but showed a lack of phylogeographical structure. This pattern may be the result of repeated recolonization and expansion from several areas. Such genetic differentiation may also be attributed to the relatively long-term isolation of *S. perennis* in Pleistocene refugia in Europe, which resulted in independent separation of different cpDNA phylogenetic lineages and variation in the nucleotide composition of cpDNA.

Discussion. The lack of strong phylogeographical structure makes it impossible to indicate the centre of haplotype diversity; however, refugia located in the Carpathians, Sudetes or Alps are the most probable sites where *S. perennis* existed in Europe. This lack of structure may also indicate a high level of gene flow in times when the landscape and fen systems were not fragmented in numerous geographically-isolated populations. This makes it difficult to speculate about the relationships between Asiatic and European plant populations and the origin and distribution of this species in Europe. Today, it seems to be restricted due to the occurrence of plants which clearly reflects the genetic variability from the ancient period.

Introduction

The distribution of organisms and their genetic structure are the consequences of repeated ~~quaternary~~ Quaternary climatic changes in ecosystems. These changes have dramatically modified the vegetation and resulted in extinctions in colder areas of Europe, America and the Arctic (Hewitt, 1996, 2004; Taberlet et al., 1998). Climate change was also the primary reason for numerous plants and animals migrating to southern parts of Europe or warmer localities (before ice cover) where they survived unfavourable conditions before later beginning their remigration to the north (Ronikier, Cieślak & Korbecka, 2008; Slovák et al., 2012). Some of the most commonly-recognised southern refugia are located in the Mediterranean, on the Balkan Peninsula and in isolated mountain ranges (e.g. the Carpathians and Alps). However, separation of Central and Northern Europe or Northern Russia by a broad belt of lowlands could harbour numerous plants that inhabited similar sites or ecosystems in the Pleistocene (Schönswetter, Popp & Brochmann, 2006; Paun et al., 2008; Urbaniak, Kwiatkowski & Ronikier, 2018). The Alps were covered by ice during this period (Mojski, 1993), which restricted the distribution of plants to isolated nonglacial sites (nunataks or plants migrating to the Alps as secondary migrants being located close to the area's peripheral refugia (Stehlik, 2000, 2003; Schönswetter et al., 2005; Ronikier et al., 2008). Brockmann-Jerosch & Brockmann-Jerosch (1926) suggested that it was possible for species to survive in the glaciated Alps in scattered localities (i.e. the nunataks on the top of the Alps); however, it is also possible that species migrated into the Alps from more southern refugia or from Eastern Europe (Schönswetter, Popp & Brochmann, 2006). This scenario was confirmed for *Ranunculus pygmaeus*, which probably migrated to the valleys of the Alps via the Carpathians from source populations in Siberia (Schönswetter, Popp & Brochmann, 2006). Such migrations were possible due to alternating warm and glacial periods in the quaternary that allowed gene exchange and plant migration between European mountain ranges (e.g. the Alps, Carpathians and Sudetes) (Pawłowski, 1928; Ronikier, Cieślak & Korbecka, 2008). In contrast to the Alps, the Carpathians and Sudetes were only locally or partially glaciated during the quaternary (e.g. lower massifs generally remained ice-free). The existence of numerous valleys and several ranges over 2000 m above sea level meant that they offered a wide spectrum of sites or ecosystems as potential refugia for organisms in fragmented subranges in several regions in Europe. Outside of the Alps, the Sudetes, Bohemian Forest and Carpathians seemed to play an important role~~s~~ as botanical crossroads

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for plants migrating from Siberia, the Arctic or the Caucasus (Schönswetter, Popp & Brochmann, 2006; Ronikier, Cieślak & Korbecka, 2008).

A phylogeographical hypothesis for analysing historical climatic processes that influenced population genetic differentiation has been intensively studied in the Alps and Carpathians, thus linking together studies in the European mountain ranges (e.g. the Alps, Carpathians, Sudetes and Bohemian Forest) (Schönswetter et al., 2005; Albach, Schönswetter & Tribsch, 2006; Ronikier, Cieślak & Korbecka, 2008; Ronikier et al., 2008; Alvarez et al., 2009). However, in contrast to the Alps, the phylogeographical history of plants in the mountain ranges of Central Europe remains poorly understood (Ronikier, 2011). In addition, research considering the widespread circum-boreal plant species in the northern hemisphere inhabited populations in the Sudetes, Bohemian Forest and Harz mountains are also scarce (Alsos et al., 2005; Kramp et al., 2009; Wróblewska, 2013; Jermakowicz et al., 2015). With the exception of studies dealing with *Saxifraga* (Bauert et al., 1998; Vargas, 2001; Lienert et al. 2002; Oliver, Hollingsworth & Gornall, 2006; Winkler et al., 2012; Winkler et al., 2013) and *Salix* sp. (Mirski et al., 2017), no studies have assessed plants inhabiting lowland or mountain peat bog ecosystems. Many peat bog plant species are present in a wide range of circum-boreal alpine regions and have been able to colonise large mountain areas or migrate via Beringia to North America.

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Populations dispersed in scattered localities are susceptible to negative effects from individual population history and to possible fluctuations in their size (number of individuals). Genetic drift and founder or bottleneck effects may also influence the genetic structure of populations that are often genetically isolated (Freeland, 2008; Hansen, Thomas & Arnholdt-Schmitt, 2009). The results of climatic oscillations resulted in plant distributions and contributed to population genetic diversity that can be detected by molecular approaches (Alsos et al., 2005; Wasowicz et al., 2016). These methods are based on nuclear, ribosomal or chloroplast DNA (Taberlet et al., 1991) or DNA polymorphisms (Ronikier, 2011). These methods also provide detailed insight into the processes responsible for the presently-observed distributions and describe refugia areas for selected species. They also enable the detection and enhanced description of genetic relationships between disjunctive and closed plant populations. This can help to define the microevolutionary processes that have occurred within plant populations that have changed their previous distribution range or identify divergent lineages (Ronikier, 2011).

Comment [r3]: Surely these methods identify phylogeographic patterns which can be used to test hypotheses on processes such as climate change.

Therefore the present study used DNA sequence data from chloroplast markers to investigate the genetic structure of individuals representing populations of the peat bog plant species, *Swertia perennis*, in the Polish lowlands, Carpathians, Sudetes, Bohemian Forest and Alps. The combination of samples from various localities together with cpDNA markers should permit the identification of different haplotype lineages across the 1300 km disjunct~~ive~~ distribution range in Europe. This study also aimed to assess whether (like in other temperate mountain species) ~~late or~~ postglacial migration (e.g. from Central Asia) may be a valid explanation for the present distribution of *S. perennis*. The study also compared the genetic relationships between plant populations growing in geographically~~-~~ isolated sites in natural or semi-natural patches in the landscape (Oostermeijer et al. 1996). These populations are commonly spatially isolated and thus likely differ genetically from each other (Young et al., 1996). Therefore all of the studied *S. perennis* individuals were from populations that had a relatively wide but discontinuous distribution and were treated as isolated populations in accordance with the work of Lienert & Fischer (2004).

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Comment [r5]: Now there is a need to state the two hypotheses: migration from ice-free areas of the Alps, or migration from Eastern Europe, and what these hypotheses would predict in the phylogeographic signal.

Material and methods

The species

S. perennis Linnaeus, Sp. Pl. 226, 1753, syn.: *Gentiana palustris* All. Fl. Pedem. 1: 100, 1785 from the Gentianaceae family inhabits lowlands but can also be found at higher altitudes. The species is distributed globally across the Northern hemisphere, but also shows discontinuous distribution from Asia to North America. It inhabits wetlands, especially peat bogs in calcareous fens or high mountains; however, it is also present in wet meadows, creek shores or wet rocks. It is a species of circum-boreal distribution, inhabiting Arctic, Siberian and Northern, Atlantic and Central European provinces (Hultén, 1968; Hultén & Fries, 1986; Meusel et al., 1978). In Europe, the species can be found in all mountain range systems of Alpine orogeny, including the Pyrenees, Caucasus, Dinaric Alps, Carpathians and Herzynian mountains (Sudetes, Bohemian Forest), as well as in peat bogs in Central Europe and Eastern lowlands. *S. perennis* is a long-lived perennial rhizome herb that usually produces one erect stem that grows to around 10–50 cm tall. It is a diploid (2n = 28) organism that flourishes in July or August (Löve & Löve, 1986; Pawlikowski & Wołkowycki, 2010). The flower nectar chambers are visited by various insects including species of Coleoptera, Lepidoptera, Diptera (especially Syrphidae) and Hymenoptera (especially *Bombus* and Vespidae). The plants develop up to 50 winged seeds in ovate fruit capsules (Lienert & Fischer, 2004). The species sometimes forms large populations in favourable habitats; however, as with numerous other peat bog plants, they are, but is also sensitive to habitat fragmentation and destruction, In the last century, fens in Poland and Switzerland that were inhabited by various wetland specialists (e.g. *S. perennis*) were strongly reduced in number and size due to direct destruction of wetlands, drainage and fertilisation (Lienert et al., 2002; Pawlikowski & Wołkowycki, 2010).

Study area and sampling

One plant sample per *S. perennis* population was collected from the Carpathians, Alps, Sudetes, Black Forest, Bohemian Forest and Polish lowlands close to the Lithuanian and Belarusian border (Fig. 1). In total, 10 plant samples were collected from the Carpathians, eight from the Sudetes, three from the Alps, two from the Bohemian Forest, one from the Black Forest and four from the Polish lowlands (Table 1). Fresh plant leaves were collected in the field, placed into plastic bags and immediately preserved using a drying agent (silica orange gel).

DNA isolation and sequencing

The genomic DNA was isolated using the DNeasy Plant Mini Kit (Qiagen; Hilden, Germany), according to the manufacturer’s protocol. Dried plant leaves were previously frozen using liquid nitrogen and disrupted from using Mixer Mill MM400 (Retsch; Haan, Germany). The quality and quantity of the DNA was determined using 1% TBE agarose gel.

In similar as Groff, Hale & Whitlock (2015), we sequenced three markers from the chloroplast genome of *S. perennis*: the *trn*L-*trn*F Intergenic Spacer, *trn*L Intron and the *trn*H(GUG) – *psb*A spacer. All three chloroplast DNA regions are widely used for phylogenetic studies at all taxonomic levels (Drábková et al., 2004). The *trn*LF region is often considered evolutionary conservative but employed in phylogeny and taxonomy, and some studies have found intraspecific variation in this biogeographically informative gene regions that has been contradicted (Taberlet et al., 1991; Brunsfeld & Sullivan, 2005; Shaw et al., 2005; Fujii & Senni, 2006; Shaw et al., 2007; Groff, Hale & Whitlock, 2015). Additionally, the *trn*H-*psb*A region of the cpDNA is often more variable than the *trn*LF region (e.g. Shaw et al., 2005). The *trn*L-*trn*F Intergenic Spacer together with *trn*L Intron were tested: with “c” and “f” primers (Taberlet et al., 1991) and *trn*H(GUG) – *psb*A with *trn*H(GUG) and *psb*A primers (Shaw et al., 2005). The DNA extracts were used to PCR and seqencing reactions. PCR reaction mix included (in the total volume of 20 µl): 1U Taq recombinant polymerase (Thermo-Fisher Scientific), 10X Taq Buffer, 1 mM $MgCl_2$, 0.5 µM of each primer, 0.4 mM dNTP and 1 µl DNA template. PCR cycle was performed with a Veriti Thermal Cycler (Life Technologies, Carlsbad, CA, USA) with the following parameters: 8 min at 95°C, followed by 30 cycles of 45 s at 95°C, 45 s at annealing temperature (49.2°C – *trn*L, 51.2°C – *psb*A) and 1min at 72°C, followed by a final extension step of 10 min at 72°C. Prior to sequencing, PCR products were purified using GeneMATRIX PCR/ DNA Clean Up Purification Kit (Eurx, Gdańsk, Poland). Sequencing, post-reaction purification and reading were done by Genomed (Warsaw, Poland) using an ABI 377XL Automated DNA Sequencer (Applied Biosystems, Carlsbad, CA, USA). All sequences are available in GenBank (accession numbers - *trn*H(GUG) – *psb*A spacer: KY798346 - KY798347, KY817321 - KY817346; *trn*L-*trn*F Intergenic Spacer, *trn*L Intron: KY798346 - KY798348, KY906142 - KY906166). All molecular analyses has been done at Department of Botany and Plant Ecology Wrocław University of Environmental and Life Sciences.

cpDNA data analyses

The cpDNA sequences were aligned using DNA Baser Sequence Assembler v4 (Heracle BioSoft, 2014) and checked for nucleotide variation using BioEdit ver. 7.1.11 (Hall, 1999). Combined both cpDNA region, a widely used for phylogenetic analysis at all taxonomic levels, were concatenated and analysed together. Prior to the phylogenetic analyses, the cpDNA sequences were aligned using Muscle software (Edgar, 2004a; Edgar 2004b). We performed maximum parsimony (MP) and Bayesian inference (BI), to infer the phylogenetic relationships among selected individuals from European *S. perennis* populations. Maximum parsimony were conducted using PAUP* 4.0b10 (Swofford, 2002) and involved heuristic strategy with 1000 replicates of random addition of sequences. Bootstraps for MP analyses based on 1,000 replications of full heuristic searches with the tree-bisection-reconstruction (TBR) branch-swapping algorithm, and those for NJ analyses (Saito & Nei, 1987) under the JC model (Jukes & Cantor, 1969). The BI analyses were performed using MrBayes 3.1.2. (Ronquist & Huelsenbeck, 2003). The substitution models used for each codon position in the BI analyses were GTR+G (1st codon position), GTR+I (2nd codon position), and GTR+I+G (3rd codon position), as estimated based on Aikake's information Criterion (AIC), selected by MrModeltest 2.3 (Nylander et al., 2004) using PAUP* 4.0b10

(Swofford, 2002). The parameters of the substitution models for codon position were unlinked. The Markov chain Monte Carlo iteration was performed and stopped at 1,000,000 generations. The first 25% of generations were discarded as burn-in, whereas the remaining trees were used to calculate a 50% majority-rule tree and to determine the posterior probabilities of individual branches. The remaining trees were used to produce a majority-rule consensus tree and to calculate posterior probabilities (PP).

Haplotype network of the studied cpDNA sequences were constructed by TCS v1.21:2 (Clement, Posada & Crandall, 2000). Results were also analyzed by DnaSP (Rozas et al., 2003): the haplotype diversity (Hd) and nucleotide diversity (π). Arlequin 3.5.1.2 (Excoffier & Lischer, 2010) was used for detection of genetic variation among groups, among plants representing populations within groups and molecular variation of haplotype distribution (Fst) (Weir & Cockerham, 1984).

In total, 28 sequence of *S. perennis* individuals and sequence of five other species used as an outgroup: *Frassera speciosa*, *Lomatogonium rotatum*, *Comastoma tenellum* and *Gentianella amarella* were studied.

Results

Molecular phylogenetic analyses using cpDNA sequence data

Alignment of the *trn*LF region of cpDNA revealed a variation in length among individuals from European populations. A total of 15 variable sites with insertions or deletions were identified. These sequences were 827–838 base pairs in length, of which 33 characters were parsimony informative (Table 2). Alignment of the *trn*H(GUG)–*psb*A cpDNA locus also revealed a variation in length among individuals as well as the studied sites. A total of 19 sites with insertions or deletions (Table 3) were identified. The sequences were 410–428 base pairs in length, of which 38 characters were parsimony informative. Several indels at 658–665 in the *trn*LF region and 70–87 in the *trn*H–*psb*A region were neglected because they varied inconsistently with the substitution and because indels typically mutate more frequently than substitutions (Alsos et al., 2005).

The aligned lengths of the 33 sequences of the *trn*LF and *trn*H–*psb*A regions varied from 1485 to 1503 base pairs. The whole dataset contained 36 variable sites that were dispersed randomly across the entire analysis area. The results showed that maximum parsimony (MP) analyses were congruent with Bayesian interference (BI) analyses. The concatenate sequence data were more informative than single trees based on the *trn*LF and *trn*H–*psb*A regions, and the results are presented in Fig. 2. The topologies of the trees were congruent, and only one arbitrarily-selected tree (from 12 trees) is shown with bootstrap proportions (BP) from MP and BI at the nodes.

Phylogeographic analysis of the concatenate sequence data resulted in more than 200 parsimonious trees (CI = 0.94, RI = 0.92). The dataset did not reveal any distinct regions of *S. perennis* within Europe, and no congruent groups were identified using TCS software (Fig. 3). Several individuals from distinct populations had unique sequences; however, there was no clear connection with geographical structure. Several of the most common haplotypes were found in individuals in different populations from various regions (e.g. in individuals from the Polish lowlands, Carpathians and Sudetes) (Fig. 3). In clade (BS = 67, BP = 0.98) are placed individuals represented 11 populations from almost all study regions. To verify the results, numerous additional resequencing reactions were performed on the same or additional *S. perennis* samples.

When assessing genetic polymorphisms at the species level, *S. perennis* possessed high levels of plastid DNA diversity ($H_D = 0.841$) and nucleotide diversity ($\pi = 0.00154$). Analysis of molecular variance (AMOVA) indicated a high degree of differentiation among individuals from populations (Fst = 0.649) and a high level of differentiation (64.93) among groups of populations (Table 4). Differentiation among individuals from populations within groups was lower and reached 35.07. This confirms that the determined haplotypes are dispersed randomly across the whole of Europe.

Discussion

Genetic variability in plant populations is influenced by complex historical processes (e.g. Pleistocene glaciations, migrations, bottleneck effects and gene flow) and biological processes, the results of which can be observed using genetic analyses. However, it is difficult to define the same rules for all plant species, particularly with respect to plant migration. A good example is the history of *S. perennis* in Central and Western Europe, as this species is believed to have migrated there from Central Asia as another species from the Gentianaceae family. This study was not able to confirm this hypothesis using the data acquired. In fact, the findings suggest a discontinuous expansion range from various directions (e.g. Central Asia and Northern Euro–Asian territories). The findings also illustrate cpDNA variation between individuals from different *S. perennis* populations in Europe, with numerous unique haplotypes dispersed across this geographical area. A clear phylogeographic structure of *S. perennis* was not detected in Europe, and the results illustrate distinct structural differences between geographical haplotype lineages and the complicated nature of preglacial and postglacial plant dispersal (Abbott & Brochmann, 2003). The observed haplotypes were dispersed randomly across the study area and did not form closely-related lineages among geographical regions (Figs. 2 and 3). These results show that the *S. perennis* haplotypes probably originated from different localities and not only from one direction (e.g. Central Asia). They could also have arisen before they spread to their current localities, from where they dispersed and mutated. Plants collected from Hala Cebulowa and Hala Miziowa were situated approximately 500 m apart, and their haplotypes differed with regards to *trn*H–*psb*A length and nucleotide variation in the *trn*H–*psb*A and *trn*LF regions. Similarly, the haplotypes detected in plants from Karkonosze populations (Sudetes mountains) were located about 1 km from each other (Kocioł Wielkiego Stawu–Kocioł Małego Stawu and Kocioł Łomniczki–Kopa) and they also differed in terms of their *trn*H–*psb*A and *trn*LF length or nucleotide variation. Other haplotypes were specific only for the sites or localities in which they were grown and did not form groups of similar sequences (Fig. 2). Closely-located populations of *S. perennis* appear to be genetically isolated and form isolated population systems. This is in accordance with the work of Lienert et al. (2002) who suggested that geographical and genetic distances between *S. perennis* populations were not correlated and that gene flow between close populations was not higher than between more distant populations.

The genetic diversity of cpDNA and the lack of phylogeographical structure may be attributed to the range formation history, its shifting during the ice age and the emergence of a group of boreal mountain plants (Ronikier, 2011). During subsequent glaciations and cold temperatures, alpine plants expanded their range and warm interglacial alpine plants and boreal and subarctic species (from subpolar areas in front of the glacier) retreated to higher mountain localities and refugia areas in the far north. This may have led to distinctive geographical disjunctions. Today, *S. perennis* is found in European mountains, Northern Eurasia and North America (in peat bogs and boreal forests), as well as in isolated boreal (Hultén, 1968; Meusel et al., 1978; Hultén and Fries, 1986).

Similar haplotypes identified in this study may also form part of a residual lineage of haplotypes that colonised peat bog areas in mountains. The five most similar haplotypes (17, 18, 19, 26 and 28, presented on Fig. 3) are dispersed across several disjunctive areas without any geographical correlation. The current fragmented distribution of *S. perennis* seems to be a residual effect after more homogenous distribution. It is also possible that cpDNA mutates rapidly, seed production or dispersal does not occur or is scarce and gene flow is scattered or nonexistant. At the contact zones between migrating fronts, significant haplotype mixing has occurred, as all populations are colonising new territories. It is possible that the migration of *S. perennis* occurred in a similar way or in several phases. Mountain plants could have survived the last Wistulian glaciation; however, after the end of the cold period, new plants (possibly from Siberia) may have expanded to their present European territory. This may explain the difference in cpDNA nucleotide composition in individuals from distinct populations of *S. perennis*. It is also possible that the species both survived glaciations in situ and remigrated several times, therefore broad-fronted repeated recolonization and glacial survival can shape genetic variation (Tausch et al., 2017).

Conclusions

In conclusion, the ~~nonexistant~~non-existent phylogeographical structure of *S. perennis* in the study areas of the European range may be the result of numerous overlapping factors, such as multidirectional gene flow in the dispersal history, long-distance dispersal during postglacial recolonization and survival in several detached refugia (Beatty & Provan, 2011; Cain, Milligan & Strand, 2000; Jiménez-Mejías et al., 2012; Sanz et al., 2014). The low level of variation in the structure patterns is similar to that of other plant taxa with northern distribution and may indicate the long-term process of gene flow among the populations (Eidesen et al., 2007a; Eidesen et al., 2007b; Ehrich, Alsos & Brochmann, 2008; Westergaard et al., 2010; Alsos et al., 2012). It is also possible that plant populations occurred in Pleistocene refugia or migrated during the Holocene, and this was enough time for them to form divergent cpDNA. The lack of evidence for phylogeographic structure may indicate a high level of gene flow in the recent past. The variation in cpDNA nucleotide composition in individuals from distinct populations may also reflect genetic variability from ancient periods when the landscape and fen systems were not fragmented. The gene flow today is probably much smaller than in previous times or is ~~nonexistant~~non-existent. Habitat fragmentation for ~~stenotypic specialists from~~ peat bog~~s~~ specialists may have significantly reduced genetic variability and led to absent or minimal gene flow between populations.

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References

Abbott RJ, Brochmann C (2003) History and evolution of the Arctic flora: in the footsteps of Eric Hultén. *Molec Ecol* 12:299–313

Albach D, Schönswetter P, Tribsch A (2006) Comparative phylogeography of the *Veronica alpina* complex in Europe and North America. *Molec Ecol* 15:3269–3286

Alsos IG, Engelskjøn T, Gielly L, Taberlet P, Brochmann C (2005) Impact of ice ages on circumpolar molecular diversity: Insights from an ecological key species. *Molec Ecol* 14: 2739–2753

Alsos IA, Ehrich D, Thuiller W, Eidesen PB, Tribsch A, Schönswetter P, Lagaye C, Taberlet P, Brochmann C (2012) Genetic consequences of climate change for northern plants. *Proc R Soc B* 279: 2042-2051

Alvarez N, Thiel-Egenter C, Tribsch A, Holderegger R, Manel S, Schönswetter P, Taberlet P, Brodbeck S, Gaudeul M, Gielly L, Küpfer P, Mansion G, Negrini R, Paun O, Pellecchia M, Rioux D, Schüpfer F, Van Loo M, Winkler M, Gugerli F (2009) History or ecology? Substrate type as a major driver of spatial genetic structure in Alpine plants. *Ecol Letters* 12:632–640

Bauert M, Kalin M, Baltisberger M, Edwards P (1998) No genetic variation detected within isolated relict populations of *Saxifraga cernua* in the Alps using RAPD markers. *Molec Ecol* 7:1519–1527

Beatty GE, Provan J (2011) Comparative phylogeography of two related plant species with overlapping range in Europe, and the potential effects of climate change on their intraspecific genetic diversity. *BMC Evol Biol* 11:29

Brockmann-Jerosch H, Brockmann-Jerosch M (1926) Die Geschichte der Schweizerischen Alpenflora. In Schroeter C (eds) *Das Pflanzenleben der Alpen. Eine Schilderung der Hochgebirgsflora* (The plant life of the Alps. A description of the high mountain flora). Raustein, Zürich, pp:1110–1207

Brunsfeld SJ, Sullivan J (2005) A multi-compartmented glacial refugium in the northern Rocky Mountains: Evidence from the phylogeography of *Cardamine constancei* (Brassicaceae). *Conserv Genet* 6:895–904

Cain M, Milligan BG, Strand AE (2000) Long-distance seed dispersal in plant populations. *Am J Bot* 87:1217–1227

Clement M, Posada D, Crandall K (2000) TCS: a computer program to estimate gene genealogies. *Molec Ecol* 9:1657–1660

Drábková L, Kirschner J, Vlcek C, Paces V (2004) *Trn*L–*trn*F Intergenic Spacer and *trn*L Intron Define Major Clades Within *Luzula* and *Juncus* (Juncaceae): Importance of Structural Mutations. *J Molec Evol* 59:1–10

Edgar RC (2004a) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucl Acids Res* 32:1792–97

Edgar RC (2004b) MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* 5:113

Ehrich D, Alsos IG, Brochmann C (2008) Where did the northern peatland species survive the dry glacials? Cloudberry (*Rubus chamaemorus*) as an example. *J Biogeogr* 35:801–814

Eidesen PB, Carlsen T, Molau U, Brochmann C (2007a) Repeatedly out of Beringia: *Cassiope tetragona* embraces the Arctic. *J Biogeogr* 34:1559–1574

Eidesen PB, Alsos IG, Popp M, Stensrus Ø, Brochmann C (2007b) Nuclear versus plastid data: complex Pleistocene history of a circumpolar key species. *Molec Ecol* 16: 3902–3925

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Res* 10: 564–567.

Freeland J (2008) *Ekologia Molekularna (Molecular ecology)*. PWN, Warszawa

Fujii N, Senni K (2006) Phylogeography of Japanese alpine plants: Biogeographic importance of alpine region of Central Honshu in Japan. *Taxon* 55:43–52

Groff PA, Hale AM, Whitlock BA (2015) Chloroplast Lineages in Disjunct Western North American Populations of *Swertia perennis* (Gentianaceae). *Syst Bot* 40:220–228

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl Acids Symp Ser* 41:95–98

Hansen LD, Thomas NR, Arnholdt-Schmitt B (2009) Temperature responses of substrate carbon conversion efficiencies and growth rates of plant tissues. *Physiol Plant* 137:446–458

Heracle BioSoft (2014) DNA Sequence Assembler v4 www.DnaBaser.com

Hewitt GM (1996) Some genetic consequences of ice ages and their role in divergence and speciation. *Biol J Linn Soc* 58:247–276

Hewitt GM (2004) Genetic consequences of climatic oscillations in the Quaternary. *Phil Trans R Soc Lond B* 359:183–195

Hultén E (1968) Flora of Alaska and Neighboring Territories. Stanford Univ Press, Stanford

Hultén E, Fries M (1986) Atlas of North European Vascular Plants North of the Tropic of Cancer 1–3. Koeltz Scientific Books, Königstein

Jermakowicz E, Wróblewska A, Brzosko E, Mirski P, Hirse T (2015) Phylogeographical structure of the boreal-montane orchid *Malaxis monophyllos* as a result of multi-directional gene flow. *Bot J Linn Soc* 178:138–154

Jiménez-Mejías P, Luceño M, Lye KA, Brochmann C, Gussarova G (2012) Genetically diverse but with surprisingly little geographical structure: the complex history of the widespread herb *Carex nigra* (Cyperaceae). *J Biogeog* 39:2279–2291

Jukes TH, Cantor CR (1969) Mammalian Protein Metabolism. In Munro MN (eds) *Evolution of protein molecules*. Academic Press, New York, pp 21–132

Kramp K, Huck S, Niketić M, Tomović G, Schmitt T (2009) Multiple glacial refugia and complex postglacial range shifts of the obligatory woodland plant *Polygonatum verticillatum* (Convallariaceae). *Plant Biol* 11:392–404

Lienert JM, Fischer J, Schneller M, Diemer (2002) Isozyme variability of the wetland specialist *Swertia perennis* (Gentianaceae) in relation to habitat size isolation and plant fitness. *Amer J Bot* 89:801–811

Lienert J, Fischer M, Diemer M (2002) Local extinctions of the wetland specialist *Swertia perennis* L. (Gentianaceae) in Switzerland: a revisitation study based on herbarium records. *Biol Conserv* 103: 65–76

Lienert J, Fischer M (2004) Experimental inbreeding reduces seed production and germination independent of fragmentation of populations of *Swertia perennis. Basic Appl Ecol* 5: 43–52

Löve A, Löve D (1986) Chromosome Number Reports 93. *Taxon* 35:897–899

Meusel H, Jäger E, Rauschert S, Weinert E (eds) (1978) Vergleichende Chorologie der Zentraleuropäischen Flora Karten Band II. G Fischer, Jena

Mirski P, Brzosko E, Jędrzejczyk I, Kotowicz J, Ostrowiecka B, Wróblewska A (2017) Genetic structure of dioecious and trioecious *Salix myrsinifolia* populations at the border of geographic range. *Tree Gen & Genom* 13:15–23

Mojski JE (1993) Europa w plejstocenie (Europe in Pleistocene). PAE, Warszawa

Nylander JAA, Ronquist F, Huelsenbeck JP, Nieves–Aldrey JL (2004) Bayesian phylogenetic analysis of combined data. *Syst Biol* 53:47–67

Oliver C, Hollingsworth PM, Gornall RJ (2006) Chloroplast DNA phylogeography of the arctic-montane species *Saxifraga hirculus* (Saxifragaceae). *Heredity* 96:222–231

Oostermeijer JGB (1996) Population size, genetic variation and related parameters in small isolated plant populations: a case study. In (eds) Serre J, Margules CR, Poschlod P, Henle K Species Survival in Fragmented Landscapes. Kluver Academic Publ, pp: 61-68.

Paun O, Schönswetter P, Winkler M, Tribsch A (2008) Historical divergence vs contemporary gene flow: Evolutionary history of the calcicole *Ranunculus alpestris* group (Ranunculaceae) in the European Alps and the Carpathians. *Molec Ecol* 17:4263–4275

Pawlikowski P, Wołkowycki D (2010) Nowe stanowiska niebielistki trwałej *Swertia perennis* subsp *perennis* (Gentianaceae) na torfowiskach północno-wschodniej Polski (New localities *Swertia perennis* subsp *perennis* (Gentianaceae) on peat bogs in north-eastern Poland). *Fragm Flor Geobot Polon* 17:25–36

Pawłowski B (1928) Die geographischen Elemente und die Herkunft der Flora der subnivalen Vegetationsstufe im Tatra-Gebirge (The geographical elements and the origin of the flora of the subnival vegetation stage in the Tatra Mountains). *Bull Int Acad Polon Sci CI Sci Math Ser B Sci Nat* 1928:161–202

Ronikier M, Cieślak E, Korbecka G (2008) High genetic differentiation in the alpine plant *Campanula alpina* Jacq (*Campanulaceae*): evidence for glacial survival in several Carpathian regions and longterm isolation between the Carpathians and the Alps. *Molec Ecol* 17:1763–1775

Ronikier M, Costa A, Aguilar JF, Feliner GN, Küpfer P, Mirek Z (2008) Phylogeography of *Pulsatilla vernalis* (L) Mill (Ranunculaceae): chloroplast DNA reveals two evolutionary lineages across Central Europe and Scandinavia. *J Biogeog* 35(9):1650–1664

Ronikier M (2011) Biogeography of high-mountain plants in the Carpathians: An emerging phylogeographical perspective. *Taxon* 60(2):373–389

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–1574

Rozas J, Sánchez, Del Barrio JC, Messeguer X, Rozas R (2003) DnaSP, DNA polymorphism analyses by coalescent and other methods. *Bioinformatics* 19: 2496–2497

Saito N, Nei M (1987) The neighbor–joining method a new method for reconstruction phylogenetic trees. *Molec Biol Evol* 4:406–425

Sanz M, Schönswetter P, Vallès J, Schneeweiss GM, Vilatersana R (2014) Southern isolation and northern long-distance dispersal shaped the phylogeography of the widespread, but highly disjunct, European high mountain plant *Artemisia eriantha* (Asteraceae). *Bot J Linn Soc* 174:214–226

Schönswetter P, Stehlik I, Holderegger R, Tribsch A (2005) Molecular evidence for glacial refugia of mountain plants in the European Alps. *Molec Ecol* 14:3547–3555

Schönswetter P, Popp M, Brochmann C (2006) Rare arctic-alpine plants of the European Alps have different immigration histories: the snow bed species *Minuartia biflora* and *Ranunculus pygmaeus*. *Molec Ecol* 15:709–720

Shaw J, Lickey EB, Beck JT, Farmer SB, Liu W, Miller J, Siripun KC, Winder CT, Schilling EE, Small RL (2005) The tortoise and the hare II: Relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *Amer J Bot* 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. *Amer J Bot* 94:275–288

Slovák M, Kučera J, Turis P, Zozomová-Lihová J (2012) Multiple glacial refugia and postglacial colonization routes inferred for a woodland geophyte *Cyclamen purpurascens*: patterns concordant with the Pleistocene history of broadleaved and coniferous tree species. *Biol J Linn Soc* 105:741–760

Stehlik I (2000) Nunataks and peripheral refugia for alpine plants during quaternary glaciation in the middle part of the Alps. *Bot Helvet* 110:25–30

Stehlik I (2003) Resistance or emigration? Response of alpine plants to the ice ages. *Taxon* 52:499–510

Swofford DL (2002) PAUP* – Phylogenetic Analysis Using Parsimony (*and other methods). Version 40b10. Sinauer Associates Sunderland, USA

Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molec Biol* 17:1105–1109

Taberlet P, Fumagalli L, Wust-Saucy AG, Cosson JF (1998) Comparative phylogeography and postglacial colonization routes in Europe. *Molec Ecol* 7:453–464

Tausch S, Leipold M, Poschlod P, Reisch C (2017) Molecular markers provide evidence for a broad-fronted recolonisation of the widespread calcareous grassland species *Sanguisorba minor* from southern and cryptic northern refugia. *Plant Biol* 19(4):562–570.

Urbaniak J, Kwiatkowski P, Ronikier M (2018) Postglacial history and current population genetic diversity of a central-European forest plant *Hacquetia epipactis*. *Preslia* 90:39–57.

Vargas P (2001) Phylogenetic and evolutionary insights into the *Saxifraga pentadactylis* complex (Saxifragaceae): variation in nrITS sequences. *Nord J Bot* 21:75–82

Wasowicz P, Pauwels M, Pasierbiński A, Przedpelska-Wasowicz E, Babst-Kostecka AA, Saumitou-Laprade P, Rostanski A (2016) Phylogeography of *Arabidopsis halleri* (Brassicaceae) in mountain regions of Central Europe inferred from cpDNA variation and ecological niche modelling. *PeerJ* 4:e1645

Weir BS, Cockerham CC (1984) Estimating F – statistics for the analysis of population structure. *Evolution* 38: 1358–1370.

Westergaard K, Jøgensen MH, Gabrielsen TM, Alsos IG, Brochmann C (2010) The extreme Beringian/Atlantic disjunction in *Saxifraga rivularis* (Saxifragaceae) has formed at least twice. *J Biogeogr* 37:1262–1276

Winkler M, Tribsch A, Schneeweiss GM, Brodbeck S, Gugerli F, Holderegger R, Abbott RJ, Schönswetter P (2012) Tales of the unexpected: phylogeography of the arctic–alpine model plant *Saxifraga oppositifolia* (Saxifragaceae) revisited *Molec Ecol* 21:4618–4630

Winkler M, Tribsch A, Schneeweiss GM, Brodbeck S, Gugerli F, Holderegger R, Schönswetter P (2013) Strong nuclear differentiation contrasts with widespread sharing of plastid DNA haplotypes across taxa in European purple saxifrages (*Saxifraga* section *Porphyrion* subsection *Oppositifoliae*). *Bot J Linn Soc* 173:622–636

Wróblewska A (2013) The phylogeographical and population genetic approach to the investigation of the genetic diversity patterns in self-incompatible clonal and polyploid *Linnaea borealis* subsp. *borealis*. *Bot J Linn Soc* 173:64–76

Young A, Boyle T, Brown T (1996) The population genetic consequences of habitat fragmentation for plants. *Trends Ecol. Evol.* 11(10): 413–418.