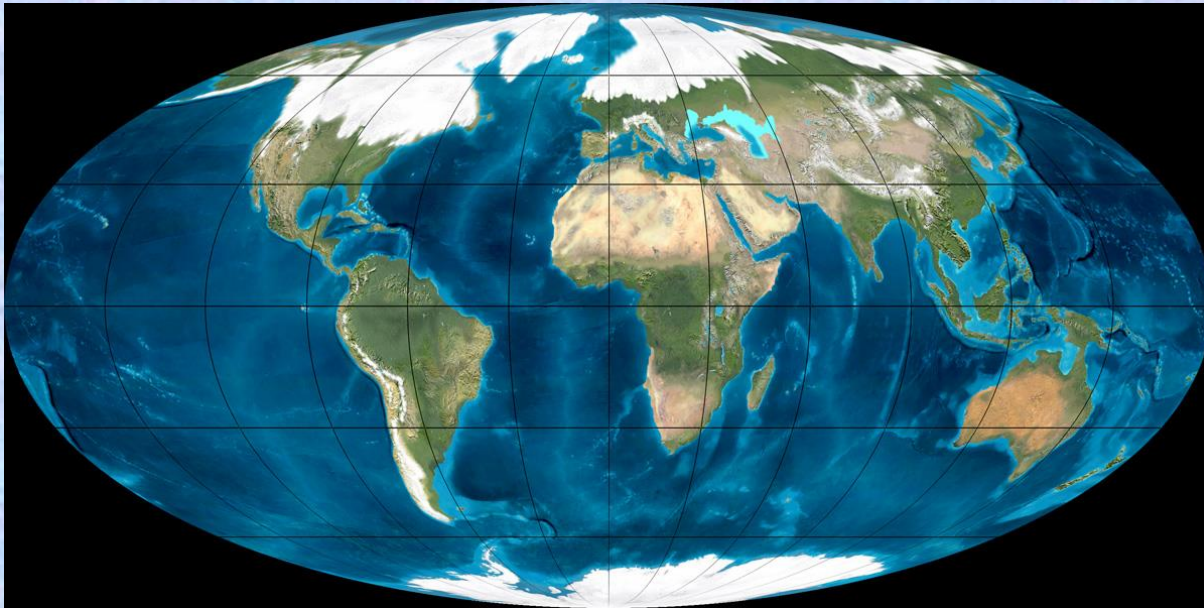


# Glacial refugia and diversification of forest species in central Europe. The case study on *Cyclamen purpurascens* Mill.

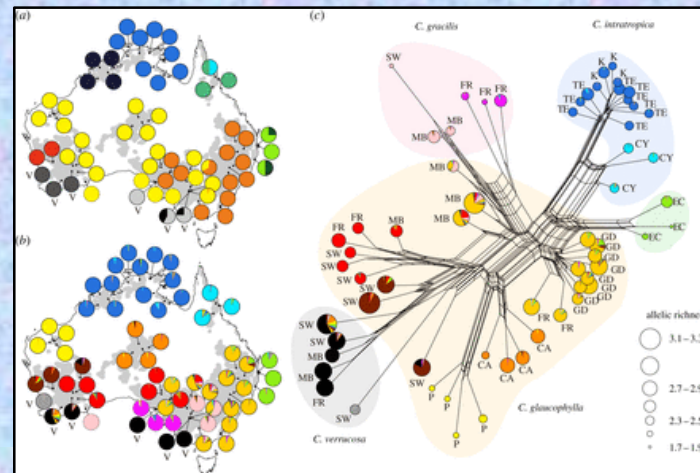


**Marek Slovák**

Department of Botany, Charles University, Praha, Czech Republic & Plant Science and Biodiversity Centre, Institute of Botany, Slovak Academy of Sciences, Bratislava, Slovakia

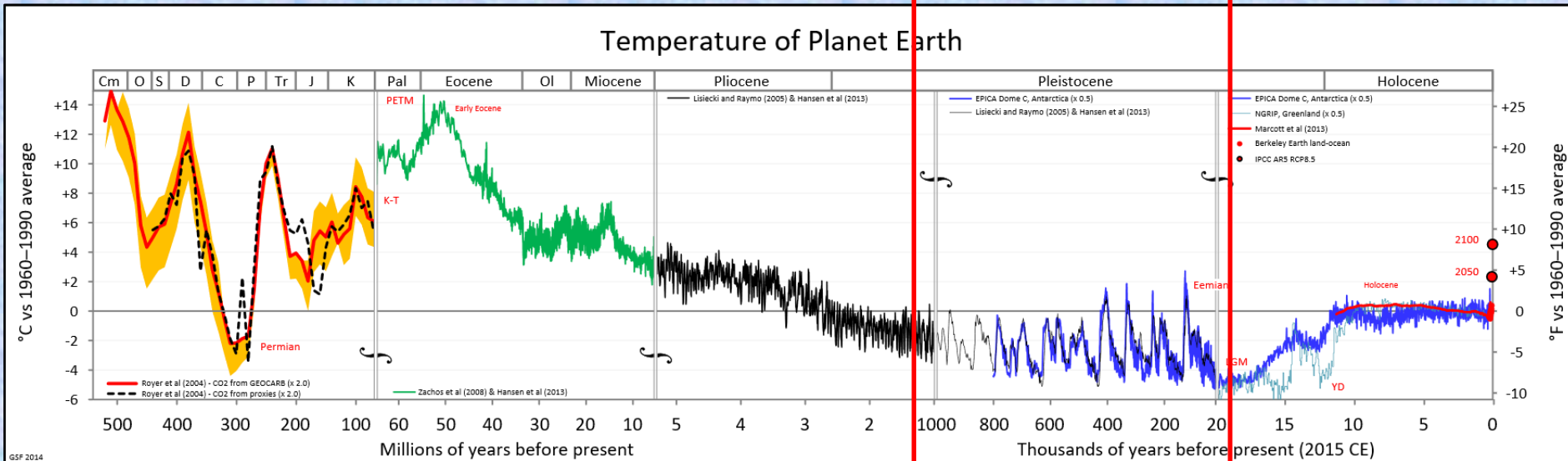
# Phylogeography (AVISE J.C., 2000)

“**Phylogeography** is a field of study concerned with the principles and processes governing the **geographic distributions** of **genealogical lineages**, especially those within and among closely related species. As the word implies, phylogeography deals with **historical, phylogenetic components** of the **spatial distributions** of gene lineages. ... In particular, phylogeography provides an empirical and conceptual **bridge** between the traditionally separate disciplines of **population genetics** and **phylogenetic biology**.”



# Paleoclimatology

## Pleistocene climatic oscillations



<https://en.wikipedia.org/wiki/Paleoclimatology>

- climate on the Earth has been cooling for ca. 60 million years (Ma)
- the Antarctic ice sheet forming ca. 35 Ma
- and the Arctic icecap growing from ca. 3 Ma
- The Quaternary Period series of Ice Ages (glacials) and interstadials (interglacials)
- These oscillations are paced by regular eccentricities in the Earth's orbit around the sun every **100 000**, **41 000** and **21 000** years

# Definitions, aims and questions

- the influence of the **Quaternary climatic oscillation** on distributing and evolution of closely related taxa
- Single species phylogeographic studies or multispecies (comparative) phylogeographic studies
- identification of the **glacial refugia and migration routes**



- Identification of the gene flow, **contact and break zones**, among populations and species
- identification of the **diversity hotspots** important for nature conservation

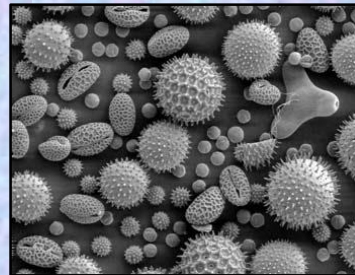
# How to trace phylogeographic history of plants?

## Paleo-botanical evidence - fossil records



- macrofossils
  - charcoal fossils
  - conserved vegetative or generative organs

- fossil pollen assemblages



## Genetic evidence - DNA

- mutations in DNA
- genetic pattern, genetic lineages at intraspecific level
- among and within population diversity



# Molecular markers

## Ideal molecular marker for phylogeography

- **sufficient variability but enabling utility across various taxonomic levels (homology)**
- **easy for laboratory preparation and data processing**
- **lack of the recombination**
- **harbor enough informative characters**
- **high mutation rate to provide sufficient variation also at infraspecific level**

# Molecular markers

- **uniparental inheritance - organellar DNA**
- chloroplast (cpDNA), mostly maternal inheritance in angiosperms
- mitochondrial DNA (mtDNA), mostly maternal inheritance in angiosperms
  - No recombination but mostly lower mutation rate
  - **classical markers** - RFLP, PCR-RFLP, cpDNA microsatellites, sequences of coding and non coding regions
  - **NGS based markers** - whole plastome sequences
- **bi-parental inheritance - nuclear DNA (nrDNA)**
  - Presence of recombination but higher mutation rate, hybridisation!
  - **classical markers** - AFLP, Isozymes, nuclear microsatellites, sequences of coding and non coding regions (ITS, single copy genes)
  - **NGS based markers** - hundreds or thousands of protein coding genes (e.g. Hybseq?), whole genome resequencing (*Arabidopsis* only)
  - Combination of nuclear and plastid markers

# Study design

➤ **Ideal case is to collect samples across whole distribution area**

➤ **literature survey and herbarium specimens revision**

➤ **number of populations analysed**

- **depends on distribution range and frequency of studied species in sampled area**
- **depends on mating systems**
- **ideally as many as possible**

**.....but, in reality, sampling depends on your funds ;-)**

➤ **to cover **entire ecological amplitude** of studied species**

- **altitudinal range, habitat types, bedrock types, **populations at the margin of species distribution or with disjunctive location****

➤ **number of analysed individuals per population**

- **ideally as many as possible, but the best is 10 and more**
- **standardly 5 to 10**
- **if less than 5 no possible inference on the population genetic structure, diversity and divergence**



# Last Glacial Maximum in Europe



**Maximum extent of glaciation during the Last Glacial Maximum (20 000 - 18 000 BP), recolonization ca since 13 000 BP**

# Postglacial re-colonization of Europe

Three types of postglacial re-colonization (HEWITT, Biol. J. Linn. Soc. 68: 87-112, 1999):

- Re-colonisation of most of Europe from Balkans and/or from S. Carpathians
- Re-colonization from Iberian, Apennine and Balkan refugia
- Re-colonization from Iberian and Balkan refugia (or from more eastern areas)



# Phylogeographic history of high mountain plants



## Nunatak

- survival of species at rocky elements of a ridge, mountain, or peak not covered with ice or snow within an ice field or glacier

## Tabula rasa



- complete extinction and postglacial re-colonisation



# Phylogeographic history of high mountain species in Europe

Molecular Ecology (2005) 14, 3547–3555

doi: 10.1111/j.1365-294X.2005.02683.x

## Molecular evidence for glacial refugia of mountain plants in the European Alps

P. SCHÖNSWETTER,\* I. STEHLIK,† R. HOLDEREGGER† and A. TRIBSCH\*

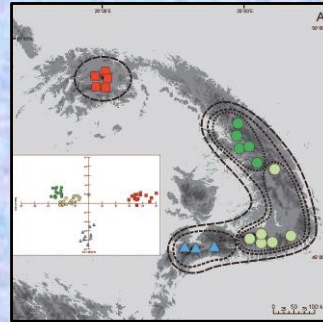
\*Institute of Botany, University of Vienna, A-1030 Vienna, Austria and National Centre for Biogenetics, Natural History Museum, University of Oslo, N-0318 Oslo, Norway; †Institute of Systematic Botany, University of Zurich, CH-8008 Zurich, Switzerland and Department of Botany, University of Toronto, Toronto, Canada M5S 3B2; †Division of Environmental and Evolutionary Biology, University of St Andrews, St Andrews KY16 9TH, UK and WSL Swiss Federal Research Institute, CH-8903 Birmensdorf, Switzerland

### Abstract

Many mountain ranges have been strongly glaciated during the Quaternary ice ages, and the locations of glacial refugia of mountain plants have been debated for a long time. A series of detailed molecular studies, investigating intraspecific genetic variation of mountain plants in the European Alps, now allows for a first synopsis. A comparison of the phylogeographic patterns with geological and palaeoenvironmental data demonstrates that glacial refugia were located along the southwestern, southern, eastern and northern borders of the Alps. Additional glacial refugia were present in central Alpine areas, where high elevation plants survived the last glaciation on ice-free mountain tops. The observed intraspecific phylogeographies suggest general patterns of glacial survival, which conform to well-known centres of Alpine species diversity and endemism. This implies that evolutionary or biogeographic processes induced by climatic fluctuations act on gene and species diversity in a similar way.

**Keywords:** Alpine plant species, comparative phylogeography, glacial refugia, historical biogeography, Quaternary glaciation

Received 3 April 2005; revision accepted 22 June 2005



Ecology Letters, (2009) 12: 632–640

doi: 10.1111/j.1461-0248.2009.01312.x

### LETTER

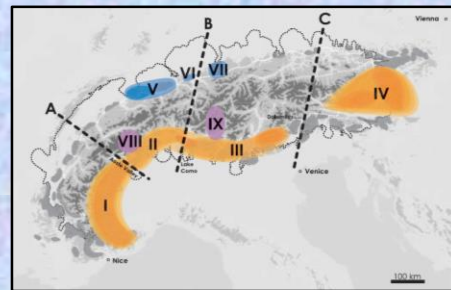
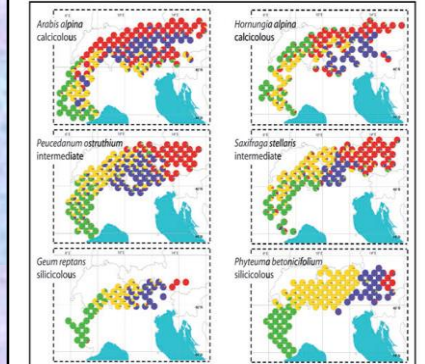
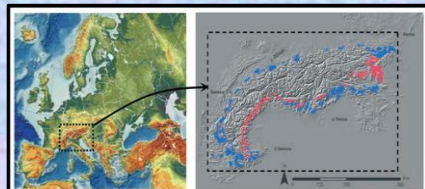
## History or ecology? Substrate type as a major driver of spatial genetic structure in Alpine plants

### Abstract

Climatic history and ecology are considered the most important factors moulding the spatial pattern of genetic diversity. With the advent of molecular markers, species' historical fates have been widely explored. However, it has remained speculative what role ecological factors have played in shaping spatial genetic structures within species. With an unprecedented, dense large-scale sampling and genome-screening, we tested how ecological factors have influenced the spatial genetic structures in Alpine plants. Here, we show that species growing on similar substrate types, largely determined by the nature of bedrock, displayed highly congruent spatial genetic structures. As the heterogeneous and disjunctive distribution of bedrock types in the Alps, decisive for refugial survival during the ice ages, is temporally stable, concerted post-glacial migration routes emerged. Our multispecies study demonstrates the relevance of particular ecological factors in shaping genetic patterns, which should be considered when modelling species projective distributions under climate change scenarios.

### Keywords

Alps, amplified fragment length polymorphisms, comparative phylogeography, ecological requirements, genetic structure, geology, glacial refugia, recolonization pathways, soil reaction.



TAXON 60 (2) • April 2011: 373–389

Ronikier • Carpathian phylogeography: An emerging perspective

## Biogeography of high-mountain plants in the Carpathians: An emerging phylogeographical perspective

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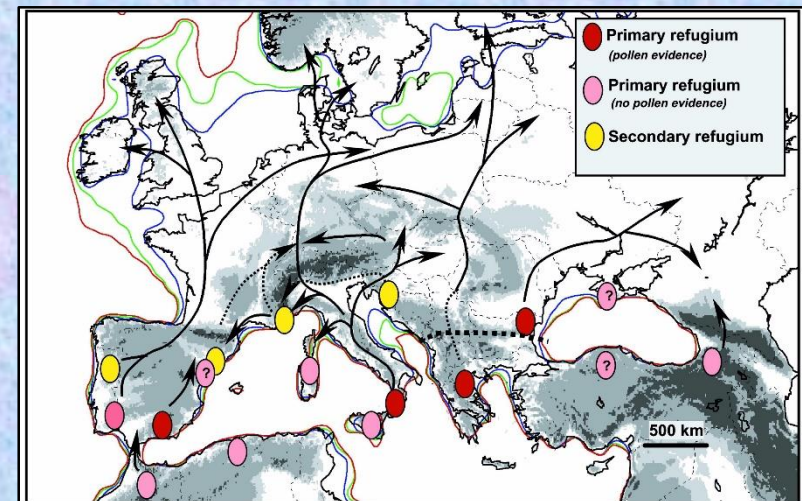
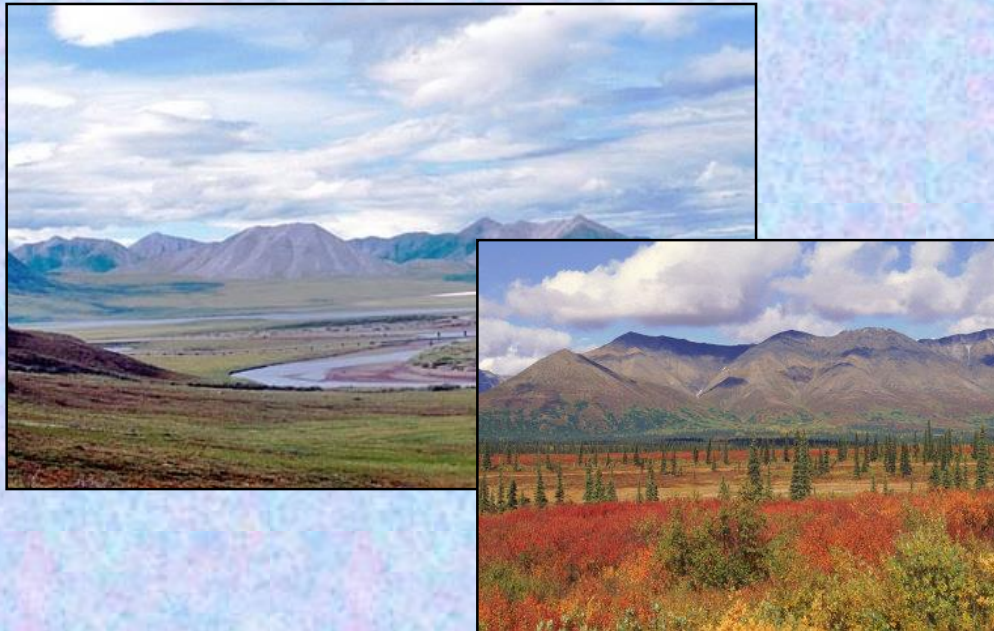
**Abstract** The Carpathians belong to the main elements of the European Alpine System and have an important biogeographical location between the Balkan ranges in the South, the Alps in the West and the Scandinavian range in the North. However, until recently this area was rarely included in detailed phylogeographical studies that could bring insights into its biogeographical history. Links with other mountain ranges and contemporary genetic structure of populations. Here, available phylogeographical studies on high-mountain plants that include data concerning the Carpathians are reviewed in order to (1) discuss regional phylogeographical structure and divergence of the Carpathian populations from those in other European mountain ranges, and (2) outline further perspectives of the Carpathian phylogeography. Analysis of available studies revealed the complexity of the biogeographical history of high-mountain plants. The studies show a deep phylogeographical structure in the Carpathians, mostly conforming with classical biogeographical boundaries, and suggesting a long-term isolation and restricted gene flow between the main Carpathian regions. For some species, though, recent dispersal events among isolated mountain ranges were detected. Such contrasting patterns were found at a larger geographical scale as well (e.g. between the Carpathians and the Alps). Several examples suggest the importance of the Carpathians in migration of arctic-alpine plants from the East towards the North. In most reviewed studies, however, the Carpathians are only marginally represented and detailed intraspecific studies based on dense population coverage in all disjunct areas of species' ranges are clearly needed to obtain reliable information and confirm the preliminary phylogeographical patterns emerging from the overview presented here.

**Keywords:** Alps; Balkans; Carpathians; conservation; European mountains; genetic structure; high-mountain plants; phylogeography; Sudetes

# Phylogeographic history of temperate (boreal) forest species

## Classical paradigm

- survival in south European peninsula, postglacial re-colonisation of northern region
- more northern located regions only tundra and step species



# Evidence of extra-Mediterranean refugia for temperate species. Review on fossil data



Quaternary Science Reviews 23 (2004) 2369–2387



## Trees or no trees? The environments of central and eastern Europe during the Last Glaciation

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Received 27 January 2004; accepted 5 June 2004

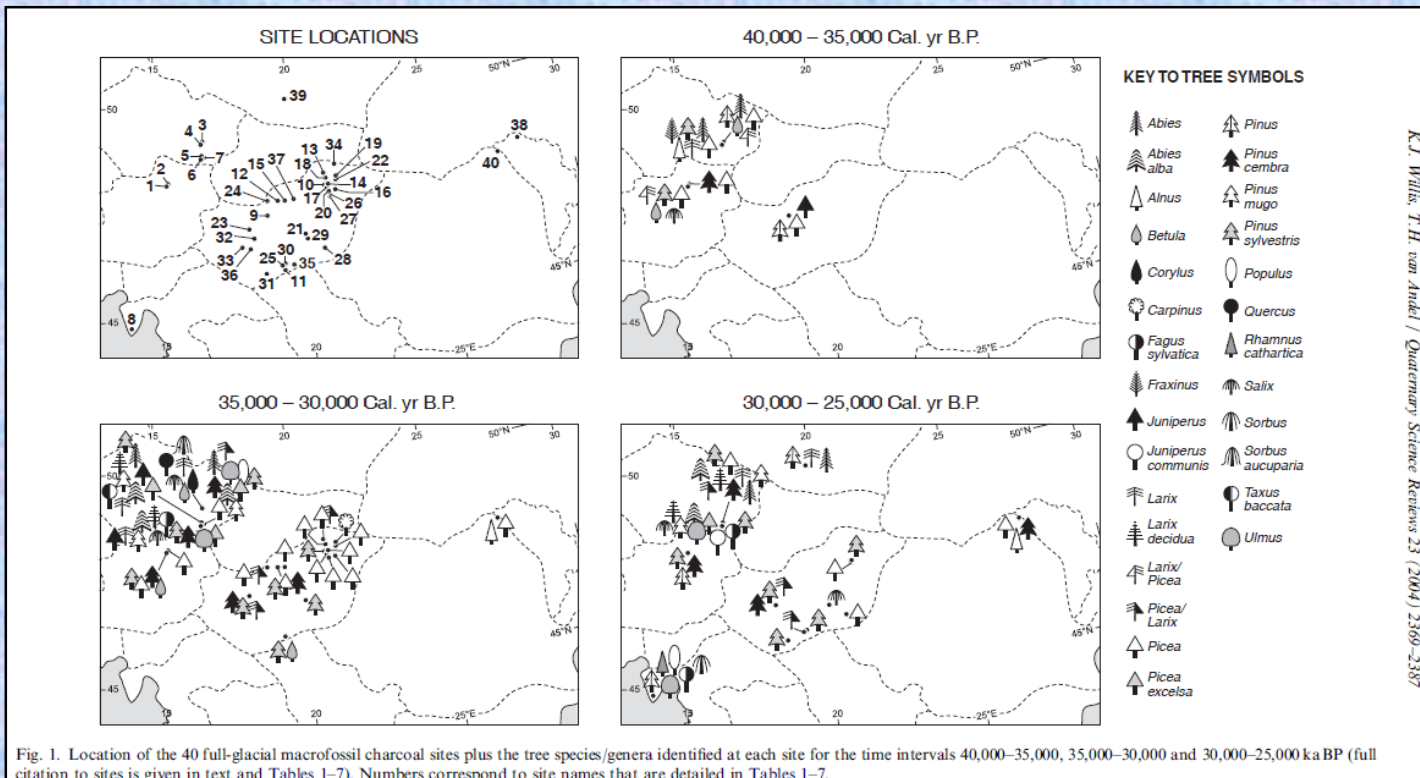


Fig. 1. Location of the 40 full-glacial macrofossil charcoal sites plus the tree species/genera identified at each site for the time intervals 40,000–35,000, 35,000–30,000 and 30,000–25,000 ka BP (full citation to sites is given in text and Tables 1–7). Numbers correspond to site names that are detailed in Tables 1–7.

# Evidence of extra-Mediterranean refugia for temperate species. *Fagus sylvatica* - fossil and genetic data



Research

## A new scenario for the Quaternary history of European beech populations: palaeobotanical evidence and genetic consequences

Donatella Magri<sup>1</sup>, Giovanni G. Vendramin<sup>2</sup>, Bernard Comps<sup>3</sup>, Isabelle Dupanloup<sup>4</sup>, Thomas Geburek<sup>5</sup>, Dušan Gömöry<sup>6</sup>, Małgorzata Latalowa<sup>7</sup>, Thomas Litt<sup>8</sup>, Ladislav Paule<sup>6</sup>, Joan Maria Roure<sup>9</sup>, Ioan Tantau<sup>10</sup>, W. O. van der Knaap<sup>11</sup>, Rémy J. Petit<sup>12</sup> and Jacques-Louis de Beaulieu<sup>13</sup>

<sup>1</sup>Dipartimento di Biologia Vegetale, Università 'La Sapienza', P. le Aldo Moro 5, 00185 Rome, Italy; <sup>2</sup>CNR, Plant Genetics Institute, Florence Division, National Research Council, Polo Scientifico Sesto Fiorentino, Via Madonna del Piano, Edificio D, I- 50019 Sesto Fiorentino, Florence, Italy; <sup>3</sup>Laboratoire d'Ecologie Génétique, Université Bordeaux I, F-33405 Talence, France; <sup>4</sup>Computational and Molecular Population Genetics Laboratory, University of Bern, CH-3012 Bern, Switzerland; <sup>5</sup>Department of Genetics, Federal Research and Training Centre for Forests, Natural Hazards, and Landscape, Hauptstrasse 7, A-1140 Vienna, Austria; <sup>6</sup>Technical University, Faculty of Forestry, SK-960 53 Zvolen, Slovakia; <sup>7</sup>Department of Plant Ecology, University of Gdansk, PL-80-441 Gdańsk, Poland; <sup>8</sup>Institute of Paleontology, University of Bonn, Nussallee 8, D-53115 Bonn, Germany; <sup>9</sup>Department de Biologia Vegetal i Ecologia, Universitat Autònoma de Barcelona, E-08193 Bellaterra (Barcelona), Spain; <sup>10</sup>Babes-Bolyai University, Dep. M. Kogalniceanu nr. 1, RO-400084 Cluj-Napoca, Romania; <sup>11</sup>Institute of Plant Sciences, University of Bern, Altenbergrain 21, CH-3012 Bern, Switzerland; <sup>12</sup>INRA, UMR Biodiversité, Gènes & Ecosystèmes, 69 Route d'Arcachon, F-33612 Cestas Cedex, France; <sup>13</sup>UMR CNRS 6116, EuroMediterranean Centre for Biodiversity and Evolutionary Research, F-13545 Aix-en-Provence, France



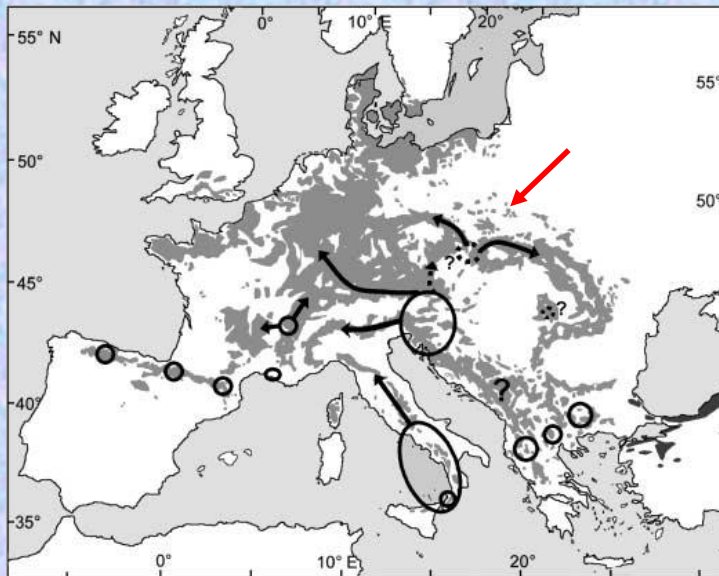
### Summary

- Here, palaeobotanical and genetic data for common beech (*Fagus sylvatica*) in Europe are used to evaluate the genetic consequences of long-term survival in refuge areas and postglacial spread.
- Four large datasets are presented, including over 400 fossil-pollen sites, 80 plant-macrofossil sites, and 450 and 600 modern beech populations for chloroplast and nuclear markers, respectively.
- The largely complementary palaeobotanical and genetic data indicate that: (i) beech survived the last glacial period in multiple refuge areas; (ii) the central European refugia were separated from the Mediterranean refugia; (iii) the Mediterranean refuges did not contribute to the colonization of central and northern Europe; (iv) some populations expanded considerably during the postglacial period, while others experienced only a limited expansion; (v) the mountain chains were not geographical barriers for beech but rather facilitated its diffusion; and (vi) the modern genetic diversity was shaped over multiple glacial-interglacial cycles.
- This scenario differs from many recent treatments of tree phylogeography in Europe that largely focus on the last ice age and the postglacial period to interpret genetic structure and argue that the southern peninsulas (Iberian, Italian and Balkan) were the main source areas for trees in central and northern Europe.

Key words: beech (*Fagus sylvatica*), chloroplast markers, fossil pollen, glacial refugia, nuclear markers, plant macrofossil, Quaternary.

*New Phytologist* (2006) doi: 10.1111/j.1469-8137.2006.01740.x

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# Evidence of extra-Mediterranean refugia for temperate species. Fossil evidence

*Preslia* 80: 307–324, 2008

## Forest vegetation of the last full-glacial period in the Western Carpathians (Slovakia and Czech Republic)

Lesní vegetace v období posledního vrcholného glaciálu v Západních Karpatech

Vlasta Jankovská<sup>1</sup> & Petr Pokorný<sup>2\*</sup>



Data presented in this article, together with that in the literature on the Weichselian full-glacial vegetation of central-eastern Europe, point to the importance of the Western Carpathians as a large-scale forest refugium, where many elements of forest biota survived the Last Glacial Maximum. Due to orographic precipitation and high mesoclimatic humidity in the mountain valleys of this mountain range, this region could have been covered during most of the Weichselian full-glacial by montane forests. *Larix*, *Pinus cembra*, *Pinus sylvestris* and *Picea* dominated a rather dense forest canopy during those times. Current analogy of this vegetation occurs in certain areas of Siberia – those with a continental and still relatively humid climate. Isolated patches of some broadleaf trees like *Corylus avellana*, *Alnus glutinosa*/*A. incana*, *Tilia*, *Ulmus*, *Quercus*, *Fagus*, *Carpinus*, *Abies* and *Acer* may have existed in more favourable places (e.g., the limestone region of Southern Slovakian Karst), but pollen analyses above cannot prove this firmly. Around the



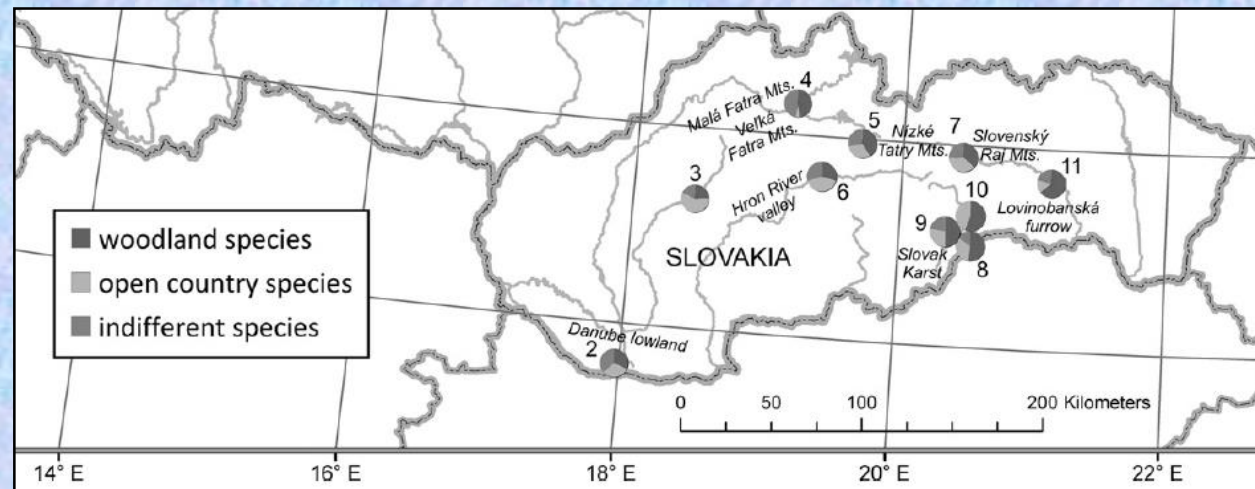
# Evidence of extra-Mediterranean refugia for temperate species. Molluscs fossil evidence

Direct evidence of central European forest refugia during the last glacial period based on mollusc fossils

Lucie Juříčková \*, Jitka Horáčková, Vojen Ložek

Quaternary Research 82 (2014) 222–228

Although there is evidence from molecular studies for the existence of central European last glacial refugia for temperate species, there is still a great lack of direct fossil records to confirm this theory. Here we bring such evidence in the form of fossil shells from twenty strictly forest land snail species, which were recorded in radiocarbon-dated late glacial or older mollusc assemblages of nine non-interrupted mollusc successions situated in the Western Carpathians, and one in the Bohemian Massif. We proposed that molluscs survived the last glacial period in central Europe in isolated small patches of broadleaf forest, which we unequivocally demonstrate for two sites of last glacial maximum age.



# Evidence of extra-Mediterranean refugia for temperate species. Various plant and animal species

Review | [Open Access](#)

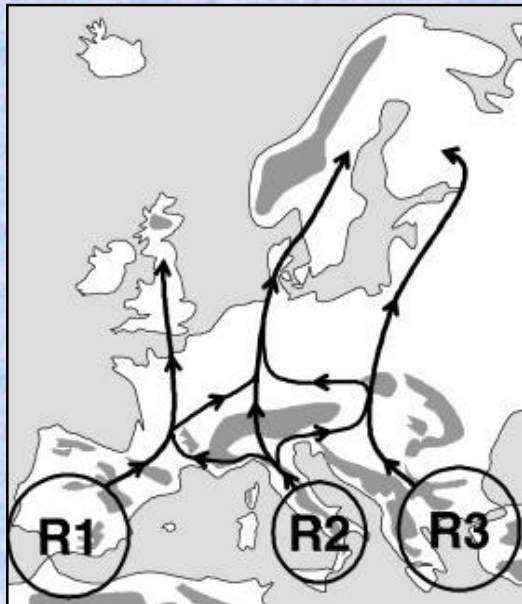
## Extra-Mediterranean refugia: The rule and not the exception?

Thomas Schmitt ✉ and Zoltán Varga

*Frontiers in Zoology* 2012 9:22

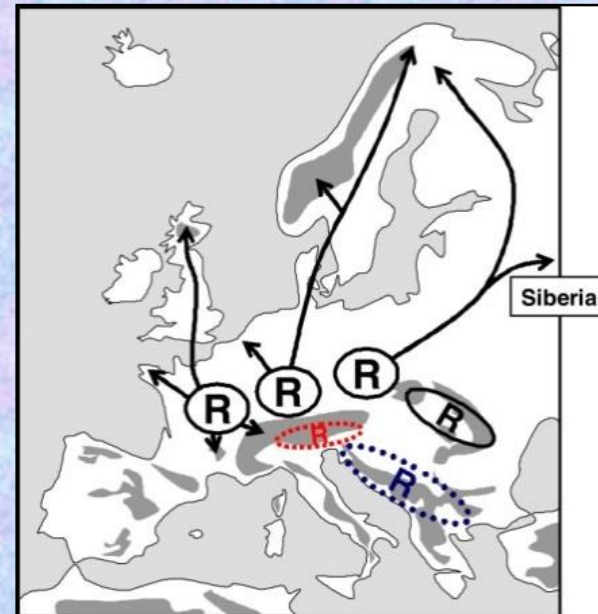
<https://doi.org/10.1186/1742-9994-9-22> | © Schmitt and Varga; licensee BioMed Central Ltd. 2012

Received: 29 June 2012 | Accepted: 28 August 2012 | Published: 6 September 2012



Schmitt, Varga 2012 *Front. Zool.* “classical” refugia

VS.



Schmitt, Varga 2012 *Front. Zool.* “cryptic” northern refugia

# Evidence of extra-Mediterranean refugia for temperate species. Various plant and animal species

Opinion

Cell  
PRESS

## Cryptic or mystic? Glacial tree refugia in northern Europe

P.C. Tzedakis<sup>1</sup>, B.C. Emerson<sup>2,3</sup>, and G.M. Hewitt<sup>3\*</sup>

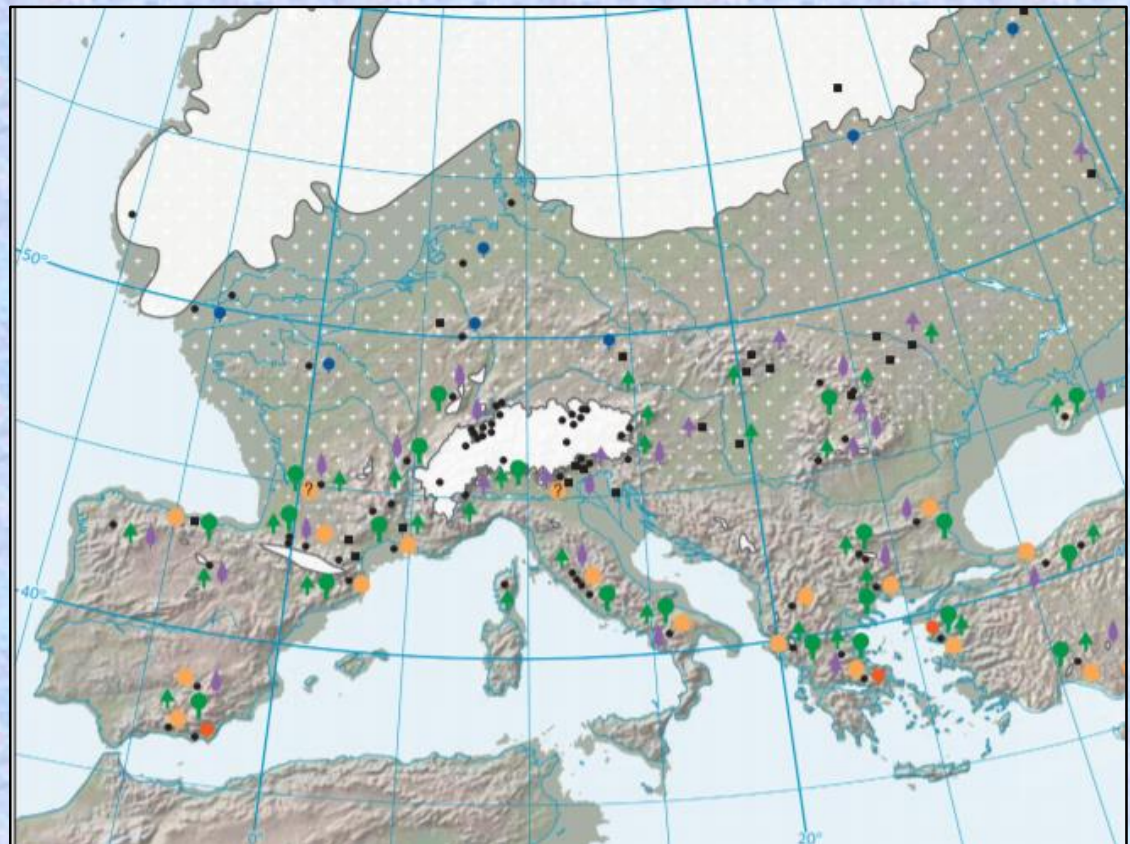
<sup>1</sup>Department of Geography, University College London, London, WC1E 6BT, UK

<sup>2</sup>Island Ecology and Evolution Research Group, Instituto de Productos Naturales y Agrobiología (IPNA-CSIC), C/Astrofísico Francisco Sánchez 3, La Laguna, Tenerife, Canary Islands, 38206, Spain

<sup>3</sup>School of Biological Sciences, University of East Anglia, Norwich, NR4 7TJ, UK

### Key:

- Boreal dwarf shrubs
- ▲ Boreal/Mountain conifers
- ◆ Boreal/Mountain summergreen trees
- ▲ Pinus
- Juniperus
- Temperate trees
- Mediterranean sclerophylls
- Combined macrofossil and pollen sites
- Pollen sites
- Macrofossil sites



# Evidence of extra-Mediterranean refugia for temperate species. Genetic evidence - forest species

## Glacial survival in northern refugia? Phylogeography of the temperate shrub *Rosa pendulina* L. (Rosaceae): AFLP vs. chloroplast DNA variation

HANA DANECK<sup>1</sup>, TOMÁŠ FÉR<sup>1\*</sup> and KAROL MARHOLD FL.S<sup>1,2</sup>

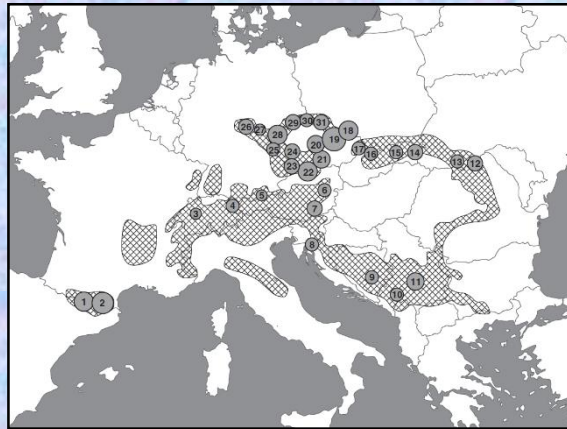
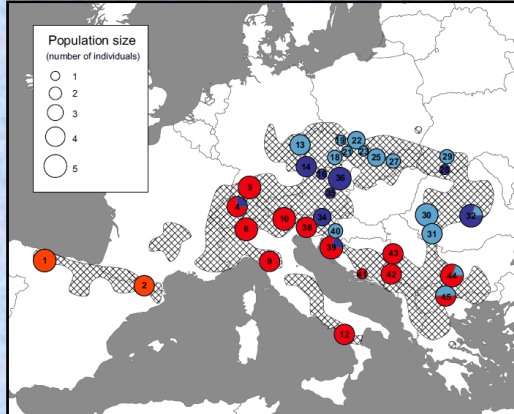
Preslia 83: 237–257, 2011

237

## Phylogeography of *Lonicera nigra* in Central Europe inferred from molecular and pollen evidence

Fylogeografická studie druhu *Lonicera nigra* se zaměřením na střední Evropu – kombinace molekulárních a paleopolynologických dat

Hana Daneck<sup>1</sup>, Vojtěch Abraham<sup>1</sup>, Tomáš Fér<sup>1</sup> & Karol Marhold<sup>1,2</sup>



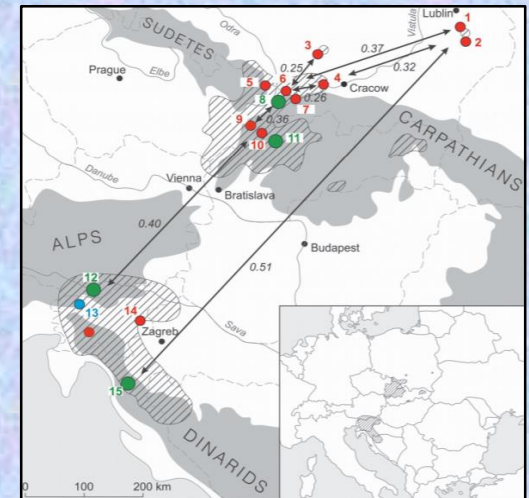
Preslia 90: 39–57, 2018

39

## Postglacial history and current population genetic diversity of a central-European forest plant *Hacquetia epipactis*

Postglaciální šíření a současná genetická diverzita populací středoevropské lesní byliny *Hacquetia epipactis*

Jacek Urbaniak<sup>1</sup>, Paweł Kwiatkowski<sup>2</sup> & Michał Ronikier<sup>3</sup>



# Evidence of extra-Mediterranean refugia for temperate species. Genetic evidence - mountain plants with large ecological amplitude in Europe

**MOLECULAR ECOLOGY**  
 Molecular Ecology (2016) 25, 3929–3949  
 doi: 10.1111/mec.13721

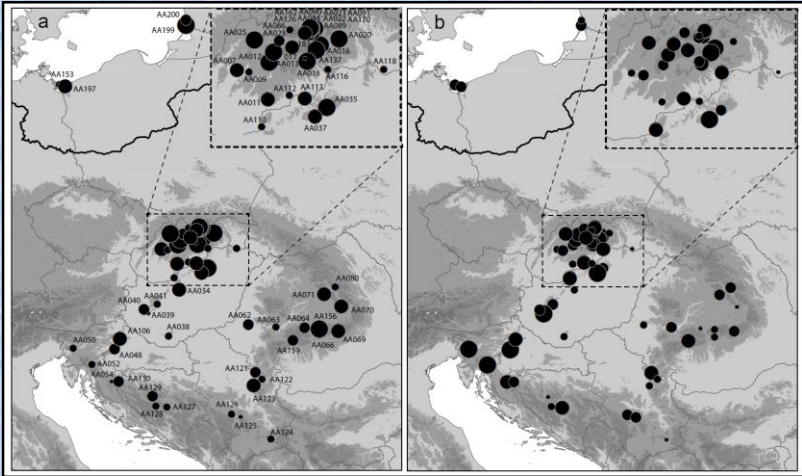
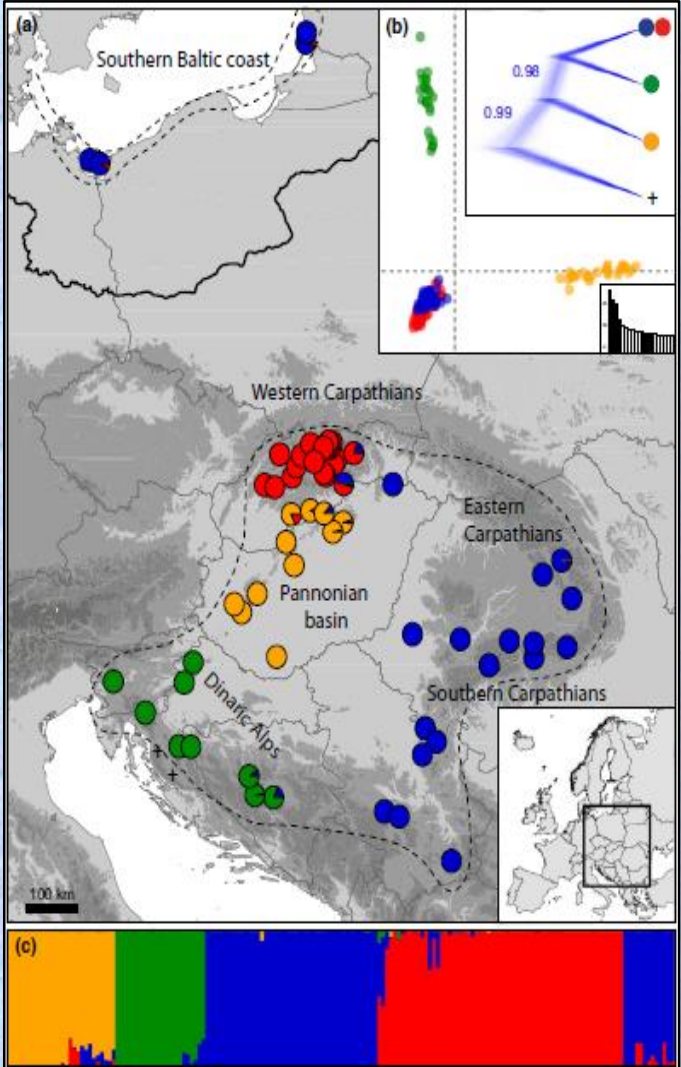
**Northern glacial refugia and altitudinal niche divergence shape genome-wide differentiation in the emerging plant model *Arabidopsis arenosa***

FILIP KOLÁŘ,<sup>1,†</sup> GABRIELA FUXOVÁ,<sup>1</sup> ELIŠKA ZÁVEŠKÁ,<sup>§</sup> ATSUSHI J. NAGANO,<sup>\*,\*\*</sup>†† LUCIE HYKLOVÁ,<sup>†</sup> MAGDALENA LUCANOVÁ,<sup>†</sup> HIROSHI KUDOH<sup>‡</sup> and KAROL MARHOLD<sup>1,††</sup>

<sup>1</sup>Natural History Museum, University of Oslo, PO Box 1172 Blindern, Oslo NO-0318, Norway, <sup>†</sup>Department of Botany, Faculty of Science, Charles University in Prague, Prague CZ-128 01, Czech Republic, <sup>‡</sup>Institute of Botany, The Czech Academy of Sciences, Průhonic CZ-252 43, Czech Republic, <sup>§</sup>Institute of Botany, University of Innsbruck, Innsbruck AT-6020, Austria, <sup>\*</sup>Center for Ecological Research, Kyoto University, Kyoto JP-520-2113, Japan, <sup>\*\*</sup>Faculty of Agriculture, Ryukoku University, Shiga JP-512-8577, Japan, <sup>††</sup>IST PRESTO, Suitama JP-332-0012, Japan, <sup>†††</sup>Institute of Botany, Slovak Academy of Sciences, Bratislava SK-845 23, Slovak Republic

**Abstract**  
 Quaternary climatic oscillations profoundly impacted temperate biodiversity. For many diverse yet undersampled areas, however, the consequences of this impact are still poorly known. In Europe, particular uncertainty surrounds the role of Balkans, a major hotspot of European diversity, in postglacial recolonization of more northerly areas, and the Carpathians, a debatable candidate for a northern 'cryptic' glacial refugium. Using genome-wide SNPs and microsatellites, we examined how the interplay of historical processes and niche shifts structured genetic diversity of diploid *Arabidopsis arenosa*, a little-known member of the plant model genus that occupies a wide niche range from sea level to alpine peaks across eastern temperate Europe. While the northern Balkans hosted one isolated endemic lineage, most of the genetic diversity was concentrated further north in the Pannonian Basin and the Carpathians, where it likely survived the last glaciation in northern refugia. Finally, a distinct postglacial environment in northern Europe was colonized by populations of admixed origin from the two Carpathian lineages. Niche differentiation along altitude-related bioclimatic gradients was the main trend in the phylogeny of *A. arenosa*. The most prominent niche shifts, however, characterized genetically only slightly divergent populations that expanded into narrowly defined alpine and northern coastal postglacial environments. Our study highlights the role of eastern central European mountains not only as refugia for unique temperate diversity but also sources for postglacial expansion into novel high-altitude and high-latitude niches. Knowledge of distinct genetic substructure of diploid *A. arenosa* also opens new opportunities for follow-up studies of this emerging model of evolutionary biology.

**Keywords:** approximate Bayesian computation, *Arabidopsis*, niche differentiation, phylogeography, RADseq





**Phylogeography of the alpine violet  
(*Cyclamen purpurascens* Mill.) - northernmost glacial refugia  
and an endemic subspecies in the Western Carpathians?**

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# *Cyclamen purpurascens* Mill.

## Life span

- long lived perennial tuberous geophyte

## Morphology and phenology

- morphologically rather invariable, fragrant and pale pink to purple flowers, cordate leaves with variable silver-grey ornamentation on the upper side
- unique phenology within the genus – evergreen, summer flowering

## Karyology

- diploids  $2n=2x=34$

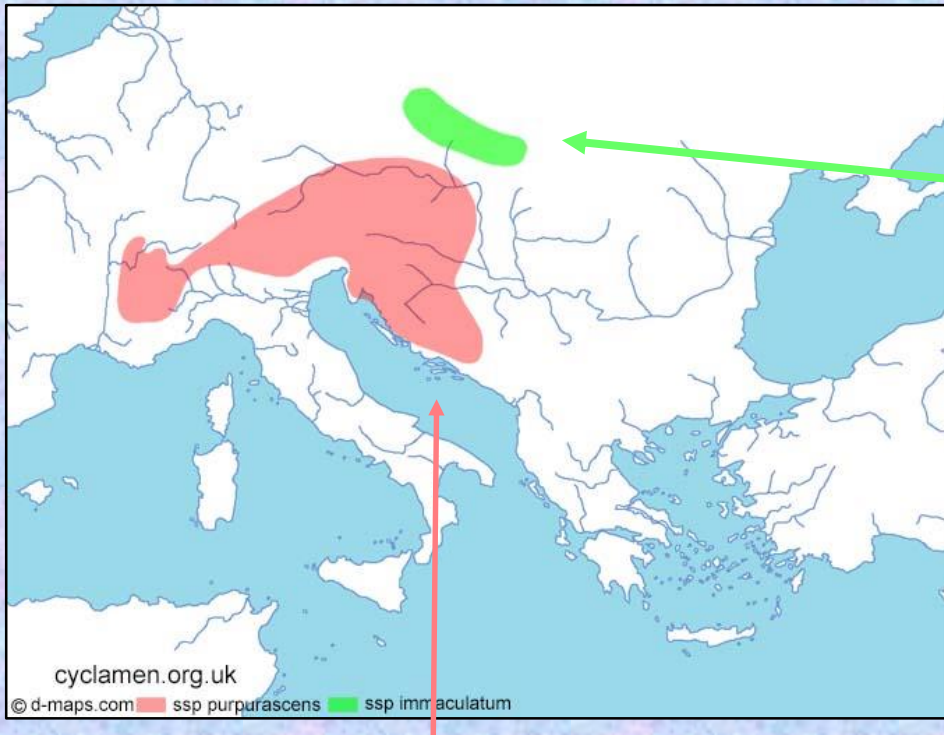
## Habitats and native distribution

- the only *Cyclamen* species extending to continental and sub-Mediterranean climate area
- deciduous broadleaved or mixed from forests from the montane to the subalpine belts, calcareous bedrock
- Alps, Dinarides, Jura Mts., SE Moravia, SW Pannonia, and Western Carpathians

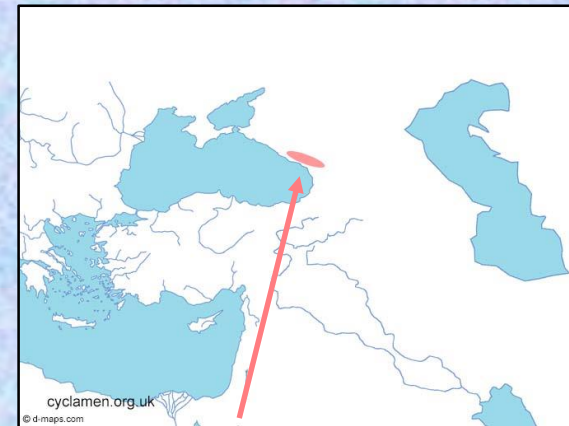
## Taxonomy

- closest relatives - *C. fatrense* Halda & Soják; uncertain taxonomic status and *C. colchicum* from the Caucasus
- leaf lamina without silver-grey ornamentation on the upper side
- stenoendemic distribution in Western Carpathians

# Distribution map of *C. purpurascens* s.l. and its relatives



*C. fatrense*



*C. purpurascens*

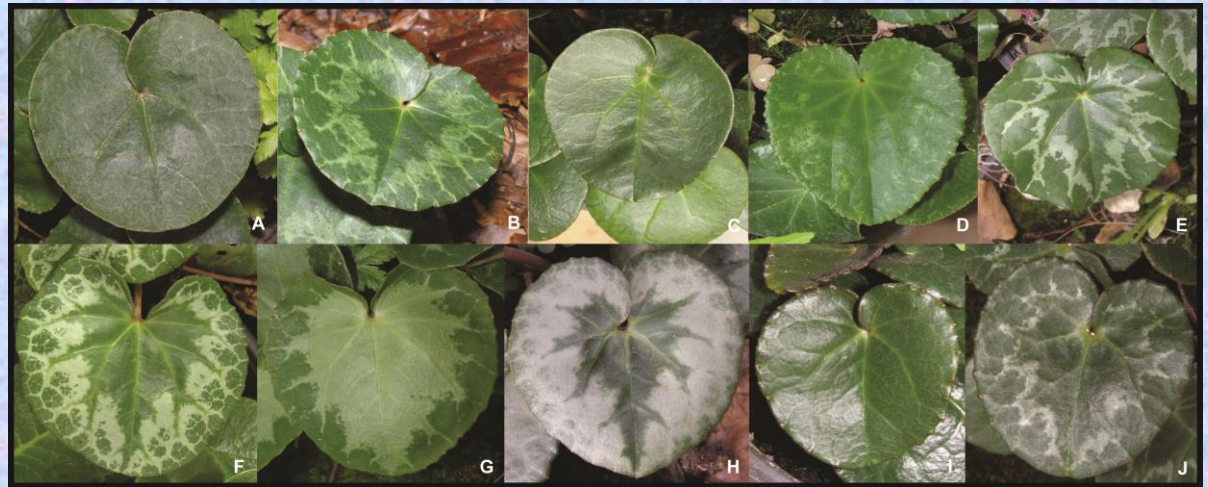


*C. colchicum*



## Aims of the study

- to test hypotheses regarding glacial survival and postglacial colonisation routes of *C. purpurascens*, and to explore how they are congruent with the histories inferred for temperate trees
- to study overall genetic, morphological and karyological variation of this species with special focus on the Western Carpathian populations considered to represent a narrow endemic taxon *C. fatrense*



# Material & Methods

## Sampling strategy

- to cover entire native distribution area of the species
- 68 populations and 399 individuals

## Genetic analyses

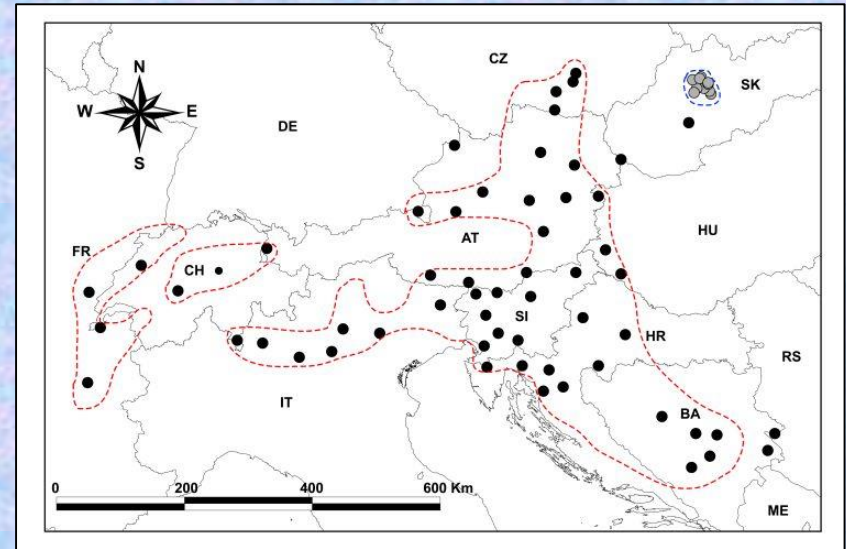
- amplified fragment length polymorphism - AFLP (68 populations, 399 individuals), cpDNA sequences of *trnD-trnT* intergenic spacer (82 individuals)

## Multivariate morphometric analyses

- principal component analyses - PCA, canonical discriminant analyses CDA (46 populations, 903 individuals; 16 morphological characters)

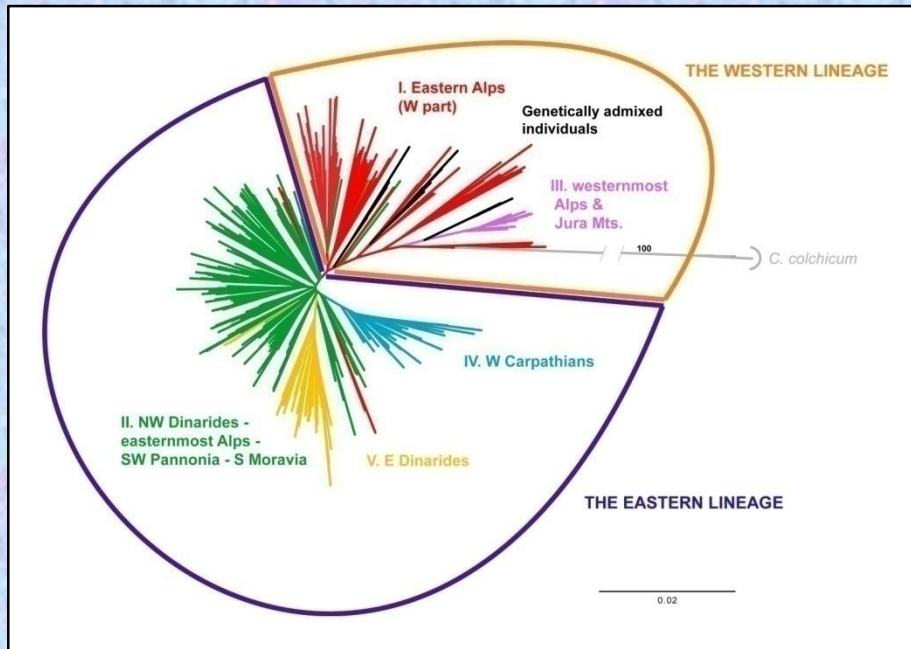
## Karyological analyses

- direct chromosome counting
- genome size and DNA ploidy level estimation: propidium iodide flow cytometry (29 populations, 34 individuals)



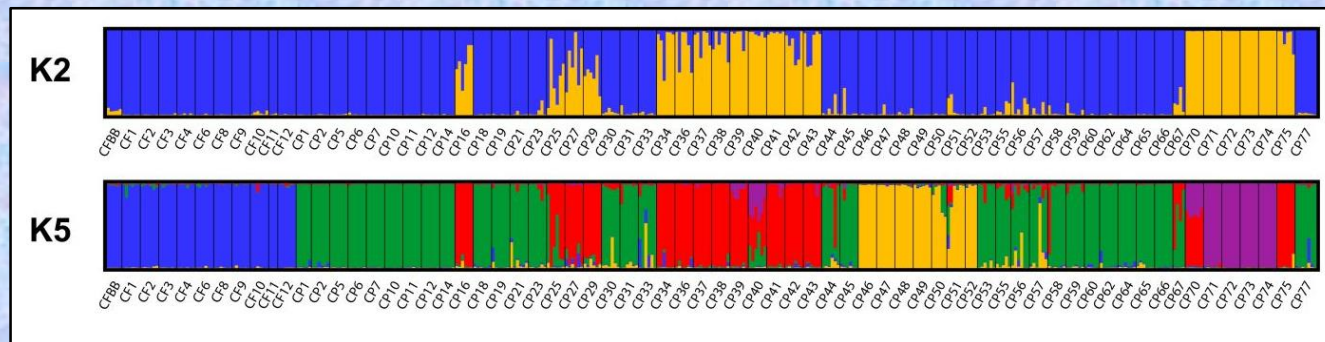
# Genetic analyses (AFLP)

## Bayesian clustering at $K = 2$ and $K = 5$ , neighbour joining tree



Unrooted neighbour-joining tree based on the AFLP data of 396 individuals of *Cyclamen purpurascens* and five individuals of *C. colchicum*

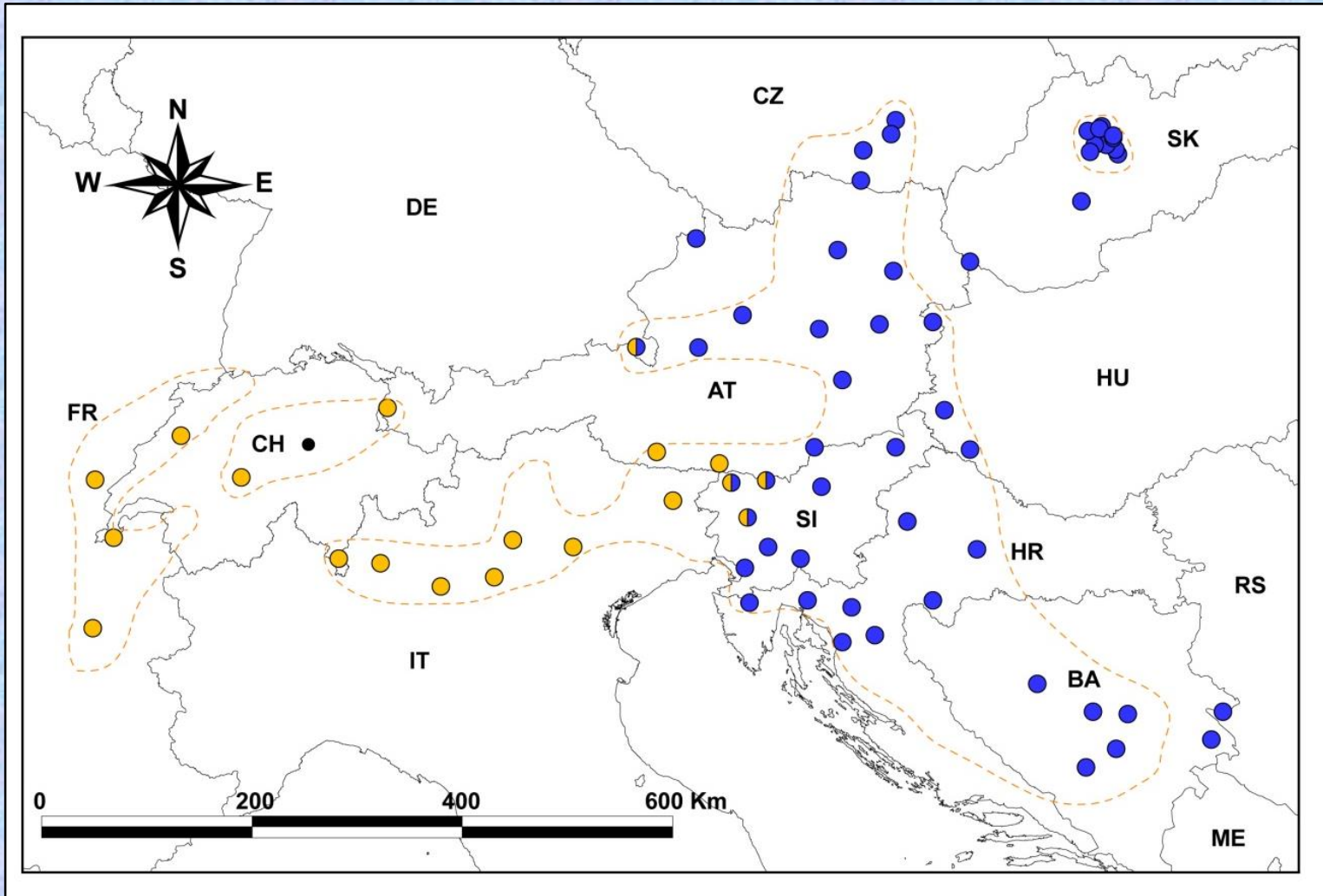
Five genetic groupings, as inferred from the Bayesian clustering at  $K = 5$ .



Genetic structure in *C. purpurascens* (396 individuals, 68 populations), as resolved by the Bayesian clustering of AFLP genotypes at  $K = 2$  and  $K = 5$  (denoted as lineages I–V). Each individual is represented by a vertical bar, coloured proportionally according to the cluster assignment.

# Genetic analyses, AFLP

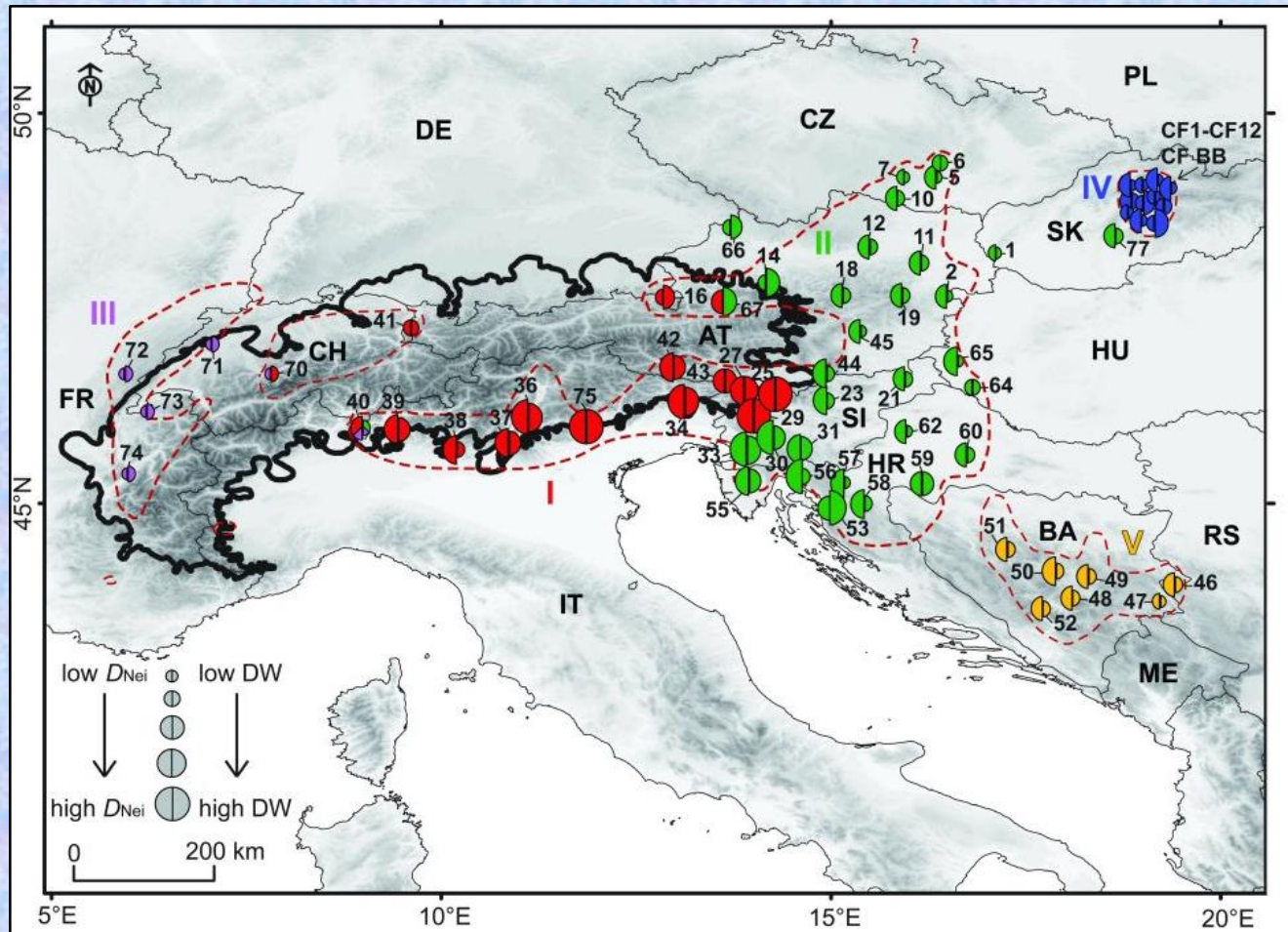
## Geographic distribution - STRUCTURE at $K = 2$



Sample sites of 68 populations of *Cyclamen purpurascens*. Genetic groupings based on AFLP data (corresponding to the Bayesian clusters at  $K = 2$ ) are indicated by symbol colour: **western lineage**, **eastern lineage** (multicoloured symbols indicate genetically admixed populations). The dashed yellow outlines indicate the indigenous distribution range of this species, summarised according to literature sources and herbarium specimens.

# Genetic analyses (AFLP)

## Geographic distribution - STRUCTURE at $K = 5$



Genetic groupings based on AFLP data (corresponding to the Bayesian clusters at  $K = 5$ ) are indicated by symbol colour: **lineage I - the western parts of the Eastern Alps**, **lineage II - Northwestern Dinarides, easternmost Alps, Southwestern Pannonia and Southern Moravia**, **lineage III - the westernmost Alps and Jura Mts**, **lineage IV - the Western Carpathians (*C. fatrense*)**, and **lineage V - (the Eastern Dinarides** (multicoloured symbols indicate genetically admixed populations). The symbol size indicates the level of genetic diversity ( $D_{Nei}$ , left symbol) and rarity (frequency-down-weighted marker values, DW1, right symbol). The thick black line shows the maximum extent of the ice sheet during the last glacial maximum (according to Penck & Brückner, 1909).

# AFLP-based genetic diversity and divergence in 5 phylogroups

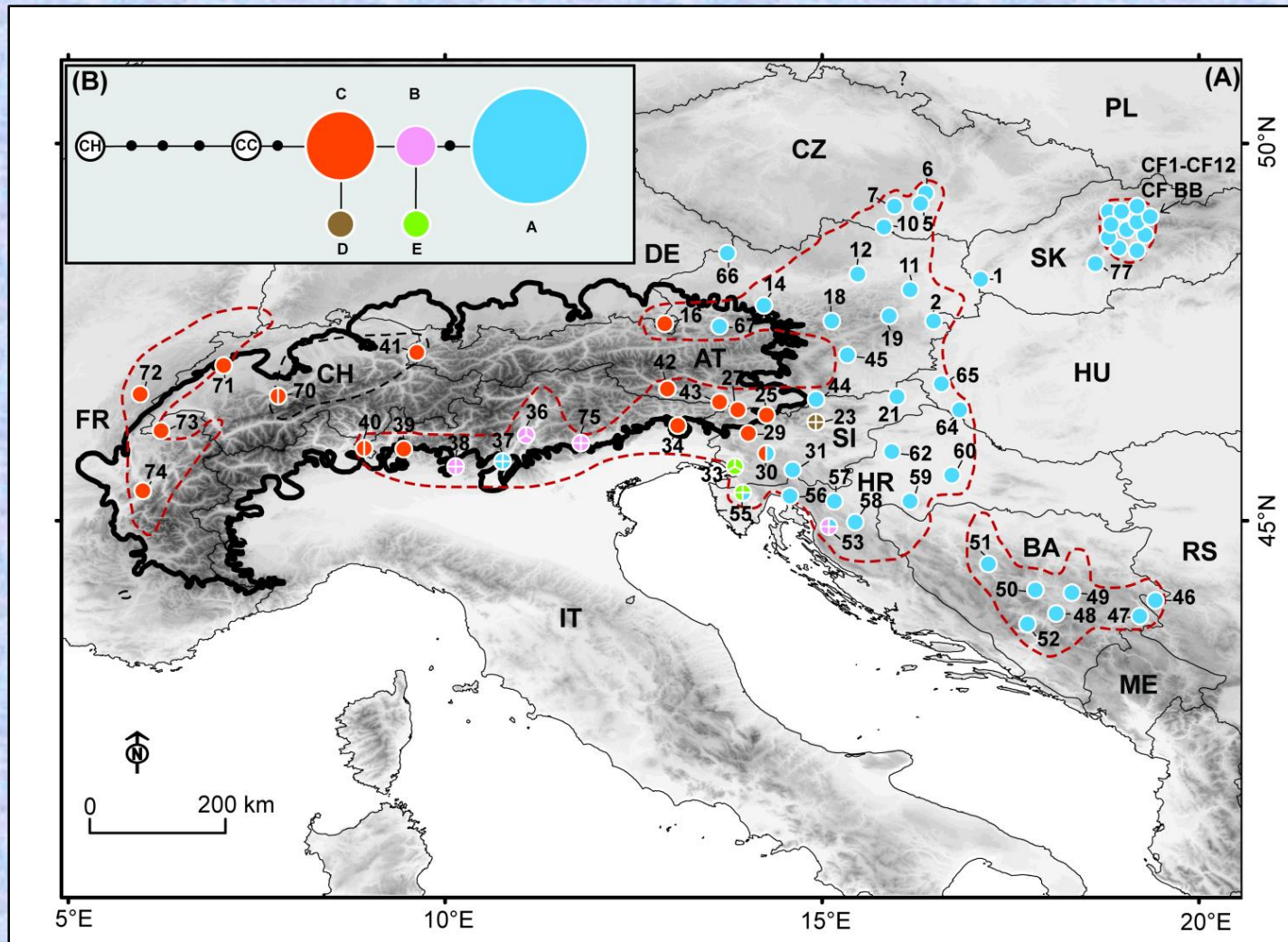
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**Table 2.** AFLP diversity and divergence parameters estimated for the five phylogroups (corresponding to the Bayesian clusters at  $K = 5$  and excluding admixed populations) of *Cyclamen purpurascens*

Phylogroup	$n_{\text{pop}}$	$n_{\text{ind}}/n_{\text{gen}}$	$n_{\text{pr}}/n_{\text{pr-f}}$	$D_{\text{Nei}}$	DW1
I, Eastern Alps (western part)	13	78/77	15/0	0.0798 <sup>AD</sup>	5.08 (2.47–10.53)
II, north-western Dinarides, eastern-most Alps, southern Moravia, south-western Pannonia	30	177/176	13/0	0.0637 <sup>BD</sup>	2.77 (1.54–6.48)
III, western-most Alps and Jura Mountains	4	24/10	0/0	0.0128 <sup>C</sup>	2.60 (2.43–2.99)
IV, Western Carpathians	11	62/55	5/0	0.0316 <sup>C</sup>	2.13 (0.63–5.40)
V, eastern Dinarides	7	39/39	0/0	0.0603 <sup>ABD</sup>	2.29 (1.84–2.54)

$n_{\text{pop}}$ , number of populations;  $n_{\text{ind}}/n_{\text{gen}}$ , number of individuals/number of AFLP genotypes;  $n_{\text{pr}}/n_{\text{pr-f}}$ , number of private and private fixed markers;  $D_{\text{Nei}}$ , average Nei's gene diversity (letters in superscript indicate which values are statistically different at  $\alpha = 0.05$  in a Tukey–Kramer test; different letters stand for significantly different values, identical letters for those not significantly different); DW1, average rarity index, with minimum and maximum values in parentheses.

# Analyses of cpDNA sequences



The geographical distribution of the cpDNA haplotypes of *C. purpurascens*. The thick black line shows the maximum extent of the ice sheet during the last glacial maximum (according to Penck & Brückner, 1909). (B). The maximum parsimony network of the cpDNA haplotypes based on *trnD-trnT* sequences of *C. purpurascens* (haplotypes A – E), *C. colchicum* (CC) and *C. hederifolium* (CH). The symbol sizes are proportional to the haplotype frequency, the lines represent mutational steps, and the dots are unsampled haplotypes.

# Conclusions on genetic pattern

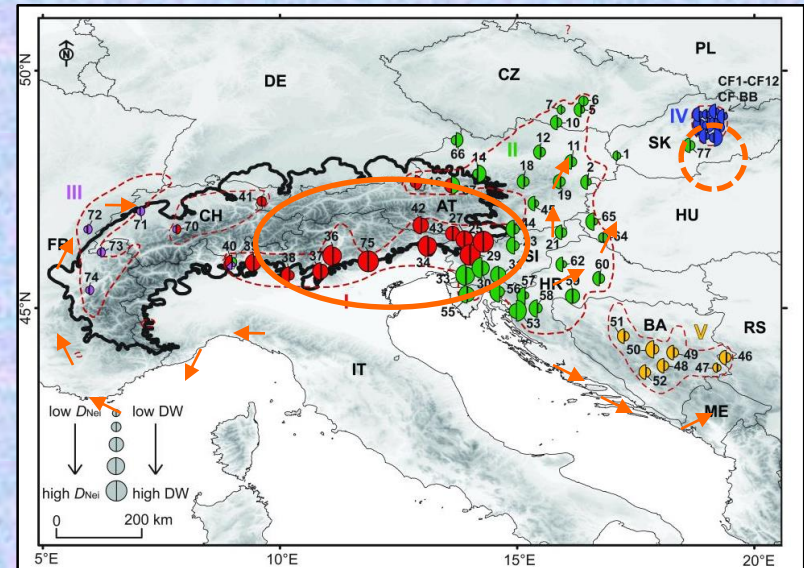
- AFLP ( $K = 2$ ) and cpDNA sequences - two genetic groups (western and eastern) with little overlap
- more detail AFLP structure ( $K = 5$ ) - five genetic groups (lineages I, II, III, IV and V) with non- random geographic distribution
- the highest  $D_{nei}$  and rarity (DW1) values and rare cpDNA haplotypes found in populations from southern, south-eastern Alps and western Dinarides (lineages I and II) = glacial refugia
- marginal lineages III, IV and V genetically less variable = postglacial colonization
- lineage III strongly genetically depauperated (long term isolation, sub-optimal ecological conditions and/or altered mating systems)

**W Carpathian populations (*C. fatrense*) : no unique cp DNA haplotype; separated AFLP lineage IV - clearly homogeneous; higher  $D_{nei}$  and rarity (DW1) values in several population = the northernmost refugia ?**



# Conclusions on phylogeography

- center of genetic diversity is in **SE Alps and NW Dinarides = the most probable glacial refugia** for *C. purpurascens*
- postglacial migrations towards W Alps, Jura Mts., Central Alps, SW Pannonia, SE Moravia and E Dinarides
- Carpathian populations **most probably survived glaciation in W part of Carpathians**
- postglacial colonization routes congruent at least with several temperate trees (e.g. *Fagus sylvatica*)



# Summary

Concept of **three main Mediterranean refugia (only)** is rather simplistic – even within these refugia the distribution of genetic variation is structured (“Refugia within Refugia”)

There were **glacial tree refugia in Central Europe** and in the **West Carpathians**, in particular; question remains whether during the LGM there was extensive taiga woodland or just occurrence of boreal and temperate trees in favorable microhabitats surrounded by the continuous permafrost

(Widespread) occurrence of broadleaved trees in this area during the LGM is a matter of dispute

# Karyological variation

## Chromosome number, DNA ploidy level and absolute DNA content

- all analysed individuals are karyologically uniform
- diploids with  $2n = 2x = 34$
- absolute DNA content ranging between  $2C = 6.63 - 6.92$  pg, with a maximum divergence of  $4.6\%$

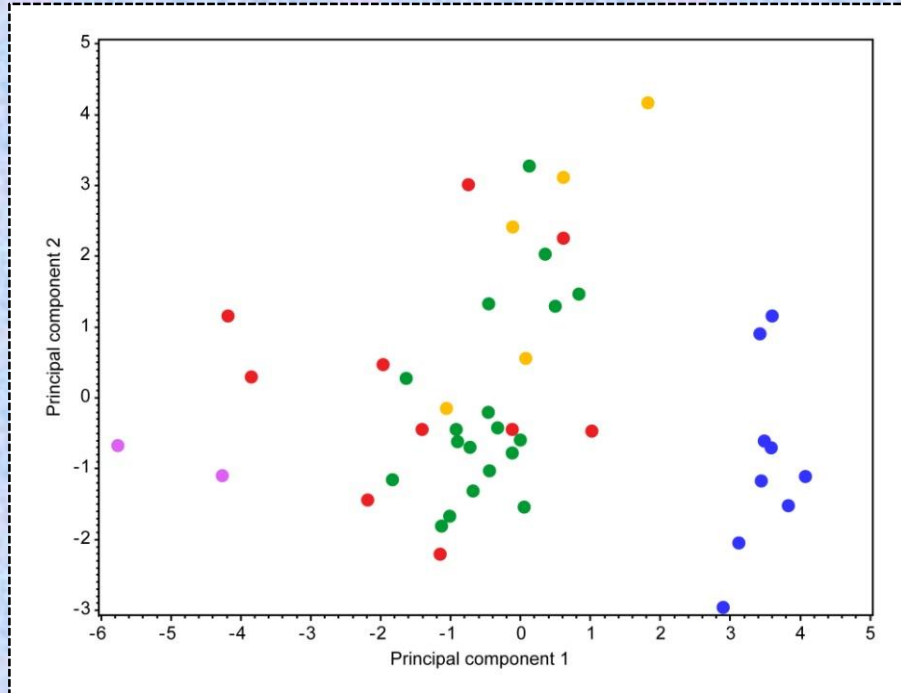
## Conclusions

- negligible difference in absolute DNA content = lack of karyological variation

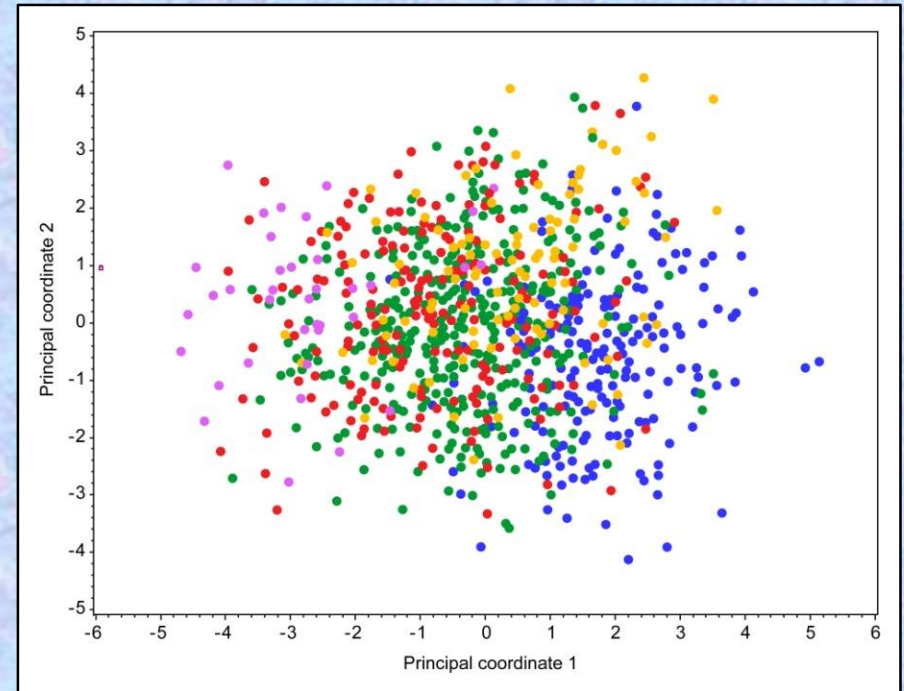


# Morphological analyses

## Multivariate morphometrics – PCA's



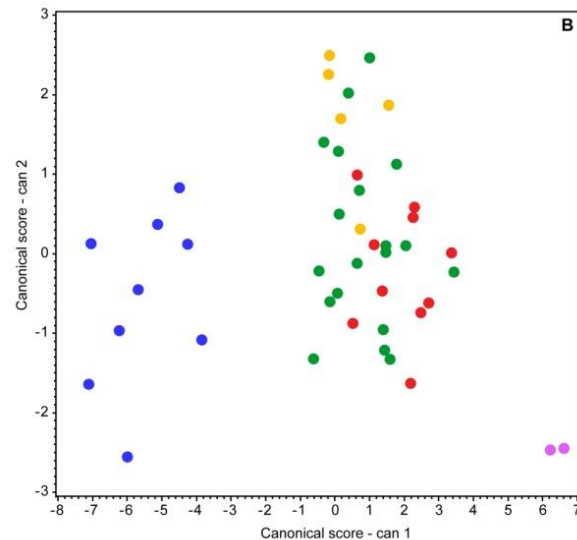
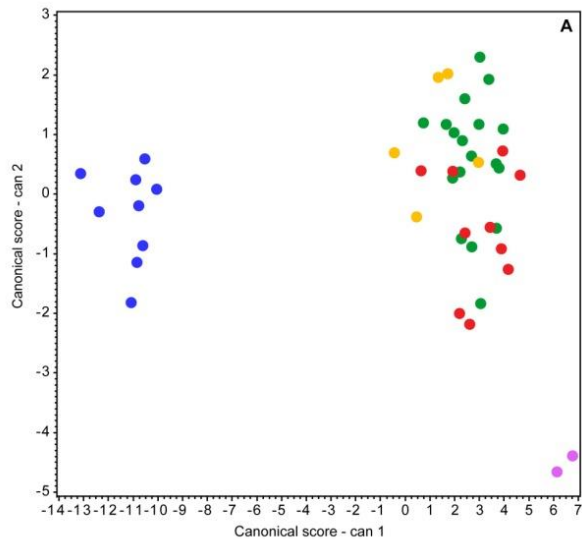
Principal component analysis (PCA) based on 45 populations and 16 morphological characters. Different colours indicate genetic groupings as resolved by Bayesian analysis at  $K = 5$  (lineage I, lineage II, lineage III, lineage IV, lineage V).



Principal component analysis (PCA) based on 886 individuals and 16 morphological characters. Different colours indicate genetic groupings as resolved by Bayesian analysis at  $K = 5$  (lineage I, lineage II, lineage III, lineage IV, lineage V).

# Morphological analyses

## Multivariate morphometrics – CDA's



Canonical discriminant analysis (CDA) of populations of *C. purpurascens* s.l. with five predefined groups, as resolved by Bayesian clustering of AFLP data at  $K = 5$  (lineage I, lineage II, lineage III, lineage IV, lineage V).

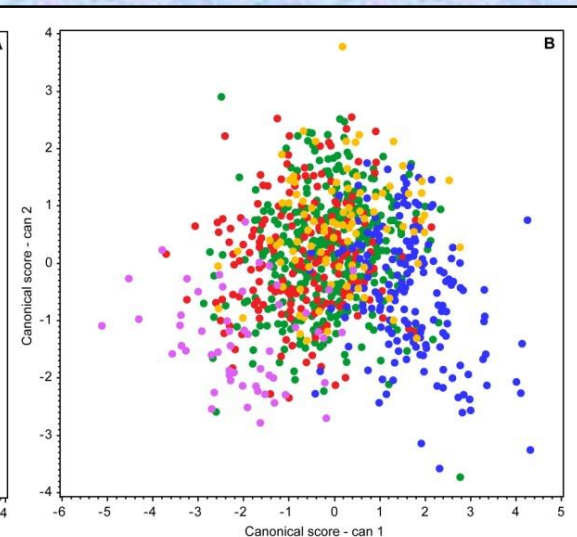
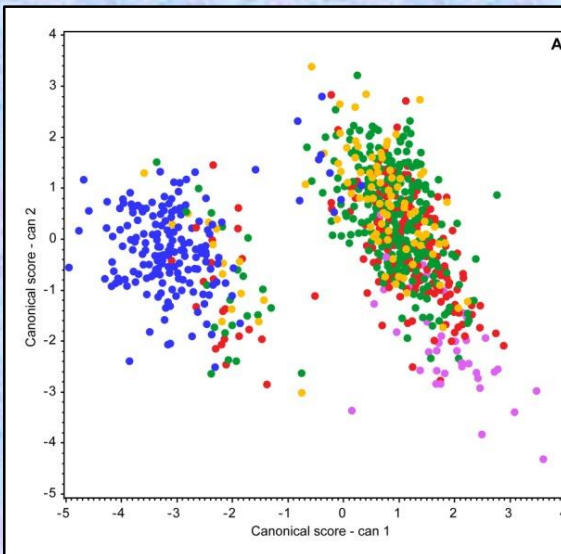
A. CDA based on 45 populations and 16 morphological characters.

B. CDA based on 45 populations and 15 morphological characters (excluding the ornamentation on the upper leaf surface).

Canonical discriminant analysis (CDA) of individuals of *C. purpurascens* s.l. with five predefined groups, as resolved by Bayesian clustering of AFLP data at  $K = 5$ . (lineage I, lineage II, lineage III, lineage IV, lineage V).

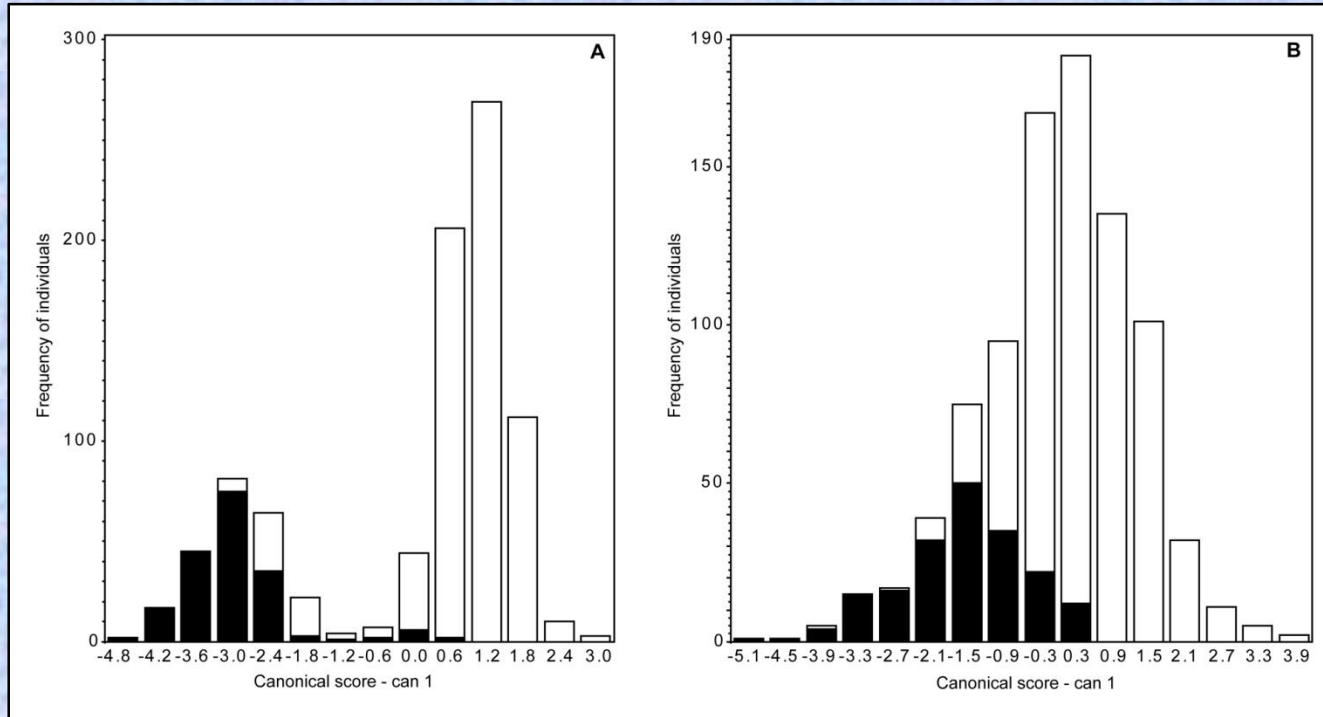
A. CDA based on 886 individuals and 16 morphological characters.

B. CDA based 886 individuals and 15 morphological characters (excluding the ornamentation on the upper leaf surface).



# Morphological analyses

## Multivariate morphometrics – CDA's



Histograms of the canonical discriminant analysis (CDA) of individuals of *C. purpurascens* – Alps, Dinarides, Jura Mts., SW Pannonia, SE Moravia - empty (phylogroups I, II, III and V) and W Carpathians - solid (phylogroup IV).

A. CDA based on 886 individuals and 16 morphological characters.

B. CDA based on 886 individuals and 15 morphological characters (excluding the ornamentation on the upper leaf surface).

# Morphological analyses

## Conclusions

- two morphological (morphotype ) groups distinct at population level
- no morphological structure at individual level
- lineage III (W Alps, Jura Mts.) showed some trend to morphological differentiation (smaller floral parts), problematic interpretations due to limited number of analyzed populations

### W Carpathian populations (*C. fatrense*)

- **clearly separated** at the **population** level
- **not separated** at the **individual** level
- differs by **combination** of several morphological characters
- the most important diagnostic characters: absence of ornamentation on their upper leaf surface, the shape of the leaves and sepals, the width of the petals, the position of the widest part of the leaf lamina

# Final conclusions



## Overall genetic, karyological and morphological variation

- morphologically and genetically more variable than it was expected
- karyologically rather invariable
- five genetic (AFLP) groups, but only two differs also morphologically
- populations from W Carpathians (lineage IV) - the **most differentiated from all lineages**, separated homogeneous AFLP lineage and distinct morphological group at population level
- populations from W Alps and Jura Mts. (lineage III) – tendency to morphological and genetic diversification, overlaps (AFLP and morphological admixture) with adjacent lineages I and II



# Final conclusions

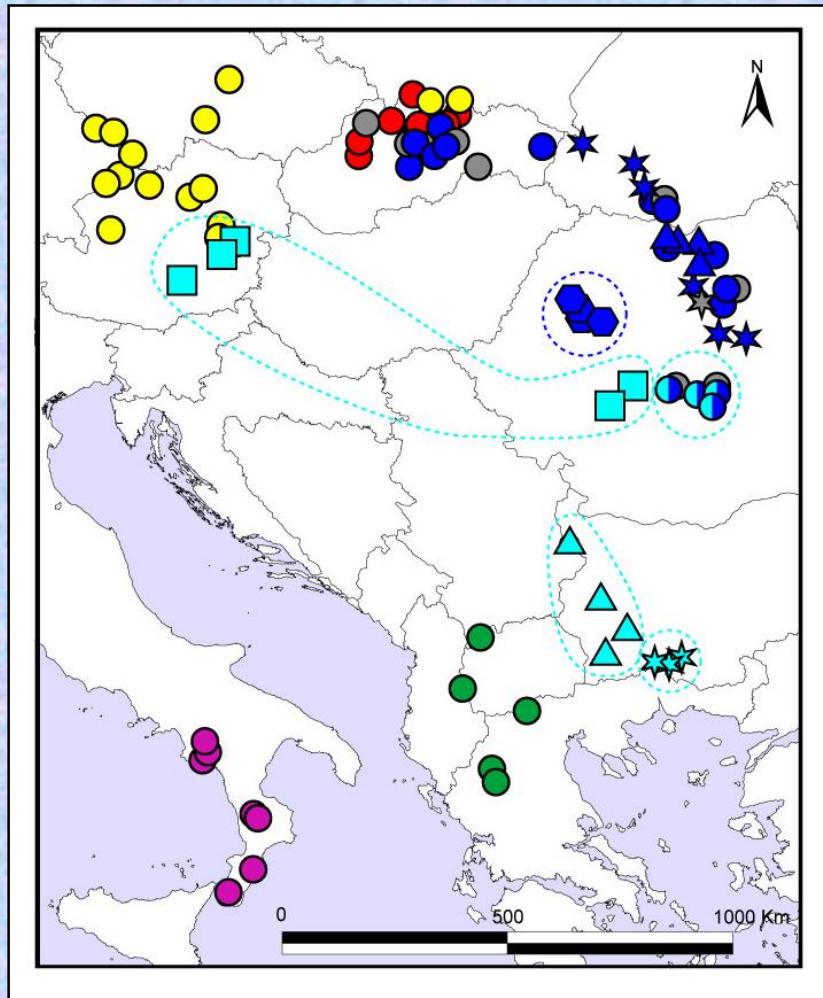
## Single heterogeneous species or several infraspecific taxa???

- two subspecies unequal concerning their morphological, genetic variation and distribution area
- *C. purpurascens* Mill. subsp. *purpurascens* - genetically and morphologically heterogeneous (AFLP lineages I, II III and V; five cpDNA haplotypes), distribution Alps, Dinarides, Jura Mts., SE Moravia, SW Pannonia
- *C. purpurascens* subsp. *immaculatum* (Hrabětová) Halda et Soják. - genetically and morphologically rather homogeneous (AFLP lineage IV, single cp DNA haplotype), distribution = W Carpathians

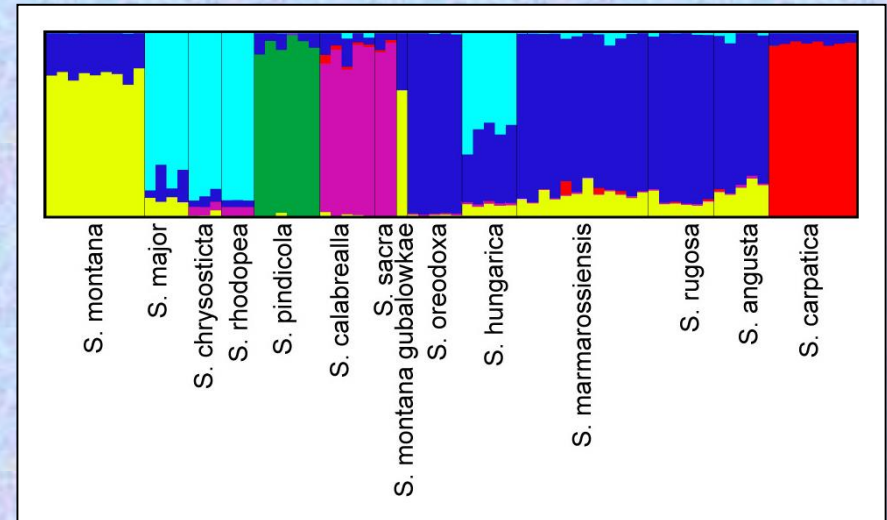
# Future work - evolution and phylogeography of the genus *Soldanella*



# Future perspective - evolution and phylogeography of the genus *Soldanella*



Colour of symbols follow group delimitation based on STRUCTURE analysis, different symbols indicate recently recognised taxa, orange arrows show alpine populations and dashed line indicates well delimited genetic lineages



Genetic structure based Bayesian model based clustering analysis based on 74 individuals and 12,902 filtered SNPs.

- *S. carpatca* is the most divergent taxon
- populations from southern Carpathians, north-eastern Alps and south-eastern Balkan form one lineage
- rest of Carpathian populations form single clade with shallow genetic structure