Evolutionary and biogeographical significance of refugia for arctic-alpine plants

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Overview

- Introduction
  - When and where did Arctic and Alpine species evolve?
  - Where do new adapted species/genotypes evolve today?
  - How fast do species evolve?
  - What is a refugium?
  - Inferring processes with genetic & species diversity data

- Examples
  - Fossil data
  - Biogeographical evidence
  - Molecular evidence

- Conclusion
  - Theoretical concept of refugia in evolutionary research
  - Open questions
When and where did Arctic and Alpine species evolve?

Cooling and parallel onset of alpidic orogenesis (including uplift of Tibetan plateau) in Miocene and Pliocene

Pleistocene cycles; glacial / interglacials

→ Adaptive evolution started well before glacializations
Maximum ice extent ~20,000 years ago:
One mainly glaciated area
One mainly unglaciated area

Modified from Abbott & Brochmann 2003; Brochmann et al. 2004
Forestation and fragmentation of the Arctic tundra c. 6ka BP

Crawford et al., 2003, Ann. Bot. 91
When and where did Arctic and Alpine species evolve?

Composition of the European mountain flora

Biogeographic elements:
- "in situ"-element
- Asian element
- arctic-alpine element

Speciation and formation
- Adaptation from (Tertiary) lowland taxa
- Allopatric differentiation of adapted species
- Adaptive radiations within/among mountains
- Polyploidisation & hybrid speciation
- Immigration of adapted species and anagenetic evolution

(Reisigl & Keller, 1987)
Where do new adapted species/genotypes evolve today?

Higher speciation rates expected:

- High levels of genetic drift (small & isolated populations)
  - range margins
  - islands (isolated mountains)
  - specialized species with disjunct distribution

- High selection pressure
  - ecologically extreme habitats
  - high competition

- Hybrid evolution (intra/interspecific)
  - secondary contact zones
  - unstable environments & high disturbance

- Higher mutation rates

→ could be "anywhere", no a priori hypothesis suggested
Where do new adapted species/genotypes evolve today?

New plant species start as neo-endemics!

Endemic plant species in Europe

Hendrych 1981. Preslia 53
Where do new adapted species/genotypes evolve today?

Low levels of endemism in the North

Fig. 10. Levels of current vascular plant endemism (black part of pie) in the previously glaciated North Atlantic regions (“single-region endemics”, data from Tables 1 and 2; cf. Fig. 1) and in alpine Eurasian regions situated outside the main area of glaciation (data from Myers & Giller, 1988). Pie size increases proportionally with the total number of species in each region. The lines connecting North Atlantic regions illustrate the three main patterns observed for North Atlantic endemics that are shared among two or more regions (“several-region endemics”). The alpine regions outside the main area of glaciation are (from the west) the Pyrenees (14% endemism), the Alps (32%), the Balkan Mountains (34%), the South Greek Mountains (37%), the Carpathians (12%), the Caucasus (25%), and the Ural Mountains (8%).

Brochmann et al. 2003. Taxon 53
Diploids tend to unglaciated areas, whereas polyploids inhabit once glaciated Areas

(example: *Asplenium ceterach*)
Higher rates of polyploidy in once glaciated areas of the Arctic

Figure 1. Frequency and level of polyploidy in the arctic flora along the south-north gradient, based on 1719 of the chromosome-counted taxa in the Panarctic Flora checklist (excluding most Cyperaceae etc., see text). The taxa are classified into zonal groups according to the south-north zonal subdivision of the Arctic in Elvebakk et al. (1999). See Table 1 for definition of zonal groups.

→ higher rates of polyploid evolution in once glaciated areas !?
How fast do new Arctic and Alpine plants evolve?

Once glaciated areas host only few endemics
Endemics are almost exclusively (Allo)Polyploids
North Atlantic: Brochmann et al. 2004
Eastern Alps: Tribsch 2004

Almost no restricted diploid species that evolved postglacially, although the Holocene was a unusually long stable period

→ 20,000 years are not enough for extensive speciation (except for hybrid speciation)
Where do plant species speciate and where do they and their genes survive climatic instabilities?

As climatic cycles are too fast to allow for speciation at diploid level, do species evolve in refugia or do they just survive there?

As endemism of species is not randomly distributed, so can we explain biogeographic patterns found today with geography of refugia only?

Where did those genetically variable populations survive that were source for those living today? Only in refugia?
What is a *refugium*?

“We define a *refugium* as a geographical area of relative ecological stability that has provided habitats during cold and warm, wet and dry stages during climatic instabilities for survival of populations, more or less *in situ*. Thus, a *refugium* is (more or less) situated in the same place during all stages of the Quaternary, for example, in mountain ranges that never have been fully glaciated.“

*Tribsch & Schönswetter 2003, Taxon*

Modified after Haffer 1969 & Tallis 1991

A *refugium* must provide:

- a buffer for climatic changes
- stability throughout time, ideally throughout [e.g.] the Pleistocene
- survival of large, genetically variable (diploid) populations that were the source for populations found today
A potential refugium offering longterm stability is most likely found in "southern" mountaneous regions.
Climate 1: species is widespread
Climate 2: species range is restricted
If there is evidence that in a potential refugium stable populations have lived throughout climatic cycles it should be called **realized refugium**
All populations originate from refugia, where they survived effectively.
Potential peripheral refugia of mountain plants in the European Alps during the Last Glacial Maximum (20,000 years ago). Blue areas: refugia on calcareous bedrock; red areas siliceous bedrock. Broken black line: maximum extent of the ice sheet, white broken line: snow line.

Potential ‘Arctic’ refugia

European Mountains
Central Asian Mountains
Rocky Mount. & Cascades
Beringia

? Taymyr, Ural, Putorana
? Northeast. N-America

Potential source areas
of ‘arctic lineages’
Inferring historical patterns from species distributions and genetic diversity data

Conclusions on refugia based on:

- Distribution patterns of species, biogeographical & phylogenetical analysis
- Species richness
- Patterns of endemism

- Phylogeographic patterns
- Genetic variation of populations within species
- Distribution of endemic (private, unique, rare) alleles
Inferring historical patterns from species distributions and genetic diversity data

Conclusions on refugia based on:

- Distribution patterns of species, biogeographical & phylogenetical analysis
- **Species richness**
- **Patterns of endemism**

Phylogeographic patterns
- **Genetic variation of populations within species**
- **Distribution of endemic (private, unique, rare) alleles**
Hypothesis: same processes shape genetic and species diversity

Population 1

- high variation

Population 2

- low variation
- rare marker

Genetic variation

Genetic diversity index

Rarity index
Genetic diversity patterns and history of species

<table>
<thead>
<tr>
<th>high genetic diversity</th>
<th>low genetic diversity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>high divergence</strong></td>
<td><strong>bottlenecks/drift in small, isolated populations, in situ survival in isolated real refugium/a</strong></td>
</tr>
<tr>
<td>(many rare / endemic alleles)</td>
<td></td>
</tr>
<tr>
<td><strong>low divergence</strong></td>
<td><strong>bottlenecks due to long-distance dispersal, single immigration</strong></td>
</tr>
<tr>
<td>(few rare / endemic alleles)</td>
<td></td>
</tr>
<tr>
<td>limited gene flow, but large populations, <em>in situ</em> survival in large real refugium/a</td>
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<tr>
<td>high gene flow among populations, multiple immigration, no refugium</td>
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</table>

**Table 5.4**

Interpretation of differences between haplotypes and nucleotide diversities.

<table>
<thead>
<tr>
<th>Haplotype diversity</th>
<th>Nucleotide diversity</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (&lt;0.5)</td>
<td>Low (&lt;0.5%)</td>
<td>Recent population bottleneck. Founder effect with single or few organelle lineages. Divergence between geographically subdivided populations.</td>
</tr>
<tr>
<td>High (≥0.5)</td>
<td>High (≥0.5%)</td>
<td>Bottleneck followed by rapid population growth and mutation accumulation. Large stable population with long evolutionary history. Secondary contact between differentiated lineages.</td>
</tr>
</tbody>
</table>

After Grant and Bowen (1998).
Species diversity & patterns
(3 areas/local floras)

Species richness

Rarity index
### Species diversity patterns and history of regional floras

<table>
<thead>
<tr>
<th>Peculiar Floristic Composition</th>
<th>High Species Richness</th>
<th>Low Species Richness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Many rare/endemic taxa</td>
<td>Limited species flow, but rich floras, large long-term refugium</td>
<td>Bottlenecks/drift in small, isolated floras, small isolated refugium</td>
</tr>
<tr>
<td>Few rare/endemic taxa</td>
<td>High species flow among areas, massive immigration from various sources, no refugium</td>
<td>Bottlenecks due to long-distance colonisation, recent immigration to remote areas</td>
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Diversity & divergence and population/species/flora histories

<table>
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<th>high divergence (endemism/rarity)</th>
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<td>low divergence (endemism/rarity)</td>
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Testing hypotheses of potential refugia to discover **realized refugia** of general importance

- Evidence from fossil data
- Biogeographical evidence
- Molecular evidence
Fossil evidence for refugia

Figure 4. Schematic diagram to indicate proposed areas of refugia for temperate tree taxa in the three southern peninsulas of Europe during the last full glacial (ca. 100–16 ka). Evidence to support this model comes from numerous fossil pollen and plant macrofossil assemblages. (Redrawn from Willis & McElwain (2002) and references cited therein.)

Fossil evidence for refugia

Fig. 9 Tentative location of refuge areas for *Fagus sylvatica* during the last glacial maximum and main colonization routes during the postglacial period.

Magri et al. 2006, New Phytol. 171
Fossil evidence for refugia

Good quality of fossil records necessary

Restricted to certain plants, usually common species

Difficult to proof **continuous** presence of populations in an area

! Increasingly support for ”Northern Refugia” also for boreal and nemoral species

→ Fossils alone cannot proof presence of realized refugia
Testing hypotheses of potential refugia to discover realized refugia

- Evidence from fossil data
  - Biogeographical evidence
- Molecular Evidence
Realized refugia for species in the Southern Europe – Centres of Endemism are mainly in mountain ranges

Verlaque et al. 1997, Geobios 21
Biogeographic patterns in the Alps (Data from IntraBioDiv)

Diversity & History

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<td>limited species flow, but rich floras, large long-term refugium</td>
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<tr>
<td>not peculiar floristic composition, few rare/endemic taxa</td>
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Realized refugia for species only in E, S, SW

Potential refugia

Würm ice sheet

Species diversity s.l.

Würm glaciation centres
Biogeographical evidence for refugia for floras

Summary:

**Alps**

SW, S, E-Alps host realized refugia for vascular plant species assemblages
No realized refugia in Central and Northern Alps

**Eurasia** (see introduction)

new analyses needed, but mountains and Beringia host doubtlessly realized refugia
No refugia north of Alps/Carpathians/Pyrenees
Testing hypotheses of potential refugia to discover realized refugia

- Evidence from fossil data
- Biogeographical evidence
- Molecular Evidence
Examples from molecular data

Refugia of *Mesotriton alpestris* (Salamandridae)

Refugia of *Erebia epiphron*

**Figure 4**
Hypothetical distribution patterns of the mountain ringlet *Erebia epiphron* in western Europe during the last glacial maximum. Grey areas show mountain areas above 1000 m asl. Hatched areas: Western major group, dotted areas: eastern major group. Redrawn from Schmitt et al. [168].
Realized refugia – phylogeographical evidence in the Alps

Summary of phylogeographic data of 10 taxa of alpine vascular plants

Schönswetter, Stehlik, Holderegger & Tribsch 2005
Study taxa & results: AFLP fingerprinting

patterns of genetic diversity (& phylogeographic patterns)

Alps
*Ranunculus alpestris* s.l.
*Carex curvula*

Eurasia
*Gentiana verna* s.l.
*Thalictrum alpinum*
*Pedicularis oederi*
Genetic diversity index (Nei 1972)

Rarity index
(Schönswetter & Tribsch 2005, frequency down-weighted marker values, calculated for each population)
Alps
*Ranunculus alpestris* s.l.
*Carex curvula*
IntraBioDiv-data with 40 taxa (see Gugerli et al. 2009, PPEES)
Carex curvula s.l.
(Tribsch et al., ined.)
Carex curvula s.l.
(Tribsch et al., ined.)

data from IntraBioDiv

rarity

diversity
**Carex curvula s.l.**  
*(Tribsch et al., ined.)*

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### Rarity

- High divergence (endemism/rarity)  
  - High diversity  
  - Low diversity

- Low divergence (endemism/rarity)  
  - High diversity  
  - Low diversity
Ranunculus alpestris s.l.
(Paun et al., Molec. Ecol., 2008)
Ranunculus alpestris s.l.
(Paun et al., Molec. Ecol. 2008)

rarity

diversity
Ranunculus alpestris s.l.
(Paun et al., Molec. Ecol. 2008)

some evidence for Northern refugia
(Tribisch et al., in press)
genetic diversity & history
IntraBioDiv data set with 40 taxa

Genetic Diversity per IBD gridcell

Genetic Diversity
- 0.062
- 0.063 - 0.102
- 0.103 - 0.112
- 0.113 - 0.118
- 0.119 - 0.124
- 0.125 - 0.129
- 0.130 - 0.135
- 0.136 - 0.144
- 0.145 - 0.160
- 0.161 - 0.209

not standardized
Eurasia

Gentiana verna s.l.
Thalictrum alpinum
Pedicularis oederi
Gentiana verna s.l.
(Karin Moosbrugger, master thesis)
Gentiana verna s.l.
(Karin Moosbrugger, master thesis)

rarity
diversity
Gentiana verna s.l.
(Karin Moosbrugger, master thesis)
Pedicularis oederi & P. flammea

(Tribsch et al., ined.)
Pedicularis oederi & P. flammea

(Tribsch et al., ined.)

rarity

diversity
Thalictrum alpinum
(Tribsch et al., ined.)
Thalictrum alpinum
(Tribsch et al., ined.)
Realized refugia in Europe of general significance

“Northern” refugia common for:

- populations of alpine taxa
- alpine vascular plant assemblages
- boreal and/or temperate forests
- steppe communities?
Realized refugia in Asia of general significance

Arctic refugia common for:
- populations of alpine taxa
- alpine vascular plant assemblages (incomplete?)
- boreal and/or temperate forests (highly incomplete)
- steppe communities?
Conclusions 1

Theoretical concept of refugia in evolutionary research

The term “refugium” should be used in a defined way, discriminating between: potential refugia and realized refugia

It has to be defined for which group(s) of organisms a refugium (species or genes/alleles) is expected or real

Refugia accumulate rare alleles but not necessarily heterozygous populations, therefore genetic diversity s.str. is NOT a good predictor for refugial populations
Conclusions 2
significance of refugia in evolutionary research

There are molecular data that support a longterm (?) refugium in Northern Siberia and longterm refugia in Central Europe in the mountains

So far strong evidence for longterm refugia of evolutionary significance in areas north of the Alps and Carpathians are missing, e.g. in the periglacial areas of the ice sheets (exceptions may be for freshwater organisms, wetland species?)

The ’out of the European mountains’-colonisation of Northern Europe and the amphi-atlantic Arctic is a common phenomenon with many examples already published

Speciation on the diploid level appears to be restricted to refugia

Evolution of newly adapted genotypes/species (including hybrid speciation) may be more likely outside refugia, but only if refugia are reached through range expansion a successful speciation could happen
Open related questions

How fast can populations adapt and finally speciate during migration or in isolation?

Evolve species in refugia or do they just survive there?

Is refugial survival and limitation of geneflow really enough to explain phylogeographic and genetic diversity patterns?

Is allopatric differentiation in disjunct refugia at least sometimes associated with adaptive evolution of phylogroups/sister taxa or with reticulate evolution?

How is biodiversity in refugia affected by Global Change?
Many thanks to:
those, who have collected samples in remote places
those, who helped to reach remote places in Russia
Dorothee Ehrich (Oslo), Peter Schönswetter (Oslo & Vienna), and others from the NCB in Oslo for discussions especially about methodological issues
Virginia Mirré (Oslo) for help in the lab (AFLPs of *Pedicularis oederi*)
Karin Moosbrugger (Salzburg) for her enthusiasm in Gentians
IntraBioDiv floristic consortium for the excellent data
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