

Phylogeography in the *Viola rupestris* and *V. riviniana* complexes – a preliminary study Thomas Marcussen¹, Inger Nordal¹ and Bengt Jonsell²

Viola subsection *Rostratae* Kupffer consists of several critical species complexes in Europe. These include e.g. *V. canina* L. and the two species investigated here, *V. rupestris* F.W. Smith and *V. riviniana* Reichenb.

Viola rupestris is a paleo-tetraploid species ($2n = 20$) with a very wide Eurasian distribution from the British Isles, Scandinavia, and the Pyrenees in the west and eastwards almost to the Beringian. In North America it is replaced by the closely related *V. adunca* Sm. ($2n = 20$), sometimes considered a subspecies of *V. rupestris*, and by *V. labradorica* Schrank. ($2n = 20$) in the north-eastern States and Greenland. *Viola rupestris* belongs mainly in the Boreal zone, although reaching the sub- to low-alpine belts in the mountains and the subarctic zone in Siberia and the extreme north of Scandinavia (North Cape). Allozyme electrophoresis (AAT, AMP and PGM) suggests four different postglacial migration routes within Europe: (1) One western route, today represented by the British and Dutch enclaves, (2) one Central European route, extending from the Alps and into the southern parts of Fennoscandia, (3) one north-eastern route represented by populations from northern Finland, and (4) one isolated North Scandinavian enclave, usually referred to as ssp. *relicta* Jalas. It is possible that ssp. *relicta* has a connection westwards and, thus, that it has been recruited from transatlantic populations of *V. adunca* or *V. labradorica* (this is being checked).

Viola riviniana is an allo-octoploid species ($2n = 40$) restricted to Europe. It is very closely related to *V. reichenbachiana* ($2n = 20$) which it shares one $2n = 20$ genome. In contrast to *V. rupestris*, it is mainly a woodland species, but occasionally growing up to more than 2000 m in the Alps. Allozyme electrophoresis identifies four different entities within the *V. riviniana* complex: One (1) includes British populations only, probably representing a western immigration route (like *V. rupestris*); (2) the second group includes Central European populations. These are, apparently, substantially influenced by (unidirectional?) introgression from *V. reichenbachiana* ($2n = 20$) and may not have differed much originally from the third group, (3) represented by Fennoscandian populations, where *V. reichenbachiana* is not present. (4) The fourth group consists of populations from the Alps. It is very distinct from the others, enzymatically as well as morphologically, and should be referred to a separate species (*V. "laricicola"* sp. nov.). If *V. "laricicola"* proves tetraploid ($2n = 20$), it may be the second parent of *V. riviniana* (this is being checked).

Molecular biogeography and genetic diversity of *Pulsatilla vernalis* (L.) Miller (Ranunculaceae) – a new project Michal Ronikier

Pulsatilla vernalis is a distinct, endemic to Europe species having a very special geographic distribution (Meusel, Jäger & Weinert 1965). This particular pattern was even defined as «*Pulsatilla vernalis* type» (Pawlowski 1929). It is a high mountain species living in isolated areas of European mountains' alpine and subnival zones (mainly Alps, Pyrenees, western Carpathians). On the other hand however, this plant appears also in very different ecological conditions, in the lowlands of Central Europe and Southern Scandinavia (entering the boreal zone but not expanding farther to its northern part). On the basis of a morphological study, four varieties were distinguished (Aichele & Schwegler 1957), differentiating the populations from various parts of geographic range. Three of these varieties allow to observe some spatial distributive sequence, repeated in two isolated areas: Central Europe and Southern Scandinavia. Var. *alpina* is present in the mountains of both areas, while var. *vernalis* and *bidgostiana* in the lowlands. The fourth variety, var. *pyrenaica*, occupies isolated areas of Pyrenean chain.

The genetic diversity of populations is being studied using PCR-based AFLP method (Vos et al. 1995) generating molecular markers from the whole genomic DNA. The representative population samples for this study are collected in all

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Abb. 10: *Pulsatilla vernalis*

isolated parts of species distribution (Pyrenees, Central Massif, Alps, Rila Mts., Western Carpathians, northern Poland lowlands, Finland lowlands, Norway lowlands and mountains – Jotunheimen massif).

Basing on appropriate molecular AFLP markers, genetic variability of species will be estimated and a molecular variability pattern will be established. Research should permit a verification of several hypotheses concerning: (1) the primary centre of the species' origin; (2) probable routes of its migration and presumable history of the forming of its present range; (3) relations between single mountain parts of the distribution area; (4) history of the lowland part of the total range.

Circumpolar cpDNA phylogeography of the diploid *Cassiope tetragona* – a new project Anne-Cathrine Scheen¹, Ulf Molau², Inger Nordal³ and Christian Brochmann¹

The diploid, woody perennial *Cassiope tetragona* has a circumpolar distribution. The species is an important component of arctic vegetation and has been investigated in global change programs in connection with the International Tundra Experiment (ITEX), by experimental manipulations as well as by retrospective analyses. We presently initiate a study of molecular variation in *C. tetragona* on a circumpolar scale. Silica-dried leaf material has so far been collected from arctic Canada (extensive sampling made possible by participation in the Swedish Tundra Northwest 99 expedition), Greenland, Svalbard, Scandinavia, and one site in arctic Russia; additional field-collections as well as herbarium material will be used to cover the entire geographic distribution of the species. We plan to do (1) initial, complete sequencing of a series of non-coding cpDNA regions of a few field-collected plants from different geographic main regions, in order to identify small parts (<300 bp) with high level of variation and with sufficiently conserved flanking regions to allow for design of new internal primer pairs; and (2) full analysis of all material including partly degraded DNA from herbarium specimens, based on complete sequencing of small regions and/or fragment analysis to identify particular single- or few-nucleotide polymorphisms. Preliminary results will be presented.

Phylogeography of the high alpine cushion-plant *Androsace alpina* (Primulaceae) in the Alps Peter Schönswitter and Andreas Tribsch

Androsace alpina is a perennial cushion plant endemic to high alpine to nival habitats in siliceous mountain ranges of the European Alps where it ascends up to 4200 m. The plant can be found in major portions of the Alps, but although the distribution is rather continuous, *A. alpina* does not occur in postulated major refugial areas in the Southwestern Alps or the Eastern Alps. This distribution pattern might be interpreted as an indication for in situ survival on nunataks during the ice ages. To test this hypothesis, we investigated 259 individuals from 53 populations covering the entire distribution area of *A. alpina* using AFLP-fingerprinting. With three primer combinations used, 218 unambiguously scorable fragments were generated, 177 of which (81.2%) were polymorphic. The FST-value under the random-mating-hypothesis among all populations was 0.8 (95% C.I. 0.45–0.51), the Shannon-Index ranged from 4.26–4.50. Both a UPGMA clustering of the populations and a Principal Coordinate Analysis between pairs of individuals revealed a relatively high geographical structuring between groups of populations. An AMOVA conducted on those groups partitioned the overall variation into 57.7% variation within populations, 19.7% among populations and the comparatively high value of 22.6% among regions.

Five geographic regions were clearly separable: (1) the south-western-most part of the distribution area (Cottic and Grajic Alps), (2) the Western Alps from Mont Blanc to Splügenpass, (3) the southern part of the middle Alps from Splügenpass to the Dolomites, (4) the Eastern Alps ± east of Mt. Grossglockner and (5) one population in the Dolomites (Pordoi). The northern part of the



Abb. 11: *Androsace alpina*