

ACONITUM IN CENTRAL EUROPE: FROM LINNAEAN TAXONOMY TO MOLECULAR MARKERS

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Abstract. A role of the Linnaean taxonomy in the arising of historical-biogeographical hypotheses is envisioned. The first example concerns the presumed hybrid origin of an Eastern-Sudetic endemic *Aconitum plicatum* subsp. *sudeticum*. It was described on the basis of a unique character set including glandular hairiness of the indumentum. A PCR-RAPD+ISSR fingerprinting confirmed the supposition based on the morphological analysis. The second example is dealt with the marginal populations. They are of special interest because of the ecological and population genetic phenomena, including genetic drift and subsequent schizoendemism (endemoviciarism), a form of the peripatric speciation. *Aconitum bucovinense* occurs in two marginal, isolated populations in the Western Bieszczady Mts. (E Carpathians). They form a unique morphotype recognized by a taxonomic revision. A PCR-ISSR protocol was used to check a hypothesis on the genetic distinctness of the small, isolated populations. In the effect the lowering by 13% of genetic diversity in the marginal populations, in comparison to the core population, was noted. However, the genetic depauperation was accompanied by the existence of unique bands leading to the distinct genetic stocks in the marginal populations.

Key words: Carpathians, marginal population, peripatric speciation, reticulate evolution, schizoendemism, Sudetes

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Introduction

The genus *Aconitum* in Central Europe possesses some 10% of a total of 300-400 species (LIANGQIAN L. & KADOTA 2001; MITKA 2003), mostly known from central and eastern Asia (KADOTA 1987). In Europe its taxonomic division, in opposite to Asia, is clear. The subgenus *Lycotconum* is confined to a few species, however morphologically variable (WARNCKE 1964). In the Carpathians it is represented by three species and their hybrids (MITKA 2008). The most abundant subgen. *Aconitum* consists of two section: the diploid sect. *Cammarum* and the tetraploid sect. *Aconitum* (JOACHIMIAK *et al.* 1999, MITKA 2003, ILNICKI & MITKA 2009). *A. anthora* should be included into a separate subgen. *Anthora*, together with some other Asian species.

The aim of the present paper is to show, how the Linnaean taxonomy may aide to understand the evolution of the genus in a regional scale. The molecular DNA analyses may yield fruitful results while considered in the framework of the proper classification system, which forms a “working hypothesis” on the evolutionary relationships within a taxon (MITKA 2004).

Evolutionary links between the Sudetes and Carpathians

The two neighbor mountain systems belong to two

different orogenic systems. The Eastern Carpathians and Western Carpathians were formed at the Oligocene/Miocene boundary (26–22 myr BP), that the whole of the Carpathians were united some 14 myr BP (in the Middle Miocene), and that this event was accompanied by regression of the sea from the Alpine-Carpathian Foredeep. On the other hand, the Sudetes, a part of the Hercynides, were uplifted much earlier at the Cretaceous/Paleocene boundary during the Laramian tectonic phase some 65–60 myr BP. From that time to the present they have formed a stable land mass (see MITKA *et al.* 2007).

Taking into consideration the geological-historical context of the biota's evolution in the Central-European mountain ranges one may hypothesize that the Carpathian flora has its roots in the old, Sudetic flora (SYABRYAY 1995). A taxonomic revision of the genus *Aconitum* in Poland and adjacent countries enabled an endemic species to the Eastern Sudetes, i.e. *A. plicatum* subsp. *sudeticum* Mitka (2003), to be described. It has pilose indumentum that relates it to the Western Carpathian *A. firmum* subsp. *maninense* (Skalický) Starmühl. and *A. firmum* subsp. *moravicum* Skalický. Thus, a hypothesis was that *A. sudeticum* is a hybrid between the Sudetic *A. plicatum* and one of the Carpathian species from the *A. firmum*-group. To check the hypothesis the molecular and cytogenetic studies on the Western-Carpathian and Eastern-Sudetic *Aconitum* were carried out (MITKA *et al.* 2007). A PCR-ISSR+RAPD protocol and C-Giemza

heterochromatine staining showed the distinctness of the two species at the genome level and some similarities at the population genetic level. First of all, *A. plicatum* is an autotetraploid and *A. firmum* is an allopolyploid. The pattern of the heterochromatine bands show that *A. firmum* could be a hybrid between *A. plicatum* and a diploid species related to *A. variegatum*. The latter species could be an ancestral to the European *Aconitum* sect. *Cammarum* since it has the simplest in structure, homozygous the NOR-chromosome pairs (JOACHIMIAK *et al.* 1999). The phenetic UPGMA classification revealed the close relationships between *A. maninense* and *A. sudeticum* at the bootstrap value 58% (MITKA *et al.* 2007). Here, we used the same data to perform a population-genetic analysis with the use of a reticulate evolution algorithm. The result displayed in Fig. 1 confirms the shared genetic genomes of the Sudetic *A. sudeticum* and Carpathian *A. maninense*, which are nested within *A. plicatum* with a high bootstrap 82%. Both species are allopatric, narrow endemics. *A. sudeticum* occurs in the Hrubý Jeseník Mts. and Mt. Snežník (*locus classicus*), and *A. maninense* in the Stražovske vrchy Mts. (*locus classicus*) and the Tatra Mts. (MITKA 2003). Their geographical ranges could meet in the forelands during one of the Quaternary pleniglacials. At those periods the high-mountain species extended their areas. A secondary contact, probably occurred somewhere in the Moravian Gate, might result in the gene exchange between the Sudetic and Carpathian genetic stocks. In interglacial period the geographical ranges of the parental species and their hybrid were again restricted to the ancestral areas. However, the process was asymmetric, because the putative hybrid *A. sudeticum* occurs today only in the one, Sudetic, area. Another hypothesis is

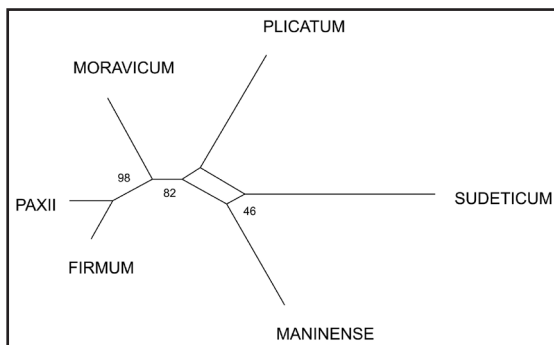


Fig. 1. Consensus Network (SplitsTree4, HUSON & BRYANT 2006) based on Reynolds's distances from 255 ISSR+RAPD bands. Bootstrap values (1000 permutations) are given.

Table 1. Genetic diversity of the two marginal and core populations of *Aconitum bucovinense* in the Carpathians. PPL95% – percentage of polymorphic loci, *h* – Nei's gene diversity, S.D. – standard deviation, *I* – Shannon's index of diversity (POPGENE ver. 1.32, YEH 1999); *DW* – rarity index (SCHÖNSWETTER & TRIBSCH 2005), *RSign* – statistical significance ($p \leq 0.05$) based on 1000 permutations (AFLPdat, EHRICH 2006). The analysis based on 258 ISSR bands (BOROŃ *et al.* 2011).

Population	Caryńska	Halicz	Core
n	23	45	21
PPL95%	50.0	70.20	67.84
<i>h</i>	0.0713	0.0745	0.0791
S.D. <i>h</i>	0.1139	0.1027	0.0833
<i>I</i>	0.1270	0.1395	0.1521
S.D. <i>I</i>	0.1737	0.1567	0.1384
<i>DW</i>	2.5739	2.4753	4.0193
<i>Rsign</i>	small	small	large

that both species: *A. maninense* and *A. sudeticum* are putative hybrids and represent opposite ends of the hybrid's morphospace. If so, the parental species could be *A. plicatum* and one of the forms of *A. firmum*, for example *A. f.* subsp. *moravicum*. To test the hypothesis more molecular DNA and cytogenetic studies on *Aconitum* in the Eastern Sudetes and Western Carpathians are needed.

Populations at the range margin

Aconitum bucovinense is a high-mountain species, Southern/Eastern Carpathian endemic. Previous phenetic studies on a whole tetraploid group of *Aconitum* in the Eastern Carpathian showed the distinctiveness of the species's populations at the range margin in the Western Bieszczady Mts. (MITKA 2002). The morphological traits specific to the region include: the type of indumentum hairiness (see Fig. 1 in MITKA 2000) and the shape of the spur. Recently, we checked a hypothesis on the peripatric speciation of *Aconitum bucovinense* in the Western Bieszczady Mts. (BOROŃ *et al.* 2011). Peripatric speciation is a form of a schizoneadism or endemoviarism. The latter terms denote continuous diversification of an ancestral taxon into derived taxa of identical chromosome number in various parts of the range. The process is dealt with founder effect and subsequent accidental elimination of genes (genetic bottleneck). In the effect the speciation process is accelerated by the isolation of relatively small, marginal populations. We checked the hypothesis

Table 2. Analysis of molecular variance (AMOVA) of populations of *Aconitum bucovinense* in the Carpathians (Arlequin software ver. 3.1, EXCOFFIER *et al.* 2006), based on 258 ISSR bands (BOROŃ *et al.* 2011).

Source of variation	Sum of squares	Variance components	Percent variation	P
Marginal vs. Core population				
Among populations	79.743	1.95674	10.34866	< 0.001
Within populations	1474.774	16.95142	89.65134	
Total	1554.517	18.90816		
Between Marginal populations				
Among populations	127.683	3.70486	19.91069	< 0.001
Within populations	983.567	14.90253	80.08931	
Total	1111.250	18.60739		

that the marginal populations are genetically uniform and deprived of the genetic diversity in comparison to the core area (BOROŃ *et al.* 2011).

The indices of population genetic diversity: percentage of polymorphic *PLP*95% and Nei's genetic diversity *h* have not differed among the core and marginal population, in spite of the Shannon's diversity index *I* that tends to be lower in the marginal populations, and rarity index *DW* that is statistically significantly greater in the core area, and statistically significantly lower in the marginal populations (Tab. 1). At the same time the among-population component of the total molecular variance is nearly twice as great in between the marginal population as between the marginal and core populations (Tab. 2).

In sum, the marginal populations differ each of other by the possessing of unique bands. However, Shannon's index of diversity is on average 13% lower in the marginal populations in comparison to the core area. However, the genetic depletion in the marginal populations is accompanied by rare bands occurrence. These population genetic phenomena are linked with special morphological characters, unique to the populations at the range margin. They need a special conservation program.

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