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Magisterská práce

Serpentine differentiation and polyploid evolution in *Knautia arvensis* agg. (Dipsacaceae)

Filip Kolář

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Serpentine differentiation and polyploid evolution in *Knautia arvensis* agg. (Dipsacaceae). [MSc. thesis]

Anotace:
Evolutionary history of an intricate polyploid complex *Knautia arvensis* agg. in Central Europe has been studied using combined approach of molecular (AFLP, DNA-sequencing) and cytological (flow cytometry, karyology) techniques. Possible evolutionary scenario has been suggested for the whole complex and for the serpentine population in particular, based on critical assessment of results obtained from the different methods.

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Introduction

The serpentine or ultramafic soils are usually easily recognized in the countryside as 'islands' of barren soil, rocks or sparse forests surrounded by more close vegetation. This is primarily caused by the specific chemical (low Ca/Mg ratio, high heavy metal content, low nutrient availability) and physical (drought, mechanical instability) properties of serpentine soils (Brady et al. 2005, Kazakou et al. 2008). Consequently, such unique combination of abiotic factors strongly influences the whole biota living there, primarily the plant life.

The peculiar flora of serpentine islands attracted botanists since the early times. The serpentines, although cover only about 1% of the world's surface (Proctor 1999) host an striking diversity of species and vegetation types. Many species living there represent elements unique either in the particular area or even worldwide (i. e. endemics). For example, more than 10% of the Californian endemic flora is restricted to serpentines, although the serpentine soils makes up less than 1% of the soil in this state (Kruckeberg 1984). Naturally, the botanists started to explore such diverse phenomenon. In contrast to the numerous descriptive studies treating species or vegetation diversity of serpentines, however, markedly fewer researches asked directly the most intriguing question – how this striking diversity have evolved?

Generally, the unique characteristics of serpentine soils can influence plant evolution in two ways (Kruckeberg 1986, 1991, Macnair & Gardner 1998):

- **Selection and sympatric speciation.** Peculiar serpentine conditions could act as a strong selective *agens* picking tolerant genotypes out of mainly non-tolerant colonizing gene pools. This disruptive-selection process often results in ecotypic differentiation (e. g. Kruckeberg 1951, 1967, Rajakaruna et al. 2003). Nevertheless, if reproductive barriers are achieved, the process could proceed to sympatric *in situ* formation of serpentine endemic (Kruckeberg 1986, Macnair & Gardner 1998, Rajakaruna 2004).

- **Isolation and allopatric differentiation.** The exclusion of many non-tolerant species from the serpentine sites makes from such localities, in fact, an islands of favourable conditions where many other species, competitively weak but tolerant, could thrive. When some strong environmental change appears the non-serpentine populations of such species can become locally extinct. The relicts surviving in island-like serpentine refugia then can allopatrically differentiate into one or several different taxa (Novák 1960, Mayer & Soltis 1994).

Kruckeberg (1986) compared the role of serpentine in plant evolution to an environmental 'trigger' which could launch almost every known evolutionary process. Interestingly, little attention has been paid to the concerted action of such serpentine 'trigger' and probably the most intriguing force in plant evolution – polyploidy. Polyploidy, as a common phenomenon plant kingdom, is especially well-known for its leading role in the plant sympatric speciation (Coyne & Orr 2004). Recent estimates suggest that probably all angiosperms underwent at least one round of genome duplication in their evolutionary history (Soltis et al. 2009). In fact, it has been suggested that at least 2–4% of all speciation events in angiosperms involve polyploidization (Otto and Whitton, 2000). In addition, polyploid taxa often exhibit a wider ecological plasticity (in comparison to their diploid relatives) what could have direct consequences in distinct edaphic (e. g. serpentine) differentiation within diploid-polyploid complexes (e. g. Ehrendorfer 1962).

In this thesis I have chosen *Knautia arvensis* agg. as a model taxon for elucidation some general patterns and processes of polyploid evolution at the serpentine localities. *Knautia arvensis* agg. is an intricate polyploid complex which has been already known for its distinct and promising pattern of ploidy-level and serpentine differentiation. It harbours two ploidy levels with more or less parapatric distribution with a contact zone running through Central Europe (Ehrendorfer 1962). In addition, a few diploid populations were discovered in areas otherwise occupied by tetraploids which inhabit relict habitats of serpentine outcrops and a subalpine glacial cirque (Štěpánek 1982, 1989). In one serpentine area in west Bohemia, however, a tetraploid cytotype has been discovered. This have lead to formulation a hypothesis on their independent *in situ* autopolyploid origin (Kaplan 1998), Collectively, the 'full factorial' diploid vs. tetraploid, serpentine vs. non-serpentine pattern in *Knautia arvensis* agg. appeared as a promising model for investigations of the combined role of polyploidy and serpentine in evolution of a particular plant group. Therefore a combined approach of molecular (AFLP, DNA-sequencing) and cytological (flow cytometry, karyology) techniques has been employed in order to disentangle the complex evolutionary history of Central European populations of *Knautia arvensis* agg.

The present thesis is based on the two following papers which are shortly summarized here and the full versions are appended below:

I. Kolář F, Štech M, Trávníček P, Rauchová J, Urfus T, Vít P, Kubešová M, Suda J. (2009): Towards resolving the *Knautia arvensis* agg. (Dipsacaceae) puzzle: primary and secondary contact zones and ploidy segregation at landscape and microgeographic scales. – *Annals of Botany* 103: 963–974

II. Serpentine differentiation and polyploid evolution in postglacial Central Europe: the story of Holocene-relic populations of *Knautia arvensis* agg. (Dipsacaceae). – *Manuscript*

I. – Cytotype differentiation and polyploid evolution in *Knautia arvensis* agg.

[*Towards resolving the* Knautia arvensis *agg. (Dipsacaceae) puzzle: primary and secondary contact zones and ploidy segregation at landscape and microgeographic scales*]

In this study, flow cytometry was employed to investigate the patterns and dynamics of ploidy variation in *Knautia arvensis* agg. The large scale flow-cytometric screening revealed the largely parapatric distribution of 2x and 4x cytotypes, with a diffuse secondary contact zone running along the NW margin of the Pannonian basin. Spatial segregation of the cytotypes was also observed on regional and microgeographic scales (i. e. even within the cytotype-mixed populations). In serpentine area in west Bohemia a sympatric growth of diploids and tetraploids has been detected, most probably representing the primary zone of cytotype contact. In addition as significant (ca 5%) difference in genome size between the non-serpentine (Pannonian) and relict (i. e. serpentine, subalpine and calcicolous) diploids served as a first evidence of different evolutionary histories of the two diploid groups.

II. – The role of serpentine in postglacial evolution of *Knautia arvensis* agg.

[*Serpentine differentiation and polyploid evolution in postglacial Central Europe: the story of Holocene-relic populations of* Knautia arvensis *agg. (Dipsacaceae*)]

In this study we aimed to describe the complex evolutionary history of *Knautia arvensis* agg. in Central Europe. Using amplified fragment length polymorphism (AFLP) fingerprinting and chloroplast DNA sequencing we have revealed a distinct genetic structure within the complex. While the non-serpentine (i. e. Pannonian) diploids clearly separated from the rest of the complex, the relict diploids and tetraploid appeared to be more closely related. Within the relict diploid group two distinct lineages (one serpentine and one calcicole) have been revealed. However, the serpentine diploids from West Bohemia remained to be intermingled within local tetraploids both in AFLP and cpDNA. This fact, together with thy cytotype distribution pattern and ecological observations suggested recurrent origin of serpentine tetraploids from local diploids. Moreover, the serpentine-typical genotypes have been found in tetraploid populations beyond the serpentine outcrops. This underlined the importance of polyploidization in creating novel and more vigorous plant genotypes and also pointed out the potential of serpentine refugia not only as s reservoir but also as a source of plant diversity for surrounding areas.

Conclusions

The both papers collectively demonstrate how the many-sided interactions among ecological differentiation and polyploid evolution produced the unique evolutionary pattern in *Knautia arvensis* agg. complex. The combined use of several molecular markers, flow-cytometric data and ecological observations revealed a complex evolutionary scenario suggesting an involvement of wide variety of evolutionary processes and mechanisms like fragmentation into Holocene refugia, repeated colonization of the area by several lineages, hybridization, allopatric speciation, and recurrent polyploidization. The key role of serpentine substrate in this scenario arises from its ability to serve as a refugium for many competitively weak taxa (as the diploid *Knautia arvensis*) which could further evolve into distinct types. The recurrent polyploidization recorded in one serpentine area followed by spread of local genotypes beyond the border of their former refugium further underlines the importance of the diversity hosted by serpentine localities.

Knautia arvensis agg. represents an ideal model complex where both polyploidy and serpentine differentiation have met to produce a fascinating evolutionary story which could tell us something about patterns and processes involved in the creation and maintenance of plant diversity. Considering the presence of both primary and secondary contact zones and ploidy-specific reproductive barriers, the *Knautia arvensis* complex also provides a unique model system for studying the evolutionary dynamics of populations exhibiting ploidy heterogeneity, and for examining ecological and genetic circumstances that govern the interactions between different cytotypes in mixed populations.

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