Review of the PhD thesis
by Pavel Trávníček: Microevolutionary processes and inter-cytotype interactions in mixed-ploidy populations

The presented thesis addresses the phenomenon of sympatric occurrence of multiple cytotypes within species. It explores patterns of spatial distribution of different cytotypes at various scales in selected study species, and aims to reveal the mechanisms of cytotype coexistence and maintenance, as well as their evolutionary implications. The thesis deals with a topic highly relevant in the field of plant evolution, since it addresses processes and patterns present in natural populations at early stage of polyploid evolution. This area has remained largely unexplored since recently; it was the advent of flow cytometric techniques that allowed rapid, non-destructive and really extensive ploidy level screening and, thus, accelerated this kind of research. Several assumptions and theories regarding the cytotype co-occurrence and interactions were postulated in the past, and it is interesting to see how they are experimentally tested nowadays.

The thesis is composed of a few introductory chapters and six scientific papers, all published in highly ranked journals (Annals of Botany, American Journal of Botany, Journal of Biogeography, Preslia, Journal of Plant Research). The presented papers are remarkable for their well-elaborated experimental design, thorough data analyses, interpretations and discussion. Since the papers went through strict review processes in the journals, they are all well-written, yielding strong, convincing results, without any weaknesses. The introductory chapters of the thesis bring 1) an overview of the current stage of polyploid research, mainly of the aspects related to the topic of the thesis; 2) brief information on the studied plant groups; and 3) a summary and perspectives for future studies. These chapters are very well focused and concise, highlighting the most relevant aspects of the studied topic. What I slightly miss is a chapter on methodology; although the methodical approach is explained in each of the presented papers, due to the space limit in the scientific papers, some more details may have been presented in the introductory chapters. I do not mean explaining or repeating some fundamental, textbook knowledge on flow cytometry, but perhaps going into more details in some specific statistical evaluation, e.g., spatial analyses.

The thesis brings several highly significant results, expanding our knowledge on cytotype sympatric occurrence and polyploid evolution, and also points to the directions of future research. What I especially liked about the thesis was its comparative aspect; it addressed similar research questions in five different polyploid species/complexes with medium to high cytotype variation, which resulted in discovering rather diverse, in some cases even contrasting, patterns, indicating that no simple generalizations can be made on the cytotype co-existence. It was illustrated that we need to accept a high diversity and complexity of the mechanisms and processes acting in natural mixed-ploidy populations. Interestingly, also some unusual, unexpected patterns contradicting traditional assumptions have been revealed. For example, it was indicated that the sympatric occurrence of different cytotypes within a population may not be a rare phenomenon, but is much more frequent than envisaged. No reproduction barriers and free mating among the cytotypes of *Pilosella echioides* was an astonishing finding, suggesting the presence

of a well-balanced system maintaining this cytotype diversity, in discordance with the minority cytotype exclusion theory. The high frequency of triploids in this species is another unique phenomenon, which may call for the re-evaluation of the role of triploids in some mixed-ploidy populations. On the other hand, spatial and ecological segregation of the cytotypes has been documented in *Senecio carniolicus*, known as one of the usual ways to avoid the minority cytotype disadvantage. The study on *Senecio carniolicus*, however, also illustrated that the diploids have broader ecological amplitude than the hexaploids, which is in contrary to the commonly observed patterns. All these findings point to the immense diversity and dynamics of mixed-ploidy species and populations.

I have a few questions for the candidate that may stimulate discussion on the observed findings.

1) The discovery of high frequency of triploids in *Pilosella echioides* is very interesting, considering the commonly observed patterns that triploids (or odd polyploids) in sexually reproducing groups are rather rare. I just wonder, is anything known about the meiosis in these triploids, about the chromosome pairing and segregation (or is such a study in progress)? It was found that these triploids display large cytotype seed variation, so I assume that they might produce viable and fully functional gametes of different chromosomal constitutions. I recall the cases of odd-polyploid *Rosa* sect. *Caninae* or *Onosma arenaria*, which are characterized by the so called stabilized asymmetric meiosis giving rise to the female and male gametes of different chromosomal constitution, and their reproduction has been called as hemisexual. It would be interesting to find out if there is something similar going on in the triploids of *P. echioides*.

2) In the studied *Gymnadenia conopsea* complex, some populations were found karyologically uniform, but other were highly variable with the co-occurrence of several cytotypes. Is this just a random pattern or could the cytotype diversity in some populations be stimulated by some ecological factors (e.g., environmental heterogeneity, pollinator diversity, habitat disturbance, etc.) favouring the origin and maintenance of new, minority cytotypes? Or are there any other hypotheses? In the case of *Pilosella echioides* it was tentatively suggested that the area with high cytotype co-existence might be explained by a primary hybrid zone or long-distance dispersal of polyploids, or by hybridization with other species. Could something similar be expected in *Gymnadenia*?

3) In the study on *Lythrum salicaria*, the cytotype variation was examined from seeds collected in 1998-2003. I am wondering, what was the germination rate of the seeds, especially those older ones? Can we exclude that this (i.e., different germination rates) has not biased the observed cytotype patterns? Also, the cytotype variability of seeds might not truly represent the variability present in adult populations. There are some examples (e.g., *Pilosella alpicola, Arabidopsis arenosa* agg.) that show that seed cytotype variation is quite different from what is present in the population of adult individuals. Can any estimates be done in respect of the relationship between seed/seedling and adult ploidy variation in this species?

4) The finding of diploid *Senecio carniolicus* exhibiting larger ecological amplitude when compared with the hexaploids is intriguing. Polyploids, due to the higher

genetic diversity, higher frequency of heterozygosity, and other genetic and epigenetic processes following polyploidization, are expected to have a broader ecological amplitude, or exhibit a habitat shift. In the studied *Senecio* species, just an opposite pattern was revealed. If one considers that these hexaploids are autopolyploids derived from diploids of the same species, this in fact means a narrowing of ecological amplitude. But if the hexaploids were of an allopolyploid origin, it could be probably easier to explain this finding. Is there any possibility that they are allopolyploids, or are there any studies (in progress or planned) that could prove their origin? Or are there any other hypotheses to explain the observed finding?

In conclusion, I am absolutely convinced that the presented goals of the thesis have been accomplished, the thesis brings valuable and significant results, and it also illustrates the ability of the candidate to conduct high-quality research studies. At this point I also wish to stress that the candidate's list of publications is impressive, as are also many other research activities and achievements. It is my pleasure to give here my recommendation for awarding the scientific-academic degree "philosophiae doctor".

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