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

Using flow cytometry, often complemented with chromosome counts, P. Trávníček estimated ploidy level of high number of individuals belonging to several populations of different, mostly Central European taxa, to explore spatial distribution of different cytotypes and to answer the question, whether multiple cytotypes can coexist in natural populations. In comprehensive introductory chapters, the author reviews our present knowledge about importance of polyploidy for plant evolution as well as about theoretical principles and a few published practical examples of co-existence of multiple cytotypes and their interactions in nature, and as a final point presents the five taxa under investigation. Apart from the introduction, the thesis is composed of six scientific papers (in three of them P. Trávníček being the first author), published in renowned international journals. It has to be stressed that not only the number of articles included in the thesis is comparatively high, but also the fact that all have already been published illustrates the quality of this thesis.

The importance of polyploidy in plant evolution has long been acknowledged and the theoretical backgrounds of different modes of polyploid formation have been tested experimentally or in natural systems. Polyploidisation has not only been important in the formation of new evolutionary lineages (e.g., species), but it has also been recognised that different ploidy levels can occur within species. However, only recently, after the introduction and expansion of flow cytometry in plant population research, several studies have demonstrated that there are many more species including multiple ploidy-levels than ever thought before. It has been shown that in several taxa different cytotypes are geographically excluded, but it has also been proven that different cytotypes can occur in sympatry within the same populations, given that different mechanisms (e.g., related to different modes of reproduction, as well as ecological differentiation) allow their co-existence. However, the number of studies addressing the latter phenomenon is limited, and I am very happy to see that this gap in our knowledge is starting to be filled. For this reason and because of the overall quality of the presented research (see below) the results obtained by P. Trávníček and co-workers and presented in his thesis can only be assessed as highly relevant.

The methods used comprise extensive field work in order to obtain the plant material (up to 6554 individuals sampled in 257 populations in the case of *Vicia cracca*!), DAPI flow cytometry of high number of samples, chromosome counts, as well as statistical analyses of cytometrically

obtained data coupled with fine-scale distribution patterns of co-occurring cytotypes. Especially the number of samples obtained and processed can be regarded as exceptional.

None of the results obtained by P. Trávníček and co-authors is particularly "ground-breaking", but all presented studies are well-done and yield strong, convincing results. Whereas in *Gymnadenia conopsea* complex (Paper I), *Pilosella echioides* (Paper II) and *Senecio carniolicus* (Papers IV and V) extensive co-occurrence of different cytotypes has been observed in different parts of the distribution area, the populations of *Vicia cracca* (Paper III) predominately consist of either diploid or tetraploid individuals, the exceptions being the populations in the secondary contact zone between the north-western tetraploid and south-eastern diploid populations. Similarly, most populations of *Lythrum salicaria* (Paper VI) are cytologically uniform, exceptions being the populations in Israel and Turkey (and one in Hungary). In the latter study the authors have shown that highly invasive populations in North America are all of tetraploid origin and that cytotype differentiation is not a cause for invasive character of this species in its secondary distribution range.

With the exception of *Senecio carniolicus*, where a small-scale geographical study (Paper V) has shown that the diploids and hexaploids in mixed populations are altitudinally and ecologically segregated, it would be interesting to know whether similar (e.g., ecological) differentiation can be observed also among different cytotypes in mixed populations of *G. conopsea* and *P. echioides*. Such a study would greatly add to understanding the obtained results. In author's opinion, what are the likely mechanisms making such coexistance in the two study systems possible?

Another aspect, which would make the results of the study of *V. cracca* (Paper III) even more interesting, would be to compare whether the cytotype differentiation is also correlated with the morphological differentiation, possibly enabling a taxonomic separation of both cytotypes (it is also not clear, whether herbarium voucher material exists for this, as well as for the other studies, and where the vouchers are deposited!). Is there any evidence of clear morphological differentiation between both cytotypes? Personally (not being very familiar with the biogeography of the study area), I would also be interested to get to know possible explanations for such a striking geographic delimitation of both cytotypes along the border of Czech Republic and Slovakia (a similar pattern is familiar to me from the study about *Knautia arvensis* by Kolar et al.).

I find the thesis well written and conclusions justified and supported by the results. There is however one statement, for which I have found no justification in the cited paper, nor in the study by Houghton-Thompson et al. (2005), namely, that the invasiveness of *L. salicaria* is triggered "by crossing of different genotypes originated from multiple introduction events (Kubátová et al. 2008)" (page 25). What is the author's explanation for such a conclusion?

However, for the reasons outlined above, and also taking into consideration the impressive publication list of P. Trávníček, I am absolutely convinced that the candidate meets the goals of the thesis, and it is therefore a pleasure to recommend that he should be awarded the scientific-academic degree "philosophiae doctor" after successful defence of the thesis.

Yours sincerely,

(Dr. Božo Frajman)