Review of the PhD thesis submitted by Václav Mahelka: Natural hybridization between two allopolyploid wheatgrasses *Elytrigia intermedia* and *E. repens* (Poaceae, Triticeae)

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I. Generally comments

Natural interspecific hybridization is recognized as the major genetic interaction among plant species. It can increase the variety of genetic combinations available to selection and affect subsequent interactions between hybrids and related taxa. In 1916 Dutch botanist Jan Paulus Lotsy was first who suggested interspecific hybridization as a major speciation factor; his position was consequently adopted by many evolutionary biologists and plant biosystematists.

Understanding the causes and consequences of interspecific hybridization under natural conditions can play an important role in understanding the impacts of artificial interspecific hybridization on economically important cultivated plants. It also aids in better predicting the potential risk of hybrid invasiveness and in preventing the genetic disintegration of rare native species through introgressive hybridization. Finally, this knowledge can also be applied to modeling the potential danger of hybridization between genetically modified plants and their native relatives, as is discussed in detail by Václav Mahelka in introduction of his thesis.

The thesis consists of three original papers and a brief general introduction, where objectives, material and methods, and summary of results are presented. The candidate is the first or alone author of all three papers, which are either already published or accepted in international journals *Folia Geobotanica*, *Weed Research*, and *Annals of Botany*. The papers include the wide scale of methods: flow-cytometry, chromosome counting, PCR, sequencing, PCR-RFLP, plant cultivation experiments, seed fertility estimation, etc.

The first paper "**Genome size discriminates between closely related taxa *Elytrigia repens* and *E. intermedia* (Poaceae: Triticeae) and their hybrid"** (Mahelka V., Suda J., Jarolímová V., Trávníček P. & Krahulec F. in *Folia Geobotanica*, 40: 367–384, 2005) is based on flow cytometry species, hybrids and various ploidy levels identification. Paper is well presented and accompanied by instructive figures making the results and hypotheses more understandable. I especially appreciate excellent accuracy and reproducibility of flow cytometry measurements completed by classical chromosome counting.

The second paper "**Recent natural hybridization between two allopolyploid wheatgrasses (*Elytrigia*, Poaceae): ecological and evolutionary implications"** (Mahelka V., Fehrer J., Krahulec F. & Jarolímová V., presented as in preparation, but currently accepted in journal *Annals of Botany*). Besides of flow cytometry measurement, hybrid origin was determined by together presence of parental nuclear DNA sequence markers (ITS) in this paper. Maternal species is there identified using chloroplast DNA sequence markers (*trn*L intron, *trn*L-F intergenic spacer, *trn*T-L intergenic spacer and *rpl*20-*rps*12 intergenic spacer). For the sequence identification, the sequencing or PCR-RFLP were used in large representative scale of samples collected along transects across the various habitats. Different frequency of hybrids was detected in contrasting habitats. The origin of nonaploids was explained in this paper. Seed fertility of hybrid and nonaploid progenies was estimated. New ploidy and aneuploidy levels were detected during these detail analyses. I consider this paper to be an excellent example study of natural hybridization and subsequent processes.

The third paper "**Response to flooding intensity in *Elytrigia repens*, *E. intermedia* (Poaceae: Triticeae) and their hybrid"** (Mahelka V. in *Weed Research* 46: 82–90. 48, 2006). This chapter seems well thought out, and could be a significant contribution to the investigation of aspects of hybrid ecology, i. e., its fitness in flooded heavy soils. The intermediate tolerance to flooding was detected in hybrid compared with its parents.

II. Questions and comments

1. Potential gene flow between GMO *Triticum* and world wide weed *Elytrigia repens* is mentioned in the introduction as a crucial practical consequence of thesis (p. 2).

Which other crops (potentially genetically modified) besides of wheat should be considered to be risk in relation to hybridization with species of *Elytrigia*?

2. Only comment: **In discussion of the first paper you mentioned the difference between your estimation of *Elytrigia repens* genome size and data presented in Kew genome size database. In our laboratory this species was also measured by Petr Šmarda; average 2C DNA content 23.8 pg was much more similar to your 23.3 pg.**

3. The part of samples you were measured with both fluorochromes. Sample/standard ratios with DAPI differ from those with PI. This is considered to be a consequence of different AT/GC genomic ratio in *Elytrigia repens* and *E. intermedia* (cf. p. 20). *Triticum aestivum* was used as an internal standard for both types of flow-cytometric analyses (p. 18). Artificial hybridization *Triticum aestivum* with *Elytrigia intermedia* is more successful than those with *E. repens* (e. g., p. 2).
If the genomic base composition is different in *Elytrigia* species studied, which of them is more similar to *Triticum aestivum*?

4. In the most of hybrids studied by yourself, the maternal parent was *Elytrigia intermedia*. Various explanations of this asymmetry present itself: one of them could be a phenological shift in combination with (e. g.) proterogyny i. e. early blossoming *E. repens*; another explanation could be the stochastic model based on the contrast in species pollen concentration in the atmosphere which reflects the contrast in occurrence of these wind-pollinated species in the nature.
Which of these (or another) hypotheses do you prefer and why?

5. The seed fertility was determined in the second paper. There no staining method was used for estimation of pollen viability. Similarly the frequency of reduced/unreduced pollen (based on microscopic measurement of pollen size) has not been studied.
Is there any problem with pollen in *Elytrigia*?

6. While DNA content of F1 hybrids seems to be in the mid between the parental DNA contents (p. 19, 20), the DNA content of nonaploid is less than 1.5 fold in comparison to hexaploid (p. 21), in spite of the fact that both hybridization and reduce/unreduce gamete fusion are very probably actual. The excellent was your subsequent molecular corroboration

of the hybrid origin of the most of nonaploid plants in your second paper. It could well explain the lower DNA content. As the most probable scenario of hybrid origin is presented fusion of unreduced gamete of *Elytrigia repens* with reduced gamete of *E. intermedia*.
Do you think that there could be any relation between this scenario and the mother role of *E. intermedia* in vast majority of hybrids?

III. Conclusion

Thesis of Václav Mahelka constitutes on my opinion a very valuable addition to clarify the role of various actual processes in the course of natural hybridization between *Elytrigia intermedia* and *E. repens*. The candidate has demonstrated very good skills in a solid molecular and karyological methods, sampling strategy, results presentation. I recommend warmly to accept this thesis for the doctor degree after its successful defence.

doc. RNDr. Petr Bureš, Ph.D. Brno, March 3, 2007