LEIBNIZ-INSTITUT FÜR PFLANZENGENETIK UND
KULTURPFLANZENFORSCHUNG (IPK)

Stiftung des öffentlichen Rechts

Internet: http://www.ipk-gatersleben.de

F.R. Blattner • IPK • Corrensstraße 3 • D-06466 Gatersleben, Germany

Prof. Karol Marhold
Faculty of Sciences
Charles University Prague
Albertov 6
CZ-12843 Prague 2

Dr. Frank R. Blattner

Taxonomy & Evol. Biology

Phone: +49 (0)39482 5202
Telefax: +49 (0)39482 5155
E-mail: blattner@ipk-gatersleben.de

Review of the PhD thesis titled

"Natural hybridization between two alloplolyploid wheatgrasses *Elytrigia intermedia* and *E. repens* (Poaceae, Triticeae)"

presented by Ing. Václav Mahelka in March 2007 at the Charles University, Prague

The PhD thesis of Ing. Václav Mahelka consists of three scientific articles dealing with different aspects of hybridization between two naturally occurring grasses of Middle Europe. The two species of *Elytrigia* overlap partly in their distribution area and come in many areas into direct contact to each other, as *E. repens* is a widespread weed in man-influenced habitats. In parts of the Czech Republic Mr. Mahelka found plants with a phenotype intermediate between both species. In the first two papers presented in his thesis, he proofed that these plants are of hybrid origin. To my knowledge this might be the first "hard" proof of hybridization between these species, although the intermediate phenotypes were long thought to be hybrids. The third paper deals with an ecological parameter discerning the species, namely tolerance against flooding.

In the first publication of the PhD thesis, titled "Genome size discriminates between closely related taxa . . . and their hybrid," Mr. Mahelka could convincingly show that the genome sizes of the parental species provide a good tool for species identification, as the species do not overlap in their 2C values (at least for the populations analyzed). *Elytrigia intermedia* possesses nearly 4 pg more DNA per nucleus in comparison to *E. repens*. Thus, genome size can also characterize hybrids by their intermediate DNA content, and likewise putative backcrosses with one of the parental species, which show a bias in genome size towards the size of their crossing partners. The hybrid theory postulated from the genome size data of paper one was corroborated with several experiments with tools from molecular phylogenetics. In his second paper with the title "Recent natural hybridization between two allopolyploid wheatgrasses . . ." Mr. Mahelka showed that plants with intermediate genome sizes were indeed hybrids, as they possess rDNA from both parental

species. The direction of the crosses, i.e. who was the maternal or the paternal parent of the hybrids, could be clarified by using maternally inherited chloroplast DNA. Some nicely designed experiments, involving PCR amplification of mixtures of parental DNA in different ratios allowed also to show, that backcrossing with one of the parental species "dilutes" the amount of rDNA from the other species in the hybrids. Thus, Mr. Mahelka could unambiguously determine both species, their hybrids, and also backcross individuals. Moreover, the putative origin of newly found nonaploid plants could be clarified with the tools described in the first two publications. In the third article Mr. Mahelka describes the effect of flooding on both *Elytrigia* species. He found that the species are differently tolerant, i.e. that *E. repens* survives even 30 days of submersion relatively well, while *E. intermedia* suffers much more from this treatment. Hybrids behave more like the latter species. I see this last paper as a first step towards understanding the ecological needs of both species and therefore to explain their occurrence in time and space.

The PhD thesis is clearly written, the experiments, as well as the interpretation of the data are very good and show no major flaws, and the results contribute not only to the knowledge of both *Elytrigia* species but also to our understanding of how frequently hybridization between theses grasses occurs, and generally how to analyze a complex hybridization zone between two species. This makes the PhD thesis of Mr. Mahelka a valuable scientific document. The logic of the experiments and conclusions drawn from them made it a pleasure to read.

Questions to the defendant

1) Ernst Mayr invented geneflow vs. reproductive isolation as the crucial parameters in his biological species concept to discern different species. Both *Elytrigia* species cross easily in nature if they come into contact, as was nicely demonstrated in this PhD thesis. Can they, thus, be maintained as two separate species?
2) In Triticeae wide crosses seem more the rule than the exception, as many taxa combine "genomes" from quite diverse and taxonomically distant diploid genera. Is a universal and reliable species concept recognizable that works in grasses and particularly in Triticeae?
3) Both *Elytrigia* species cross easily in nature. Are these taxa closest relatives (sister taxa) within the genus, and is it possible to get a reliable phylogeny of these alloploid taxa?
4) In the discussion of the contribution of hexa- and nonaploid cytotypes to hybrids (p. 41) Mr. Mahelka states that "hybridization between different cytotypes . . . can serve as raw material for evolution" (p. 42). How can hybridization between different cytotypes, all combining more or less the same genetic material as homoploid (6x) hybrids, result in new genotypes and afterwards phenotypes?
5) The two *Elytrigia* species under study comprise all in all three different Triticeae genomes (St, H, and two slightly different E genomes). Therefore, three (four) different ITS types can be expected to occur in the species and their hybrids. What is the reason for the relative high homogeneity of the ITS copies found in the plants of study two?

Gatersleben, March 06, 2007 Dr. Frank Blattner