A review of Ph.D. Theses Evolution of *Vicia cracca* L. – distribution of cytotypes, their genetic variation and growth traits by Anežka Eliášová

Dr. Radim J. Vašut, Department of Botany, Palacký University in Olomouc

The Thesis under review deals with the microevolution in polyploid complex, which is general phenomenon in plant evolution. On example polyploid complex (2x – 4x) of *Vicia cracca* the student address the questions how did polyploids arise and become established. The species complex represents one of few examples of polyploids in *Fabaceae* in Central Europe. Author studied the origin of the polyploids genetic variability and studied differences in germination and vegetative growth providing new insights on distribution patterns of cytotypes of *Vicia cracca*. The Thesis is structured into introduction, 6 main sections consisting of 5 published journal papers and 1 manuscript.

The introduction provides well-written overview on the studied topic, motivation for study and background for the main chapters. Six main chapters are corresponding to published papers in the +- best journals in the field.

Chapter 1 provides very detailed view (6554 individuals from 257 populations) on distribution of cytotypes in Central Europe, finding different distribution patterns of cytotypes on larger geographic scale. Interesting finding was the lack of triploid hybrids in the contact zone of cytotypes, finding the continuous distribution of cytotypes across the altitudinal gradient. Author suggests (based on the monoploid genome size) the autopolyploid origin of tetraploids.

Chapter 2 provides insights on population genetic diversity and mating systems in natural populations on the contact zone of diploids and supposed autotetraploids using 4 enzyme systems supported by pollination experiments and observations of meiosis. Tetraploids were found having more alleles than diploids, but not more heterozygous than diploids. The breeding system is mixed-mating with prevailing outcrossing.

Chapter 3 author evaluates the relation between seed weight and seed germination and seedling growth. Study confirmed higher seed weight and higher germination rate in tetraploids.

In chapter 4 author investigated the differences in vegetative characteristics on different water regimes of sympatrically occurring cytotypes showing no differences among cytotypes. Diploids show greater tolerance to drought.

Chapter 5 ecological plasticity of tetraploids was studied, comparing invasive North-American populations with natural European populations suggesting polyphyletic origin of NA colonizers.

Final chapter reveals more detailed the distribution patterns of cytotypes in Europe employing molecular markers. Additional contact zones were found, polyphyletic origin of tetraploids was confirmed.

Remarks and questions

p. 38 (chapter 1). The autopolyploid origin of tetraploids is briefly discussed. It is just a very minor paragraph of the whole paper/chapter. However, other work is based on these findings/conclusions. I consider it speculative. Several evolutionary young allopolyploids show same (or similar) monoploid genome size (best examples are some apomicts).
My questions are:

a) Why the author consider the same monoploid genome size as evidence for the autopolyploid origin?
b) Which other evolutionary scenario(s) can be drawn based on these findings?

p. 74 (chapter 2). Although isozymes are considered reliable genetic markers, it is also known for being influenced by several factors (including "lab factors" or biological factors). Information on sampling says that samples were collected at natural sites, but without further details.
My questions are:

a) To what extent can be the reader sure that the excellently presented population-genetic results are not biased by the technique. I mean to what extent was the sampling among localities same? Is it possible that results are influenced by collecting material in different phenological phase, contrasting ecological habitats, or by other factors (like stress from herbivores etc.).
b) Can be same/similar overall picture drawn from microsatellite data published in Chapter 6? (as it is partly hinted in discussion of chpt. 6, p. 219).

p. 215 (chapter 6) I suggest to use Figure with all haplotypes (including *V. dalmatica* and *V. tenuifolia*) in paper in order to provide the reader better overview on the relationship within the studied group.

p. 216 and p. 224 (chapter 6) Private alleles of *V. dalmatica* and tetraploid *V. cracca*. Author says "The presence of alleles found in *V. dalmatica* and several *V. cracca* tetraploid lineages but not in diploids also might suggest introgressive hybridisation between *V. cracca* and *V. dalmatica*".
Based on published results, I would be suggesting the same. However, do the same alleles among taxa really reflect the past introgression? Can author (at least putatively) exclude a bias due to homoplasy of markers?

I miss in the Thesis some concluding remarks at the end, just summarizing obtained results across studies. While reading the thesis, repeatedly in my mind appeared questions on how are the results from each papers related to new results from other papers/chapters (which I haven't stressed in the review but keep it in mind for the further discussion, if there is space for it). But I respect that it probably not the standard at the University.

Conclusion

Generally, the Ph.D. Thesis is characterised by scientific originality, great author's effort employing various scientific and statistical techniques and experiments, and finally excellent presentation of results (in well-written papers). The study significantly contributes to the field of polyploid evolution research. It shows that the author has great ability for doing the scientific research. This is all confirmed by 5 published papers in highly respected journals in the field. I therefore recommend the thesis for the defence.

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RNDr. Radim J. Vašut, Ph.D.