Written assessment

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Thesis title: Evolution of *Vicia cracca* L. – distribution of cytotypes, their genetic variation and growth traits

Overall assessment

In this thesis, Eliasova studies polyploidy in plants using a diploid-tetraploid system, *Vicia cracca*, which is native to Europe and has been introduced in North America. The main conceptual questions addressed in this thesis are related to the evolutionary origin of polyploid cytotypes, the consequence of genome duplication on the phenotype, and the effect of different ecological conditions on the relative performance of different ploidy levels. The thesis is composed of six data chapters, five of which have already been published in the last eight years. The final data chapter is to be submitted to an international journal. The different chapters reflect the conceptual and methodological progression of studying this system. In the first data chapter, the authors conduct a remarkable descriptive study of the incidence of cytotypes across the native range. The scale and detail of this study is to be commended. In the second chapter, the authors use allozymes to describe genetic diversity and venture hypotheses about the origin of these polyploids. Although technically superseded by other types of markers, including those used in Chapter 6, this chapter represented a step forward in the understanding of the system. Of particular relevance is the use of floral pollinations to evaluate seed set across ploidy levels. One aspect that limits the interpretation of these results is that no cross-pollinations were attempted, and thus, it is not possible to estimate inbreeding depression. In Chapter 3, the candidate and co-authors investigate the potential fitness consequences of the gigas effect of polyploidy on seed size. I enjoyed this chapter, as we still have limited insights on what are the functional consequences of genome doubling, and more studies of this kind are needed before seeking generalisations. The biggest effect of polyploidy seems to be on the maximum height of seedlings. It would have been good to further connect this trait to competitive ability measured directly, for example in competition experiments. A phenomenon that emerges in this chapter, and which is also seen later on, is that within ploidy, there is significant variation between populations. In Chapter 4, the candidate and colleagues conduct a common garden experiment to compare the phenotype of different ploidy levels under water stress. I would have liked to see more expliciutly how this chapter complements the previous one, particularly as some findings seem contradictory (seedling height differences). A highlight of this chapter is the measurement of seed number and above ground biomass. Again, a point is made about differneces among populations within cytotypes. In Chapter 5, the candidate and colleagues conducted an experiment to compare native and introduced cytotypes in a common garden exposed to different shade environments. Differences in phenotype and performance were observed in tetraploids depending on their origin (native or introduced). The authors speculate whether this may be explained by post-introduction evolution, although further direct evidence is still required. Issus to consider include maternal effects and the fact that the introduced tetraploids may have a different

origin than those of sampled tetraploid, native populations. Finally, in Chapter 6, Eliasova leads a study of the genetic relationships between *V. cracca* and related species using chloroplast and microsat markers. I enjoyed this chapter, which allows revisiting some exciting questions raised by the allozyme study in Chapter 2. In the final chapter, Eliasova presents evidence that cytotypes are little genetically differentiated, and that the genetic composition of tetraploids is consistent with autopolyploidy. Interestingly, they find little evidence for gene flow in sympatric populations, and some evidence of interspecific gene flow from *V. dalmatica*. The findings on the number of genetic origins remain uncertain although chloroplast patterns suggest multiple origins. Further discussion of how incomplete lineage sorting may affect this interpretations would be valuable.

Overall, this is a well-written, detailed thesis that builds on previous cytological work and establishes *Vicia cracca* as an excellent model for the study of autopolyploidisation. I particularly commend the effort in linking polyploidy with phenotypic effects with ecologically relevant consequences. Studies such as this one, are necessary to connect genome duplication to its ecological consequences, and thus underpin a comprehensive understanding of the evolutionary dynamics of genome duplication.

Questions to the candidate

1) In Chapter 2, you conducted self-pollinations to look at the effect of selfing on inbreeding. Discuss how would you redesign this study to allow you to directly measure inbreeding depression, capacity for autogamy, and the mating system of the different cytotypes.
2) Early in the thesis you rule out allopolyploidy. However, in the final chapter you detect introgression from V. dalmatica. Discuss further what would you expect if the origin of tetraploid V. cracca is actually allopolyploid, followed by extensive backcrossing to diploid V. cracca.
3) I was interested in your AMOVA test for single vs multiple origins. Can you explain the rationale as well as the expectations under the null model?
4) How did you determine allele dosage in the allozyme study?
5) The lack of multivalents in an allopolyploid may seem puzzling. Discuss in more detail why multivalent formation is not observed. Design an experiment to test whether autopolyploidy would necessarily result in multivalent formation in the first generation autopolyploid.
6) The results of Chapter 3 and 4 may seem contradictory in regard to seedling size. Is there a contradiction indeed, and how do you explain it?
7) What may be the role of maternal effects in helping you explain the results of Chapter 5? How would you experimentally control for maternal effects?
8) Do tetraploids require vernalisation to flower? In general, does this species require a cold period to flower?
9) How do you measure fitness in a perennial, clonal plant?
10) I am not sure I follow the rationale for favouring multiple origins in Chapter 6. Could you please compare and contrast the results from the microsatellite and chloroplast analyses?

Technical comments

There are numerous typos in the citations in Chapter 6.