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Evaluation of the PhD thesis by Veronika Konečná

In her PhD thesis Veronika Konečná addresses a key question in evolutionary biology and genetics: How repeatable and thus predictable is natural evolution? To do so, she has chosen two plant models (*Arabidopsis arenosa* and *Primula elatior*) that each represent multiple cases of parallel adaptation to reasonably well-defined novel environments. These novel environments are (sub-)alpine habitats and serpentine soils. Alpine habitats are characterized by strongly fluctuating temperatures, freezing, high UV irradiance, etc., and plants on serpentine soils have to contend with low nutrient concentration and high levels of metals, often exacerbated by drought. Both types of habitats show an island-like geographic distribution, as they are generally surrounded by foothill or lowland areas and non-serpentine soils, respectively. It is from these more benign habitats that alpine and serpentine sites have been repeatedly colonized by a number of taxa. In the process, the plants had to adapt to the novel, more challenging environments, facing similar selective forces in each respective type of environment. It is therefore a relevant question whether the repeated colonization of such environments has resulted in parallel phenotypic responses, and if so, whether these are based on an overlapping set of genetic changes or whether each lineage has found its unique genetic route to adaptation. Systematic studies of these questions are very rare in plants, and even more so in polyploidy plants, of which the tetraploid lineages of *A. arenosa* are an example. Thus, Veronika has identified an important gap in our knowledge about evolution and highly appropriate study systems that she investigated with state-of-the-art approaches, making this a very timely and relevant piece of work. It has resulted in five published manuscripts (one literature review, four original studies, three of which are included in this thesis) and one unpublished manuscript, and except for case study 2, Veronika has been the major contributor to each of these manuscripts. This represents a highly impressive output for a PhD thesis.

The thesis is structured around the five manuscripts mentioned above, prefaced with a general introduction that places the work in a wider context. In this general introduction, Veronika introduces the concept of parallelism in evolution, methods of studying it, and some famous examples. She discusses the possible basis and sources of genomic parallelism, before introducing her two chosen study systems. The objectives and main approaches, as well as general features of the two taxa under investigation are clearly described. She then extracts the main results from the individual case studies, draws a number of well-supported overarching conclusions and identifies limitations and an outlook for future studies. The presentation in this chapter is very clear and well structured. It provides evidence for a very good grasp of the relevant literature and Veronika's ability to place her research critically into this context of the state of the field.

Case study 1 is a literature review by Konečná et al., 2020, about the genomic basis of serpentine adaptation. The review describes the challenges faced by plants on serpentine soils, the available evidence that plants have adapted to such soils, and underlying physiological mechanisms enabling such adaptation. It goes on to discuss emerging insights into the genomic basis of adaptation to serpentine soils, raises a number of major questions to be answered by the field, and proposes an integrative approach for doing so. In terms of further synthesis, the review provides an overview of candidate convergent genes that likely contribute to serpentine adaptation in different species. As such, the review is clearly timely and relevant to the field.

Case study 2 is an original paper by Knotek et al., 2020, with Veronika as second author. It reports studies of parallel alpine adaptation in *A. arenosa*. To do so, the paper first demonstrates that the alpine populations in the four regions under study are more closely related to geographically proximal foothill populations, rather than other alpine populations. Together with knowledge about the ecology of the species, this suggests recent, independent parallel colonization of the alpine habitats in the four regions. The paper then reports phenotypic comparisons between alpine and foothill populations, both from field-grown and common-garden grown plants. This indicates that stem height and flower size have changed in parallel and in the same direction in all four instances of alpine colonization, suggesting that these changes are important for plant adaptation to high-altitude conditions.

Case study 3 by Konečná et al., 2019, addresses the parallel colonization of alpine habitats in central Europe by *P. elatior*. As for case study 2, also here Veronika reconstructed the genetic structure and evolutionary relationships between alpine and foothill *P. elatior* populations from three mountain regions, based on microsatellite markers. Populations clustered by regions, not by habitat type, suggesting again independent parallel colonization of alpine sites from foothill populations. Alpine populations across the three regions were more strongly genetically differentiated from one another than were the corresponding foothill populations, indicating – not surprisingly – that gene-flow across mountain ridges is lower than in lowland regions. Modelling also indicated substantial migration from alpine towards foothill populations, possibly by washing down of seeds. Thus, this paper establishes *P. elatior* as an alternative model for studying the genomic basis of repeated adaptation to alpine environments and lays the foundation for further investigation of this system to test the generality of the conclusions drawn from *A. arenosa*.

Case study 4 by Konečná et al., 2021, investigated the genomic basis of parallel adaptation to serpentine soils in *A. arenosa*. As in the other two studies before, the recent, independent colonization of the serpentine sites from neighbouring non-serpentine regions is demonstrated by analysis of SNP data from 78 fully resequenced individuals from five pairs of serpentine and non-serpentine populations. This is complemented by common-garden experiments, growing individuals from both types of site in either soil type; the results clearly support parallel phenotypic adaptation to the serpentine sites, with only Ca/Mg ratios in one population deviating from this parallel pattern. Veronika then combined different divergence- or environmental association-based approaches to search for candidate genomic regions and loci that have contributed to serpentine adaptation, and compared the results across the five population pairs. This identified 61 serpentine adaptation candidates, and further modelling suggested a shared origin for the vast majority of parallel adaptation candidates, i.e. a scenario where the serpentine-adapted alleles were selected from shared variation across the ancestral non-serpentine populations. The one exception to this pattern was the gene encoding the calcium channel TPC1 that showed evidence for two independent adaptive *de novo* mutations in two serpentine populations. Intriguingly, both mutations affected the same amino acid, and homology-based protein modelling indicated that this amino acid was located close to the selectivity-determining site in the channel pore. Together this provided strong evidence that these mutations had been selected independently to allow the plants to deal with the low Ca/Mg ratio in serpentine soils. Overall, this study represents a major advance in our understanding of the genomic basis of parallel adaptation in plants, with a particular focus on tetraploid plants as an added bonus. This paper is likely to have a major impact on the field.

Case study 5 by Konečná et al. is an unpublished manuscript that represents a logical extension of the work in case study 4. Here, Veronika and her co-authors grew plants from three S-N pairs in both types of soil and performed extensive phenotyping of fitness-related traits. This was complemented by growth *in vitro* on media with defined Ca/Mg ratios. Analysis of the trait data demonstrated local adaptation to the serpentine soils, with little associated cost on non-serpentine soils. Interestingly, the

magnitude of the local adaptation effect correlated with the degree of neutral genetic differentiation across the S-N pairs. While most phenotypes showed evidence of parallel phenotypic change across the three serpentine sites, some (e.g. flowering time) showed non-parallel responses. A more detailed investigation of the re-sequencing data from case study 5 at the single-gene level indicated a significant, yet only rather small degree of parallelism at the level of individual genes. However, integrating across pathways supported a scenario where different components of the same functionally connected pathways had been selected in different serpentine populations. These findings are discussed in light of the ongoing debate about the role of redundancy in parallel adaptation. This study is a very valuable complement to case study 4 and is bound to be published in a high-impact journal.

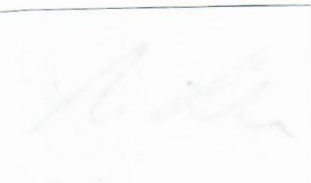
Therefore, in summary Veronika Konečná has made important novel contributions to the process and genomic basis of parallel adaptation to challenging environments in plants, with a focus on tetraploid lineages. Her findings tie in with several ongoing debates in the evolutionary-genetics literature and are therefore bound to have a strong impact in the field.

It would be interesting to hear Veronika's take on the following question:

Looking at Table 1 in case study 3, together with the relevant Methods section, it appears that two of the markers (PRIV4, PACA78) systematically deviate from HWE (significantly different in six and seven populations, respectively) in essentially all populations in which they were detected (Methods: null alleles [i.e. not amplified?] in seven and six of the populations, respectively). What could be an explanation for this, given that most of the markers seem to be in HWE throughout the populations? Is there any region in the genome for which non-random mating would be expected?

In conclusion, I recommend to accept the thesis for the defense and in case of successful defense, to award the author of the thesis, Veronika Konečná, the title Ph.D.

Yours sincerely,



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