The adaptation of organisms to a changing environment presents an ideal opportunity for studying the mechanisms of evolution. Specifically, local adaptation to the alpine environment enables us to watch the reaction of an organism to a relatively steep gradient of conditions. By understanding the processes of adaptation of an organism to a certain change of conditions, we can try to predict the reaction of different organisms to a similar change. This can be useful in the future for weakening the consequences of climate change.

To unravel the processes at the genetic level of adaptation, the approach of creating a list of candidate alleles is often taken. But only rarely, it is brought to a functional validation of the candidate adaptive alleles. In this study I am doing a functional follow up on a genomic study which revealed candidate genes supposed to contribute to an adaptation to the alpine environment. (Bohutínská *et al.*, 2021) My model system consists of natural populations of *Arabidopsis arenosa*, a wild relative of the famous model organism *A. thaliana*. This plant independently colonized the alpine environment across Europe at least five times and repeatedly formed a distinct alpine phenotype. Thus, we can work with pairs of foothill and derived alpine populations. To name a few differences, the alpine plants are shorter and have thicker leaves, bigger and more colourful flowers. Selection scans showed 151 genes possibly influencing the adaptation. I chose 2 of the genes with strongest selection signals for further study - FAR5 and MAP18.

Their alleles are not completely fixed in the gene pool of the natural populations, so it is possible to find a rare heterozygote for an alpine allele in a foothill population and vice versa. My first step was to find the rare heterozygotes among the plants grown from seeds collected from natural populations. Subsequently, I crossed these heterozygotes and in the next generation I crossed the homozygotes for a contrasting allele (e.g. alpine allele in foothill plants) and homozygotes for a native allele (e.g. foothill allele in foothill plants). Seeds from these crosses were germinated and because they are natural transgenic organisms, we were able to set a transplant experiment in Innsbruck. We planted two sets of seedlings in the alpine and foothill environment and let them grow for a few weeks. Each set comprised case (alpine plants with foothill allele and foothill plants with alpine allele) and control plants (alpine plants with alpine allele and foothill plants with foothill allele) for both genes. Based on literature on *A. thaliana* I derived the traits I focused on in phenotyping the plants. For FAR5 I analyzed the composition of suberin in roots and wounded leaves. It was shown that the amino acid position, which differs between the alpine and foothill allele of the FAR5 gene, affects the substrate specificity of the FAR5 enzyme. I mainly focused on two fatty alcohols affected by the change of specificity. (Chacón *et al.*, 2013) Expression of FAR5 was noted in roots and wounded leaves. (Domergue *et al.*, 2010) MAP18 is associated with microtubular traits and thus we focused mainly on the pollen tube growth. I also observed general growth properties and survival rate after transplantation.

By comparing the phenotypic traits of plants with various combinations of alleles and genomic backgrounds, I will examine phenotypic effects of these genes and their potential involvement in alpine adaptation. Which phenotypic shifts are associated with derived alpine allele of candidate genes, as compared to ancestral foothill allele? How do phenotypic shifts inform us about the candidate gene function? Does the function align with the function in *A. thaliana*? And lastly is there any fitness gain of plants with an allele which is native to a particular environment? My results suggest that there is a phenotypic difference between the alpine and foothill allele and that the approach of listing the candidate alleles selects alleles which are in fact influencing local adaptation. Many other questions, such as the particular fitness effect of the alpine alleles, remain yet unknown.

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