Rates of evolution in stress-related genes are associated to habitat preference in two *Cardamine* lineages

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Elucidating the selective and neutral forces underlying molecular evolution is fundamental to understand the genetic bases of adaptation. Plants have evolved a suite of adaptive responses to cope with variable environmental conditions, but we know little about which genes are involved in such responses. Here we studied molecular evolution at a genome-wide scale in two species of Cardamine with distinct habitat preferences: C. resedifolia, a species with mixed mating system found at high elevation, and the selfing C. impatiens, which prefers low elevation. We focused our analyses in genes that are involved in stress responses that distinguish the two habitats, namely cold and high irradiation.

High throughput sequencing was used to obtain gene sequences from both species. Using the available *A. thaliana* gene sequences and annotation, we identified nearly 3,000 triplets of putative orthologues, including genes involved in cold response, photosynthesis or in general stress responses. Complementary approaches, including rate of molecular evolution, codon usage and gene expression, allowed us to evaluate the possible role of positive selection in driving the evolution of *Cardamine* genes. Our analyses revealed a significantly higher rate of molecular evolution in *C. resedifolia* than in *C. impatiens*. Moreover, the rate of protein evolution was heterogeneous between functional classes and between species, with cold responsive genes evolving particularly fast in *C. resedifolia*, but not in *C. impatiens*.

Overall, our comparative genomic analyses on congeneric species unraveled evolutionary patterns associated with habitat preference of *Cardamine*. The breeding system most likely contributed, but is not sufficient alone, to explain the observed differences in the rate of protein evolution within and between species. Rather, species-specific selective pressures associated to the distinct habitats are most likely the primary causes of the different rates of protein evolution among the functional classes considered in this study.