

# Application Call 2025 - Project 6

### A miRNA taming floral homeotic genes

#### Supervisors:

Günter Theißen (main supervisor) Matthias Schleiden Institute / Genetics, Friedrich Schiller University, Jena Dr. Roy Kirsch (co supervisor) Department of Insect Symbiosis, Max Planck Institute for Chemical Ecology, Jena

#### Background:

The floral structure significantly influences the interaction of angiosperms with their environment. By specifying petal (or lodicule) and stamen identity DEF-like genes have an indispensable function in all flowering plants. Recently, a microRNA, miR5179, was identified that may control DEF-like genes in some species. Recent analyses in our lab revealed that miR5179 likely originated in the stem group of flowering plants about 200 million years ago and was conserved in several plant lineages, but has been lost independently in many other lineages. Thus miR5179 provides a fascinating conundrum: it is ancient, but not universally conserved. Why is it functionally important in some angiosperms, but dispensable in others, whereas its putative target (mRNAs of the DEF-like genes) is ultra-conserved? What is the ultimate function of miR5179 in those species in which it still exists?

#### **Project description:**

We hypothesize that miR5179 functions in the restriction of the expression of DEFlike genes. This restriction may be necessary to prevent the development of aberrant floral structures, which would compromise the interaction of the respective flowering plants with their environment, especially pollinators. To determine the function of miR5179 knock-out mutants of this miRNA in the grass species *Brachypodium distachyon* are currently being generated, using CRISPR-Cas9 technology. The work is done in cooperation with the group of Jochen Kumlehn at the IPK in Gatersleben, in which gene editing using CRISPR-Cas9 as well as transformation of grasses is well established. The phenotypes of the produced mutants will be carefully characterized with special focus on floral structure and compared to the phenotypes of the wild-type plants. Moreover, we will closely monitor the expression of miR5179 and its target genes in floral buds and flowers of wild-type and mutant plants using qRT-PCR and *in-situ* hybridization. The results of these experiments will help to elucidate as to whether miR5179 functions to tame B-class floral homeotic genes or may have another unexpected function.

### Candidate profile:

We are looking for a candidate with creativity, curiosity and an attitude of critical scientific thinking. Proven skills in molecular biology and a deep interest in plant ecology, development, and evolution are essential. The candidate should have strong communication skills and the ability and willingness to cooperate with researchers with different backgrounds (bioinformatics, developmental biology, chemical ecology, evolution). Good time management and organizational skills as well as proficiency in written and spoken English are required.

## Reading:

Aceto, S., et al. (2014) The analysis of the inflorescence miRNome of the orchid Orchis italica reveals a DEF-like MADS-box gene as a new miRNA target. *PLoS One*. 9(5):e97839.

Deng, F., et al. (2022) Molecular evolution and functional modification of plant miRNAs with CRISPR. *Trends in Plant Science*. 27(9), 890-907.

Gramzow L, Theißen G (2019) Plant miRNA Conservation and Evolution. In: de Folter S. (eds) Plant MicroRNAs. Methods in Molecular Biology, vol 1932. Humana Press, New York, NY, p. 41-50.

Gramzow, L., et al. (2020) Independent origin of *MIRNA* genes controlling homologous target genes by partial inverted duplication of antisense-transcribed sequences. *Plant J.* **101**, 401-419.

Kumlehn, J., et al. (2018) The CRISPR/Cas revolution continues: From efficient gene editing for crop breeding to plant synthetic biology. *J Integr Plant Biol*. 60(12):1127-1153.

Theißen, G., et al. (2016) MADS-domain transcription factors and the floral quartet model of flower development: linking plant development and evolution. *Development*. 143:3259-3271.

Yang, X., et al. (2012) Live and let die - the B(sister) MADS-box gene OsMADS29 controls the degeneration of cells in maternal tissues during seed development of rice (Oryza sativa). *PLoS One*. 7(12):e51435.