ALL flowers start as a dome of stem cells, the floral meristem (FM; Fig. 1). Although floral morphological diversity is seemingly infinite, the developmental trajectory of every organ in a flower is determined at initiation: a floral organ has a particular final form because it was produced by the FM at a certain developmental time point and at a certain position. The FM determines the identity, number, position, and timing of initiation of all the organs in a flower, a blueprint that is often referred to as the Bauplan. Proper establishment of the Bauplan is essential for the reproductive success of a plant and variations in the Bauplan lay the foundation for floral morphological diversity (Fig. 1).

My scientific passion is to understand the molecular developmental mechanisms of FM regulation that underline Bauplan evolution and variation. Realization of this long-term research goal requires the establishment of new model systems that have Bauplans that differ from the traditional models (e.g., Arabidopsis), but are also amenable to rigorous genetic and developmental interrogation. To this end, my PhD and postdoctoral work in *Aquilegia* and *Mimulus*, respectively, have enabled these two emerging systems to become powerful models for investigating FM regulation. My research program will begin with establishing the genetic and molecular framework that generates variation in two major aspects of the Bauplan: **1) timing variation in FM termination,** and **2) symmetry breaking in the FM**. Both aspects are major forces in shaping flowering plant evolution and diversity, yet simply cannot be investigated using traditional models. Results from my research program will not only advance our understanding of FM regulation and general Bauplan diversity, but also shed light on **fundamental aspects of development in general**, since the development of all multicellular organisms depends on the abilities of cells to assess time, position, and their identities.

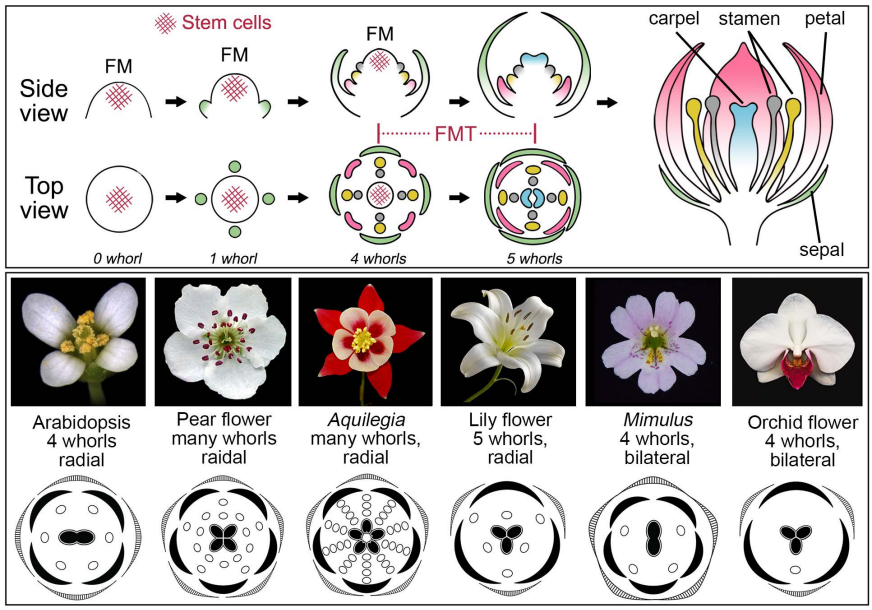

Figure 1. Upper panel: Generation of Bauplan by the FM. Lower panel: Variation in the Bauplan lay the foundation for floral morphological diversity.

DIRECTION 1 – When Is the Right Time to Stop Producing Organs?

SIGNIFICANCE:

Floral meristem termination (FMT) is the coordinated termination of stem cell activity in the FM and is **a defining feature of all FMs** [1] (Fig. 1). Each FM is responsible for the production of one flower, which always bears a finite number of organs. After a period of successive organ production, the stem cells will lose their pluripotency and differentiate into the inner-most organs of the flower. The timing of FMT essentially determines the duration of stem cell proliferation in the FM and the total number of organ whorls in the flower. When considering evolutionary changes in the Bauplan across angiosperms, several critical transitions are observed, including repeated shifts from variable to stable whorl numbers and a decrease in the total whorl numbers, with both processes directly controlled by FMT. In other words, **canalization toward relatively rapid FMT is a major force in shaping angiosperm evolution**.

Currently, however, how the timing of FMT is fine-tuned at a developmental or evolutionary level is poorly understood. Almost all of our knowledge of FM proliferation has been gained in the context of artificial selection (e.g. in agricultural crops) or mutagenesis, both of which emphasize dramatic allelic

effects that usually lead to a total cessation or over-proliferation of stem cells. Furthermore, our understanding of the regulation of FM proliferation is hindered by the fact that all currently established model systems and their close relatives invariably bear only four whorls of organs, providing no starting point for studies of natural variation in this trait.

FOUNDATION:

During my PhD, I recognized that the system developed in the Kramer lab, *Aquilegia coerulea* 'Kiragami' (Fig. 2a), is also ideal for investigating FMT variation. Using *in situ* hybridization, RNAi-based gene silencing, and transcriptome profiling of young FMs, I discovered that key genes in the FMT pathways in Arabidopsis were unlikely to be functionally conserved in *Aquilegia* [2,3]. To understand the cellular behavior dynamics during FMT, I established a quantitative confocal live-imaging system for *Aquilegia* FM, the first for a non-traditional model system [4,5]. Key findings included that carpel initiation and cessation of cell division in the FM is not synchronized, unlike what is known in Arabidopsis. Moreover, I realized that FMT can be studied as a quantitative trait, because all *Aquilegia* species share the same Bauplan except for stamen whorl numbers (SWN; Fig. 2b), and thus SWN variation can directly reflect the variation in FMT timing. I conducted QTL mapping between two sister species that differ in SWN, *A. canadensis* (Acan) and *A. brevistyla* (Abre), which revealed a complex genetic architecture underlying the natural variation in FMT timing with seven QTL [6] (Fig. 2c).

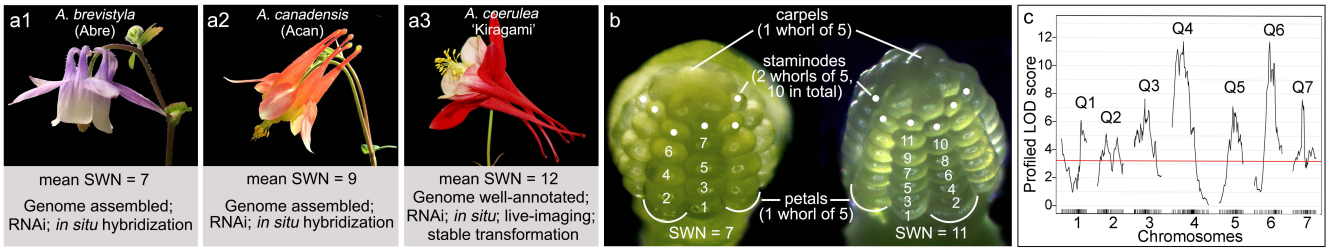

Figure 2. *Aquilegia* as model for studying FMT variation. **(a1-a3)** Three focal species and their respective resources. **(b)** Two young FM showing identical Bauplan except for SWN. **(c)** QTL mapping of SWN variation between Abre and Acan revealed seven major QTL [6].

FUTURE:

1) Fully characterize the functions of the candidate genes controlling timing variation of FMT in *Aquilegia*. The abovementioned QTL mapping has uncovered **the first list** of candidate genes that potentially control the timing variation of FMT, providing an ideal starting point to dissect the molecular basis of FMT in *Aquilegia*. We will carry out detailed expression analysis of the candidate genes in the three focal species, and fully examine the functions and genetic interactions of the candidate genes in Kiragami. 2) Investigate the molecular mechanisms giving rise to different FMT in *Aquilegia.* We will focus on the genes with the largest effect on FMT and further investigate how different alleles are associated with different whorl numbers. Alleles from Acan and Abre will be heterologously expressed in Kiragami CRISPR lines of the gene of interest to examine the level of phenotypic compensation. The potential upstream regulators and downstream targets will be revealed with yeast one-hybrid library screens and chromatin immunoprecipitation assays with sequencing, respectively.

DIRECTION 2 – Where are the Right Places to Position the Organs?

SIGNIFICANCE:

Floral symmetry is a major determinant of flower diversity and is evolutionary labile. Most flowers are either radially symmetric (i.e., have multiple axes of symmetry) or bilaterally symmetric (i.e., with a single plane of symmetry along the dorsal-ventral (D-V) axis) in their final forms (Fig. 1; 3a). Bilateral symmetry has evolved at least 130 times independently from radial symmetry and is considered a key innovation in many species-rich lineages. Although the final forms can be elaborated in countless ways, **the underlying developmental principle is the same for all bilaterally symmetric flowers:** organs initiated in the D vs. V domains follow different developmental trajectories. The D-V axis is established in

the FM as soon as it is produced by a growing tip called inflorescence meristem (IM), with cells in the FM closer to the IM defining the D domain while the ones further away defining the V domain (Fig. 3b).

Currently, it is well established that homologs of the *CYCLOIDEA* (*CYC*) gene are responsible for the elaboration of bilateral symmetry in many taxa. However, almost all studies over the past 30 years focused on simple expression correlations or, at best, functional studies of *CYC* homologs. **A mechanistic understanding** of how the D-V is established in the FM and what pre-patterns the expression of *CYC* is still missing. Currently, *Mimulus* is the **only** system with bilaterally symmetric flowers that is also amenable for rapid stable transformation (Fig. 3a).

FOUNDATION:

I established a quantitative live-imaging pipeline for FMs of *M. parishii* and uncovered striking patterns of auxin response by monitoring the expression of the synthetic reporter DR5*rev*-erRFP. Unlike the well-known DR5 patterns in the Arabidopsis FM, auxin distribution is remarkably dynamic during the earliest stages of FMs in *Mimulus* (Fig. 3d, 3e). I also identified several genes that are potentially upstream of the *CYC*-based morphogenesis program. Orthologs of these genes have been studied in Arabidopsis, but none revealed any involvement in D-V symmetry. For instance, silencing of the *Mimulus* ortholog of *CUP-SHAPED-COTYLEDON2* leads to ventralized flowers. Although it is well-known that *PIN1* is the predominant auxin efflux carrier in Arabidopsis FMs, I discovered that this role is fulfilled by the ortholog of *SISTER-OF-PIN1* (*MpSoPIN1*) in *Mimulus* FMs, and it also involves in D-V patterning (Fig. 3e).

FUTURE:

1) Elucidate the roles of auxin in symmetry breaking in the *Mimulus* FMs. The imaging analysis pipeline and the transgenic lines that I generated have laid a solid foundation for developing a quantitative understanding of DR5 and SoPIN1 distributions at high spatiotemporal resolution and how auxin influences the D-V patterning (Fig. 3e). We will also collaborate with computational biologists to construct the first theorical model for D-V establishment, which will be assisted by not only the expression data, but also cell lineage tracing data from live-imaging regarding cell geometry, growth, and division. 2) Uncover the molecular network governing D-V establishment at single cell level. Taking advantage of the structure of the *Mimulus* inflorescences that include FMs of different stages (Fig. 3b), we will conduct single-cell RNA sequencing on young inflorescences (Fig. 3c). Subpopulation of cells of interest will be enriched through fluorescence-activated cell sorting and the developmental trajectories of FMs will be computed by pseudotime ordering analysis (Fig. 3c). Novel genes will be discovered by differential expression and co-expression network analyses and validated by functional studies.

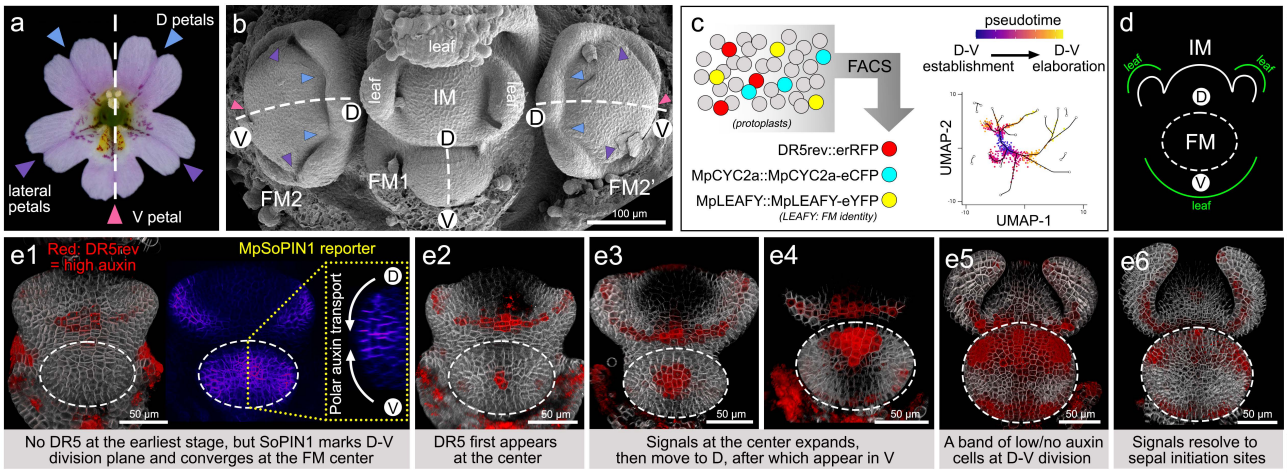

Figure 3. *Mimulus* as a model system for studying bilateral symmetry. **(a)** Front view of a *M. parishii* flower. **(b)** Top view of an inflorescence showing FMs of different stages. **(c)** Simplified workflow of single-cell RNA sequencing. **(d)** Side view illustration of an inflorescence. **(e1-e6)** DR5 expression in FMs through early developmental stages. FACS: fluorescence-activated cell sorting

[1] Min & Kramer (2023). *New Phytologist*, *238*(2), 500-505. **[2]** Min & Kramer (2017). *New Phytologist*, *216*(2), 536-548. **[3]** Min & Kramer (2020). *Scientific reports*, *10*(1), 19637. **[4]** Min *et al.* (2022). *Development*, *149*(4), dev200256. **[5]** Min et al (2022). *Bio-protocol*, *12*(12), e4449-e4449. **[6]** Min *et al.* (2022). *Journal of experimental botany*, *73*(18), 6241-6254.