EXPERT VIEW

Floral organ development goes live

Léa Rambaud-Lavigne and Angela Hay*
Max Planck Institute for Plant Breeding Research, Carl-von-Linné-Weg 10, D-50829 Köln, Germany

* Correspondence: hay@mpipz.mpg.de

Received 11 October 2019; Editorial decision 17 January 2020; Accepted 20 January 2020

Editor: Frank Wellmer, Trinity College Dublin, Ireland

Abstract

The chance to watch floral organs develop live is not to be missed! Here, we outline reasons why quantitative, live-cell imaging is an important approach to study floral morphogenesis, and provide a basic workflow of how to get started. We highlight key advances in morphodynamics of lateral organ development, and discuss recent work that uses live confocal imaging to address the regulation of floral organ number, its robustness, and patterning mechanisms that exploit stochasticity.

Keywords: APETALA1, Arabidopsis thaliana, ATML1, Cardamine hirsuta, floral organ number, live-cell imaging, petal, robustness, sepal, SUPERMAN.

Introduction

Morphogenesis is the fascinating process by which a handful of cells develop into complex, three-dimensional forms. Floral organs have some of the most diverse and complex forms found in plants. Research over the past three decades has elucidated many of the gene products and molecular mechanisms that control the unique identities of different floral organs. Moreover, it was already appreciated three decades ago that many, though certainly not all, of these gene functions are evolutionarily conserved (Coen and Meyerowitz, 1991; Causier et al., 2010). A major challenge now is to relate these gene activities to the growth of floral organs to understand what Enrico Coen calls 'the genetics of geometry' (Coen et al., 2004).

This task involves a more quantitative approach to the long-standing genotype to phenotype problem. To describe shape transitions in different genotypes requires quantitative, metric descriptions of growth patterns. To understand how growth influences and responds to the action of genes requires a quantitative framework. The problem of describing and understanding growth quantitatively has become a main topic of multidisciplinary research (Goriely, 2017). Petals and leaves have been a study system of choice to approach these questions, and cell lineage tracing has been one of the most important tools to quantify the parameters underlying growth of these organs (Poethig, 1987) (Fig. 1). By genetically marking dividing cells, their mitotic descendants can be identified as clonal sectors. Cells can be marked using endogenous transposons, such as the temperature-sensitive Tam3 transposon in snapdragon (Rolland-Lagan et al., 2003; Green et al., 2010), or by transgenic approaches, such as using a heat shock-inducible Cre–Lox system, in Arabidopsis (Sauret-Güeto et al., 2013) (Fig. 1A). Some key growth parameters can then be inferred by analysing the resulting clone patterns, for example the rate and duration of growth, the degree of anisotropy, and the main direction of growth (Rolland-Lagan et al., 2003; Green et al., 2010; Sauret-Güeto et al., 2013). Although you cannot control which individual cells will be marked, the ease of generating and analysing thousands of sectors makes this a robust technique to estimate growth. One caveat to inferring growth parameters by this method is that the geometry of the initial cell influences the size and shape of the resulting sector. For example, anisotropic growth is inferred from elongated sectors, but an elongated cell will produce an elongated sector.
Live-cell imaging, where growing plants are imaged at the cellular level repeatedly over time, can be used to address many developmental questions, as we will highlight further in this review. However, the interactions that link growth patterns to gene activities are often difficult to interpret without considering the mechanics of growth. This is because genetically specified growth of a region, such as growth rate and anisotropy, occurs within the mechanical constraints of its neighbouring regions. Therefore, the resultant growth includes the tissue deformations that arise due to this connectivity (Coen and Rebocho, 2016; Rebocho et al., 2017). Computational modelling offers a way to navigate through these issues by incorporating interactions between the main direction of growth, regional identities, and mechanics in a growing framework (Kennaway et al., 2011). Early results from working in this type of quantitative framework showed, for example, that the main direction of growth is important to generate the asymmetry that characterizes the dorsal petal lobe in snapdragon (Rolland-Lagan et al., 2003). Analysing the entire snapdragon corolla and the Arabidopsis petal gave further indications that genes control petal shape by independently specifying the properties of regional growth and also overall growth directions in the petal (Green et al., 2010; Sauret-Güeto et al., 2013). More recent advances have come from investigating how genes control leaf shape (Kuchen et al., 2012; Kierzkowski et al., 2019; Whitewoods et al., 2020). By using quantitative live imaging, comparative experimental systems, and mechanistic models, Kierzkowski et al. (2019) showed, for example, that differences between Arabidopsis thaliana and Cardamine hirsuta leaf shape result from local modification of growth during patterning and a global redistribution of growth that results from delayed differentiation (Box 1).

So, given the advantages of live-cell imaging, what do you need to know to get started?

**Going live**

A typical workflow for quantitative, live-cell imaging of floral organs is illustrated in Fig. 2. First, the shoot apex is dissected to expose the youngest floral primordia of interest (Fig. 2A). The apex is secured in position for the duration of the experiment and can be cut from the plant and cultured on growth medium to facilitate viewing (Prunet et al., 2016). Whether the sample is left intact or cultured, it is critical to compare its overall growth and development with those of control plants to understand whether the experiment causes any deviations, for example by timing the progression of floral primordia through consecutive developmental stages (Smyth et al., 1990). Confocal laser scanning microscopy is commonly used to image the live sample at cellular resolution over several time points that best fit the developmental process of interest (Fig. 2B). For example, samples are often imaged at 24 h time intervals over 2−5 d (McKim et al., 2017; Prunet et al., 2017; Montiaux et al., 2018; Ripoll et al., 2019) or up to 7−8 d (Hervieux et al., 2016; Kierzkowski et al., 2019), or at shorter 8−12 h time intervals (Hong et al., 2016; Caggiano et al., 2017; Meyer et al., 2017).

![Lineage tracing by sector analysis or live imaging. (A) Marked sectors can be induced and visualized at a range of developmental stages in order to relate growth to organ shape. For example, sectors induced at 0 days after pollination (DAP) in Arabidopsis flowers and visualized in petals at 6 DAP (left) and 12 DAP (right) are larger than sectors induced at 2 DAP and visualized in 6 DAP (left) and 12 DAP (right) petals (cartoons show independent sectors from multiple flowers superimposed on average petal shapes; scale bars=100 μm (6 DAP), 300 μm (12 DAP) (Sauret-Güeto et al., 2013)]. This method infers growth parameters from analysing sector size, shape, and orientation. This method does not usually recover all cell lineages that produce an organ. (B) Live imaging follows a growing organ, at cellular resolution, over several time points, such that complete cell lineage patterns can be mapped for a single sample. For example, cartoons show cell lineage patterns in a single Arabidopsis leaf at three successive stages of development (Kierzkowski et al., 2019). Growth parameters can be directly quantified, rather than inferred, from these cell lineage patterns.

![Lineage tracing by sector analysis or live imaging. (A) Marked sectors can be induced and visualized at a range of developmental stages in order to relate growth to organ shape. For example, sectors induced at 0 days after pollination (DAP) in Arabidopsis flowers and visualized in petals at 6 DAP (left) and 12 DAP (right) are larger than sectors induced at 2 DAP and visualized in 6 DAP (left) and 12 DAP (right) petals (cartoons show independent sectors from multiple flowers superimposed on average petal shapes; scale bars=100 μm (6 DAP), 300 μm (12 DAP) (Sauret-Güeto et al., 2013)]. This method infers growth parameters from analysing sector size, shape, and orientation. This method does not usually recover all cell lineages that produce an organ. (B) Live imaging follows a growing organ, at cellular resolution, over several time points, such that complete cell lineage patterns can be mapped for a single sample. For example, cartoons show cell lineage patterns in a single Arabidopsis leaf at three successive stages of development (Kierzkowski et al., 2019). Growth parameters can be directly quantified, rather than inferred, from these cell lineage patterns. Even if growth is isotropic. These issues can be avoided by observing individual cell lineages in a growing organ over time, and directly measuring the parameters that describe its growth (Fig. 1B). This can be achieved by live (time-lapse) imaging and subsequent segmentation of cells from each time point as described in Fig. 2. New developments in imaging and software have accelerated this approach in recent years, allowing key advances to be made in plant morphodynamics (Box 1).
These image stacks, comprising 3-D volumetric data, can then be processed with software, such as MorphoGraphX or MARS-ALT, to extract the sample contour and accurately segment the cells (Fernandez et al., 2010; Barbier de Reuille et al., 2015) (Fig. 2C). Because morphogenesis involves 3-D deformations, it is important to quantify each image as 3-D data or to summarize the 3-D data as a curved surface image. For example, MorphoGraphX can be used to extract the shape of a sample as a mesh (Fig. 2Ci–ii), and project on to this mesh the 3-D image data just below the surface, creating a curved image of the outer layer of cells (Fig. 2Ciii). This allows cell outlines to be accurately extracted without the distortions associated with a flat 2-D projection (Barbier de Reuille et al., 2015). The resulting curved surface images can then be segmented into individual cells (Fig. 2Civ). Comparing segmentation files at successive time points accurately maps the cell lineage patterns (Strauss et al., 2019). Various features, such as gene expression, amount of growth, and growth direction, can then be quantified at a cellular level over time and displayed as heat maps on curved surface images. Further information to get started with computational image processing in microscopy, with an emphasis on the Fiji image analysis platform, can be found in the detailed tutorial by Roeder (2019).

In the next sections, we will discuss work over the past 2 years that has used live imaging of floral organ development to shed light on the regulation of organ number and robustness.
Floral organ number and robustness

Floral organs acquire their distinct identities according to the ABC model of flower development (Coen and Meyerowitz, 1991). However, less is known about how the flower is partitioned into distinct whorls containing the correct number of floral organs. SUPERMAN (SUP) is a transcriptional repressor that specifies the boundary between stamens and carpels. Flowers of sup mutants form too many stamens at the expense of carpels. However, until recently, it had been impossible to distinguish whether these extra stamens originate from an organ identity change in whorl 4 or the overproliferation of whorl 3. Prunet et al. (2017) tackled this question using live confocal imaging. They showed unequivocally that extra stamens in sup flowers arise from whorl 4 cells that change identity from carpel to stamen. Extended proliferation of stem cells, rather than whorl 3 cells, in sup flowers allows the formation of further stamens (Prunet et al., 2017). Key to these results was the ability to track SUP and whorl 3 markers [APETALA3 (AP3) and PISTILLATA] at high spatial and temporal resolution in single samples. By analysing the overlap between SUP and AP3, it was clear that SUP accumulates not only in whorl 3 as previously thought, but also in whorl 4. Live imaging of sup mutant flowers showed that non-AP3-expressing cells in whorl 4 switched identity from carpel to stamen by starting to express AP3 (Prunet et al., 2017). Therefore, SUP acts in these whorl 4 cells, adjacent to the boundary with whorl 3, to repress AP3 expression and partition stamen and carpel developmental programmes in adjacent organs.

Although the number of floral organs is typically robust in A. thaliana flowers, the same is not true for its close relative, C. hirsuta. In C. hirsuta flowers, petal number varies between zero and four (Fig. 3A). Is this because petals fail to initiate or fail to grow in C. hirsuta? Monniaux et al. (2018) addressed this question using live confocal imaging. Petal formation is directed by local accumulation of auxin, and DR5 reporters can be used to mark sites of transcriptional auxin response during petal formation (Lampugnani et al., 2013). However,
these peaks of auxin activity are transitory and mark only a few cells at the sites of petal initiation. So, live imaging helps ‘catch’ these events. By mapping growth in the same samples, it was clear that DR5 expression preceded growth of floral meristem cells at petal initiation sites in A. thaliana (Monniaux et al., 2018). Yet, in contrast to A. thaliana, petal initiation sites on the floral meristem in C. hirsuta were often not marked by DR5 expression (Monniaux et al., 2018). Therefore, the number of petals in a C. hirsuta flower is affected by variation in organ initiation rather than growth. Monniaux et al. went on to show that regulatory divergence in the APETALA1 (AP1) MADS-box transcription factor can account for the species-specific difference in petal number robustness between A. thaliana and C. hirsuta (Monniaux et al., 2018). When swapped into C. hirsuta, the A. thaliana copy of AP1 was expressed in a larger domain of cells in the petal whorl than C. hirsuta AP1, resulting in robust petal number (Fig. 3A). What is still unclear is the link between AP1 divergence and destabilization of the patterning of auxin peaks that are required for petal organogenesis.

Loss of robustness makes C. hirsuta petal number sensitive to genetic, environmental, and stochastic changes (Monniaux et al., 2016; Pieper et al., 2016; McKim et al., 2017). As such, petal number is strongly influenced by natural genetic variation, but also varies in response to seasonal cues, such as day length, winter cold, and, in particular, ambient temperature. In the field, spring-flowering plants produce more petals and, in the greenhouse, petal number is increased by conditions experienced in spring, such as cool ambient temperature (McKim et al., 2017). McKim et al. used live confocal imaging to show that cool ambient temperature increases petal number via slowing growth and maturation of the floral bud (McKim et al., 2017). Extending the duration of floral meristem growth at 15 °C versus 20 °C produced larger flat regions between sepals with more space available for petal initiation (Fig. 3B). Therefore, the influence of the environment on floral meristem growth and maturation provides a developmental route for petal number to respond plastically to seasonal conditions in C. hirsuta.

Organ development can be robust but still utilize stochastic changes in gene expression to initiate cellular patterning. Sepals have a scattered pattern of highly endoreduplicated giant cells interspersed between smaller cells on their abaxial surface (Roeder et al., 2010). The A. thaliana class IV HD-ZIP transcription factor MERISTEM LAYER1 (ATML1) is necessary and sufficient for the formation of giant cells (Roeder et al., 2012; Meyer et al., 2017). However, only a subset of epidermal cells expressing ATML1 become giant. This raises the question: how does ATML1 initiate giant cell patterning? Meyer et al. tackled this question using live confocal imaging. They found that fluctuations in ATML1 concentration produce asymmetries between cells that are read out at G2 in the cell cycle as patterning information (Meyer et al., 2017). Key to this result was the use of live imaging to record dynamic patterns of pATML1::mCitrine-ATML1 gene expression. Using a genetic dosage series, the authors had shown that the proportion of giant cells was sensitive to ATML1 levels (Meyer et al., 2017). However, only by quantifying ATML1 levels in the sepal epidermis over time could they show that protein levels fluctuate in and between each cell. Using nuclear area as a proxy for cell cycle stage, it became clear that peak concentrations of ATML1 during G2 could accurately predict cell fate (Meyer et al., 2017). Therefore, cell-autonomous fluctuations generate concentration differences of ATML1 that determine the proportion of giant cells.

Future perspectives

Live imaging—what is it good for?

In this review, we have highlighted recent advances in understanding floral organ initiation and cell fate acquisition that were made using quantitative, live-cell imaging. Moreover, this approach has advanced our understanding of lateral organ development more broadly. This is because the comparative expression of multiple genes at high spatial and temporal resolution can help answer questions that are otherwise difficult to resolve. For example, Prunet et al. (2017) defined the precise expression domain of SUP by analysing cell-level overlap with AP3, and directly observed cell fate switching.
in sup mutants by following AP3 expression in individual cells over time. In another example, Caggiano et al. (2017) simultaneously imaged auxin and organ polarity genes in the shoot apical meristem to show that sites of lateral organ initiation, marked by PINFORMED1, occurred in a narrow ‘gap’ between KANADI1 and REVOLUTA expression domains. Relating gene activities, as studied in real time, to growth can answer additional questions. For example, Kierzkowski et al. (2019) resolved how CUP-SHAPED COTYLEDONS2 can both stimulate and repress growth in the leaf margin: first, it triggers auxin activity maxima to promote growth; then, it is restricted to the regions flanking these maxima where it represses growth (Box 1). In another example, Monniaux et al. (2018) showed that an auxin activity maximum precedes growth at sites of petal initiation in Arabidopsis. Quantifying fluctuations in gene expression at cellular resolution can provide further answers. For example, Meyer et al. (2017) demonstrated a fluctuation-driven patterning mechanism for cell fate in the Arabidopsis sepal by measuring ATML1 levels relative to cell cycle progression in individual cells.

Live imaging—what’s next?

Microscopes are key to live-cell imaging. All of the studies mentioned in this review used confocal laser scanning microscopes to image cell layers at the plant surface. However, the goal of long-term, minimally invasive imaging of not only surface but also internal tissues requires different microscopes. Two-photon excitation microscopy is one option to achieve deep-tissue imaging with reduced photobleaching (Grossmann et al., 2018). Light sheet fluorescence microscopy is an ideal option for long-term, time-resolved imaging due to its high-speed acquisition rates and low energy sample exposure (Grossmann et al., 2018; Ovečka et al., 2018). Although it is more challenging to image flowers with this technique compared with roots, a recent study used light sheet microscopy to image developing flowers continuously for several days at cellular resolution (Valuchova et al., 2019, preprint).

Quantitative live-cell imaging also relies on accurate cell segmentation. Making this process not only accurate but also user-friendly is an essential task. Novel segmentation algorithms based on modern machine learning concepts, such as convolutional neural networks, have the potential to reduce the manual annotation burden and address problems of 3-D cell segmentation (Wolny et al., 2020, preprint). It is also important to provide these image analysis tools via free, open-source software, such as PlantSeg (Wolny et al., 2020, preprint), ilastik (Berg et al., 2019), or MorphoGraphX (Barbier de Reuille et al., 2015). Mechanical force measurements can also be incorporated into live-cell imaging workflows. For example, MorphoRoboX is an extension of MorphoGraphX that provides control and visualization of cellular force microscopy, which is a non-invasive micro-indentation method used to measure cell stiffness (Majda et al., 2019). Exporting cell geometries directly from software such as MorphoGraphX as templates for computational models also helps to create biologically realistic simulations (Mosca et al., 2017).

In summary, we discussed the use of live-cell imaging to study floral organ development in this review, but many questions concerning flowering and flowers can, and already do, benefit from this approach. We anticipate much more flowering research going live in the near future.

Acknowledgements

We thank R. Smith and M. Tsiantis for comments, A. Runions for providing images, and N. Bhatia for discussions. We apologize to authors whose work was not mentioned due to space constraints. This work was supported by DFG FOR2581 Plant Morphodynamics grant HA6316/2-1.

References

Barbier de Reuille P, Routier-Kierzkowska AL, Kierzkowski D, et al. 2015. MorphoGraphX: a platform for quantifying morphogenesis in 4D. eLife 4, e05864.

Berg S, Kutra D, Kroeger T, et al. 2019. ilastik: interactive machine learning for (bio)image analysis. Nature Methods 16, 1226–1232.

Caggiano MP, Yu X, Bhatia N, et al. 2017. Cell type boundaries organize plant development. eLife 6, e27421.

Causer B, Schwarz-Sommer Z, Davies B. 2010. Floral organ identity: 20 years of ABCs. Seminars in Cell & Developmental Biology, 73–79.

Coen ES, Meyerowitz EM. 1991. The war of the whorls: genetic interactions controlling flower development. Nature 353, 31–37.

Coen E, Rebocho AB. 2016. Resolving conflicts: modeling genetic control of plant morphogenesis. Developmental Cell 38, 579–583.

Coen E, Rolland-Lagan AG, Matthews M, Bangham JA, Prusinkiewicz P. 2004. The genetics of geometry. Proceedings of the National Academy of Sciences, USA 101, 4728–4735.

Fernandez R, Das P, Mirabet V, Moscardi E, Traas J, Verdeil JL, Malandain G, Godin C. 2010. Imaging plant growth in 4D: robust tissue reconstruction and lineageing at cell resolution. Nature Methods 7, 547–553.

Fox S, Southam P, Pantin F, et al. 2018. Spatiotemporal coordination of cell division and growth during organ morphogenesis. PLoS Biology 16, e2005902.

Goriely A. 2017. The mathematics and mechanics of biological growth. New York: Springer.

Green AA, Kennaway JR, Hanna AI, Bangham JA, Coen E. 2010. Genetic control of organ shape and tissue polarity. PLoS Biology 8, e1000537.

Grossmann G, Krebs M, Maizel A, Stahl Y, Vermeer JEM, Ott T. 2018. Green light for quantitative live-cell imaging in plants. Journal of Cell Science 131, jcs209270.

Hervieux N, Dumond M, Sapala A, Routier-Kierzkowska AL, Kierzkowski D, Roeder AH, Smith RS, Boudaoud A, Hamant O. 2016. A mechanical feedback restricts sepal growth and shape in Arabidopsis. Current Biology 26, 1019–1028.

Hervieux N, Tsugawa S, Fruleux A, et al. 2017. Mechanical shielding of rapidly growing cells buffers growth heterogeneity and contributes to organ shape reproducibility. Current Biology 27, 3468–3479.

Hong L, Dumond M, Tsugawa S, et al. 2016. Variable cell growth yields reproducible organ development through spatiotemporal averaging. Developmental Cell 38, 15–32.

Kennaway R, Coen E, Green A, Bangham A. 2011. Generation of diverse biological forms through combinatorial interactions between tissue polarity and growth. PLoS Computational Biology 7, e1002071.

Kierzkowski D, Runions A, Vuolo F, et al. 2019. A growth-based framework for leaf shape development and diversity. Cell 177, 1405–1418.e17.

Kuchen EE, Fox S, de Reuille PB, et al. 2012. Generation of leaf shape through early patterns of growth and tissue polarity. Science 335, 1092–1096.

Lampugnani ER, Klinic A, Smyth DR. 2013. Auxin controls petal initiation in Arabidopsis. Development 140, 185–194.
Majda M, Sapala A, Routier-Kierzkowska AL, Smith RS. 2019. Cellular force microscopy to measure mechanical forces in plant cells. Methods in Molecular Biology 1992, 215–230.

McKim SM, Routier-Kierzkowska AL, Monniaux M, Kierzkowski D, Pieper B, Smith RS, Tsiantis M, Hay A. 2017. Seasonal regulation of petal number. Plant Physiology 175, 886–903.

Meyer HM, Teles J, Formosa-Jordan P, Refahi Y, San-Bento R, Ingram G, Jonsson H, Locke JC, Roeder AH. 2017. Fluctuations of the transcription factor ATML1 generate the pattern of giant cells in the Arabidopsis sepal. eLife 6, e19131.

Monniaux M, Pieper B, Hay A. 2016. Stochastic variation in Cardamine hirsuta petal number. Annals of Botany 117, 881–887.

Monniaux M, Pieper B, McKim SM, Routier-Kierzkowska AL, Kierzkowski D, Smith RS, Hay A. 2018. The role of APETALA1 in petal number robustness. eLife 7, e39399.

Mosca G, Sapala A, Strauss S, Routier-Kierzkowska AL, Smith RS. 2017. On the micro-indentation of plant cells in a tissue context. Physical Biology 14, 015003.

Ovečka M, von Wangenheim D, Tomančák P, Šamajová O, Komis G, Šamaj J. 2018. Multiscale imaging of plant development by light-sheet fluorescence microscopy. Nature Plants 4, 639–650.

Pieper B, Monniaux M, Hay A. 2016. The genetic architecture of petal number in Cardamine hirsuta. New Phytologist 209, 395–406.

Poethig RS. 1987. Clonal analysis of cell lineage patterns in plant development. American Journal of Botany 74, 581–594.

Prunet N, Jack TP, Meyerowitz EM. 2016. Live confocal imaging of Arabidopsis flower buds. Developmental Biology 419, 114–120.

Prunet N, Yang W, Das P, Meyerowitz EM, Jack TP. 2017. SUPERMAN prevents class B gene expression and promotes stem cell termination in the fourth whorl of Arabidopsis thaliana flowers. Proceedings of the National Academy of Sciences, USA 116, 7166–7171.

Rebocho AB, Southam P, Kennaway JR, Bangham JA, Coen E. 2017. Generation of shape complexity through tissue conflict resolution. eLife 6, e20156.

Ripoll JJ, Zhu M, Brocke S, Hon CT, Yanofsky MF, Boudaoud A, Roeder AHK. 2019. Growth dynamics of the Arabidopsis fruit is mediated by cell expansion. Proceedings of the National Academy of Sciences, USA 116, 25333–25342.

Roeder AH. 2019. Computational image analysis for microscopy. Teaching tools in plant biology. The Plant Cell doi: 10.1105/tpc.119.tt0819

Roeder AH, Chickarmane V, Cunha A, Obara B, Manjunath BS, Meyerowitz EM. 2010. Variability in the control of cell division underlies sepal epidermal patterning in Arabidopsis thaliana. PLoS Biology 8, e1000367.

Roeder AH, Cunha A, Ohno CK, Meyerowitz EM. 2012. Cell cycle regulates cell type in the Arabidopsis sepal. Development 139, 4416–4427.

Rolland-Lagan AG, Bangham JA, Coen E. 2003. Growth dynamics underlying petal shape and asymmetry. Nature 422, 161–163.

Sauret-Güeto S, Schiessl K, Bangham A, Sablowski R, Coen E. 2013. JAGGED controls Arabidopsis petal growth and shape by interacting with a divergent polarity field. PLoS Biology 11, e1001550.

Smyth DR, Bowman JL, Meyerowitz EM. 1990. Early flower development in Arabidopsis. The Plant Cell 2, 755–767.

Strauss S, Sapala A, Kierzkowski D, Smith RS. 2019. Quantifying plant growth and cell proliferation with morphoGraphX. Methods in Molecular Biology 1992, 269–293.

Valuchova S, Mikulkova P, Pecinkova J, Klimova J, Krumnikl M, Bainar P, Heckmann S, Tomancak P, Riha K. 2019. Imaging plant germline differentiation within Arabidopsis flower by light sheet microscopy. bioRxiv 774299 [Preprint].

Whitewoods CD, Gonçalves B, Cheng J, Cui M, Kennaway R, Lee K, Bushell C, Yu M, Piao C, Coen E. 2020. Evolution of carnivorous traps from planar leaves through simple shifts in gene expression. Science 367, 91–96.

Wolny A, Cerrone L, Vijayan A, et al. 2020. Accurate and versatile 3D segmentation of plant tissues at cellular resolution. bioRxiv 2020.01.17.910562. [Preprint].