Quantitative live imaging of floral organ initiation and floral meristem termination in *Aquilegia*

Ya Min, Stephanie J. Conway and Elena M. Kramer
DOI: 10.1242/dev.200256

Editor: Yka Helariutta

Review timeline
Original submission: 9 October 2021
Editorial decision: 1 November 2021
First revision received: 14 December 2021
Accepted: 31 December 2021

Original submission

First decision letter

MS ID#: DEVELOP/2021/200256

MS TITLE: Quantitative live-imaging of Aquilegia floral meristems reveals distinct patterns of floral organ initiation and cell-level dynamics of floral meristem termination

AUTHORS: Ya Min, Stephanie J Conway, and Elena M Kramer

I have now received all the referees' reports on the above manuscript, and have reached a decision. The referees' comments are appended below, or you can access them online: please go to BenchPress and click on the 'Manuscripts with Decisions' queue in the Author Area.

As you will see, the referees express considerable interest in your work, but have some significant criticisms and recommend a substantial revision of your manuscript before we can consider publication. If you are able to revise the manuscript along the lines suggested, which may involve further experiments, I will be happy receive a revised version of the manuscript. Your revised paper will be re-reviewed by one or more of the original referees, and acceptance of your manuscript will depend on your addressing satisfactorily the reviewers' major concerns. Please also note that Development will normally permit only one round of major revision.

We are aware that you may be experiencing disruption to the normal running of your lab that make experimental revisions challenging. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

Please attend to all of the reviewers' comments and ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion. I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

This study quantifies cellular growth characteristics through live imaging of the floral meristem of Aquilegia coerulea. This species has a unique morphology and arrangement of floral organs compared to species that have previously been quantified in the literature. The authors correlate cell growth and division with primordium emergence and examine how cell growth characteristics relate to morphogenesis. The images are beautiful, and the quantification is carefully executed and likely required a large amount of labor. The quantification of compression of cell shape is particularly interesting, as how mechanical forces affect morphogenesis is an ongoing question. The authors also find that the abaxial side of the emerging organ grows faster than the adaxial side, which could be important for organ morphology and relevant to different species. Overall, the quantification of growth and the correlation with morphology is an important contribution to our understanding plant meristem growth, how diverse flower structures are created by floral meristems, and more generally the cellular growth mechanisms that create shapes during morphogenesis.

Comments for the author

The authors state that they have three replicates, and it would be useful to include images and analysis of the additional replicates in the supplement.
Several times in the manuscript the authors say that cell division may cause organ shape or growth. It is unclear how this would occur. Cell division can be associated with growth and can when a cell reaches a size threshold, but cell division alone merely subdivides a cell with a new cell wall and does not drive growth or morphogenesis. We think saying cell divisions is associated with growth would be a more accurate description.
Please define compression in your system. Do you mean a decrease in cell width?
In the introduction, the authors explain how genes control meristem termination; however, they do not directly examine genetic mechanisms of meristem termination. Instead the authors quantify morphological mechanisms of meristem growth and termination. It would be better to reserve the speculation on how these morphological mechanisms integrate with the genetic pathways for the discussion, so the reader does not expect to see them in the results. This would also shorten the introduction, which is rather long.

Minor comments:
It may be helpful to define “apocarpous gynoedium” in line 36 of the abstract for a broad audience. Similarly it may be helpful to explain whorls of organs in line 120. Also, the meanings “indeterminate” and “determine” are could be made clearer for a broad audience in lines 56-59.
In Figure 1, a picture of the mature carpels would be helpful so the reader can understand what shape the initiating carpels will form.
In Figure 2, the curvature heatmap and cell area should be labeled above the images to make the figure easier to read. Also, it would be helpful to label some of the organs in Figure 2.
In Figure 3, the heat map scales need to be added.
The author’s ultimate goal for the future is to integrate gene expression data and morphogenesis data. They might want to cite a recent paper from Arabidopsis flower development which pioneers this integration:
Refahi, Y., Zardilis, A., Michelin, G., Wightman, R., Leggio, B., Legrand, J., Faure, E., Vachez, L., Armezzani, A., Risson, A.-E., et al. (2021). A multiscale analysis of early flower development in Arabidopsis provides an integrated view of molecular regulation and growth control. Dev Cell 56, 540–556.

Reviewer 2

Advance summary and potential significance to field

Flower meristems differ from other types of meristems by their determinate growth. During which, the meristematic activities terminate, giving rise to (relative) fixed numbers of floral organs. In this manuscript Min, Conway et al. conducted a heroic work investigating this specific developmental process in

Aquilegia, an important model species representing the early diverging Ranunculaceae family in angiosperm. Comparing to *Arabidopsis*, the *Aquilegia* flower meristem produces many more whorls of stamens/staminodes before it ends up with five (instead of two) carpels, making it an intriguing studying subject. In this study, the authors coupled an optimised *in vitro* culturing method with state-of-art *in vivo* confocal time-lapse imaging to carefully examine the termination of *Aquilegia* flower meristem. With the help of MorphoGraphX, their results elegantly illustrated the cell division and expansion behaviours during this specific developmental window. I found the results are very interesting, and it is worth noting that this work is technically challenging as flower meristems at this stage are usually fully covered by initiated sepals and petals, and such developmental process (floral termination and carpel initiation) has not even been fully resolved into celllular details in *Arabidopsis*. Moreover Ranunculaceae represents an important group of species with great diversity in their florals displays, and the methods utilised in this study may have a great potential to be applied to other models (eg. Nigella, California poppy, Cysticapnos) with distinct floral structures/phyllotaxis.

Comments for the author

The paper is precisely written and the data are also presented in a very beautiful and reader-friendly way.
Here I have only a few minor suggestions that the authors may consider:

General points:
1. This paper presents data analysis by MorphographX, and it represents cellular growth data from the epidermal cells. It was well perceived that the organ initiation process is epidermis-driven, however, this may not apply to other developmental events happened during this time window. Such differences may apply to two parts: 1) the change of the overall shape from dome to flat for the centre of meristem, would it be possibly due to differential growth in the floral receptacle underneath the meristem? 2) the process of shaping of the carpel primordia after the initiation (where the authors find differences in cell division behaviour). Would it be possible this is a developmental process driven by the internal layers, rather than the epidermis? I feel discussion on this limitation/alternative explanation could be fitted into the discussion (eg. line 377-378 for the carpels).
2. Since this paper may influence many future works on flower development in other non-model species, I found the current material and method session may better be extended with more details. Particularly, adding a supplementary figure with description showing how the explants are exactly looking, together with the culturing/imaging setup would be very helpful for potential readers to try similar method for different experimental systems. I have noticed and read the detailed protocol provided as an external link of Kramer Lab (they are very detailed and well written!), and it might be more straightforward to grab some key elements like the images of dissected explants to here.
3. This paper focuses on the cellular dynamics, and this 'dynamics' relies on the live-imaging data from an *in vitro* culturing system. This may affect the time scale. In introduction or discussion session, would it be possible to add some comments/descriptions on how long this termination process will take in nature?
How would it compare to what happened in real plants? Still I feel this should not be left without mentioning in the paper, an estimate (eg. faster or prolonged?) would be sufficient.

Minor points:
1. Page 8 - Fig 2: would it be possible to add arrows marking for which primordia they exactly are in the front view panels? It might be rather difficult for a fresh reader to judge the location and identity of emerging primordia, especially for TP1-TP3. Also, some of the MorphographX images in the right two panels were off-centered, and in different magnifications (TP2, TP6 right most panels).
2. Page 10 - Fig 3: panels A-E/A'-E' lack a scale bar for the heatmap of area expansion rate (A-E).
3. In Fig 2,3,S1: are these meristem overview 'Top views' instead of 'Front view'?

First revision

Author response to reviewers' comments

Reviewer 1 Advance Summary and Potential Significance to Field:

This study quantifies cellular growth characteristics through live imaging of the floral meristem of Aquilegia coerulea. This species has a unique morphology and arrangement of floral organs compared to species that have previously been quantified in the literature. The authors correlate cell growth and division with primordium emergence and examine how cell growth characteristics relate to morphogenesis. The images are beautiful, and the quantification is carefully executed and likely required a large amount of labor. The quantification of compression of cell shape is particularly interesting, as how mechanical forces affect morphogenesis is an ongoing question. The authors also find that the abaxial side of the emerging organ grows faster than the adaxial side, which could be important for organ morphology and relevant to different species. Overall, the quantification of growth and the correlation with morphology is an important contribution to our understanding plant meristem growth, how diverse flower structures are created by floral meristems, and more generally the cellular growth mechanisms that create shapes during morphogenesis.

Reviewer 1 Comments for the Author:

The authors state that they have three replicates, and it would be useful to include images and analysis of the additional replicates in the supplement.
Response: We have added a supplemental figure (Fig. S2) to show examples of biological replicates that were used for TP1 and TP5.

Several times in the manuscript the authors say that cell division may cause organ shape or growth. It is unclear how this would occur. Cell division can be associated with growth and can when a cell reaches a size threshold, but cell division alone merely subdivides a cell with a new cell wall and does not drive growth or morphogenesis. We think saying cell divisions is associated with growth would be a more accurate description.
Response: We agree with this comment. We have changed “driven by cell division/expansion” to “(strongly) associated with cell division/expansion” in a few places (e.g. line 196, 254), and added line 42-44 in the introduction and line 276-279 in the discussion.

Please define compression in your system. Do you mean a decrease in cell width?
Response: Yes. We have also added “If the width of a cell along a given axis is decreased during the interval, it indicates compression rather than expansion.” in line 201-203.

In the introduction, the authors explain how genes control meristem termination; however, they do not directly examine genetic mechanisms of meristem termination. Instead the authors quantify morphological mechanisms of meristem growth and termination. It would be better to reserve the speculation on how these morphological mechanisms integrate with the genetic pathways for the discussion, so the reader does not expect to see them in the results. This would also shorten the introduction, which is rather long.
Response: Thank you and we agree with this suggestion. We have removed the part mentioning the molecular pathways in the Introduction and integrated it into the Discussion (line 293-301).

Minor comments:
It may be helpful to define “apocarpous gynoedium” in line 36 of the abstract for a broad audience. Similarly, it may be helpful to explain whorls of organs in line 120. Also, the meanings “indeterminate” and “determine” are could be made clearer for a broad audience in lines 56-59.
Response: Added definitions in line 51-55, 103-104.

In Figure 1, a picture of the mature carpels would be helpful so the reader can understand what shape the initiating carpels will form.
Response: We have added a supplemental figure (Fig. S1) to show the mature organs of an *Aquilegia* flower.

In Figure 2, the curvature heatmap and cell area should be labeled above the images to make the figure easier to read. Also, it would be helpful to label some of the organs in Figure 2.
Response: We have modified the legend to specify that all the heatmaps for each column were using the same heatmap scale that is positioned above the respective columns.

In Figure 3, the heat map scales need to be added.
Response: Added – apologies for the oversight!

The author's ultimate goal for the future is to integrate gene expression data and morphogenesis data. They might want to cite a recent paper from Arabidopsis flower development which pioneers this integration: Refahi, Y., Zardilis, A., Michelin, G., Wightman, R., Leggio, B., Legrand, J., Faure, E., Vachez, L., Armezzani, A., Risson, A.-E., et al. (2021). A multiscale analysis of early flower development in Arabidopsis provides an integrated view of molecular regulation and growth control. Dev Cell 56, 540–556.
Response: Thank you for the suggestion. It is indeed very relevant, and we have cited it in the manuscript (line 69).

Reviewer 2 Advance Summary and Potential Significance to Field:

Flower meristems differ from other types of meristems by their determinate growth. During which, the meristematic activities terminate, giving rise to (relative) fixed numbers of floral organs. In this manuscript, Min, Conway et al. conducted a heroic work investigating this specific developmental process in *Aquilegia*, an important model species representing the early diverging Ranunculaceae family in angiosperm. Comparing to *Arabidopsis*, the *Aquilegia* flower meristem produces many more whorls of stamens/staminodes before it ends up with five (instead of two) carpels, making it an intriguing studying subject. In this study, the authors coupled an optimised *in vitro* culturing method with state- of-art *in vivo* confocal time-lapse imaging to carefully examine the termination of *Aquilegia* flower meristem. With the help of MorphoGraphX, their results elegantly illustrated the cell division and expansion behaviours during this specific developmental window. I found the results are very interesting, and it is worth noting that this work is technically challenging as flower meristems at this stage are usually fully covered by initiated sepals and petals, and such developmental process (floral termination and carpel initiation) has not even been fully resolved into celllular details in *Arabidopsis*. Moreover, Ranunculaceae represents an important group of species with great diversity in their florals displays, and the methods utilised in this study may have a great potential to be applied to other models (eg. Nigella, California poppy, Cysticapnos) with distinct floral structures/phyllotaxis.

Reviewer 2 Comments for the Author:

The paper is precisely written and the data are also presented in a very beautiful and reader-friendly way. Here I have only a few minor suggestions that the authors may consider:

General points:
1. This paper presents data analysis by MorphographX, and it represents cellular growth data from the epidermal cells. It was well perceived that the organ initiation process is epidermis-driven, however, this may not apply to other developmental events happened during this time window. Such differences may apply to two parts: 1) the change of the overall shape from dome to flat for the centre of meristem, would it be possibly due to differential growth in the floral receptacle underneath the meristem?
Response: While mechanical forces in the epidermis are known to play a role in cell and tissue behaviors within meristems and initiating primordia, to the authors' knowledge there is no evidence (in any system) to suggest that differential growth of the flower receptacle influences the overall shape of the meristem. In Aquilegia, there is at least 8 whorls of organs in between the receptable and the meristem (e.g. Figure 1D-F), therefore we consider it unlikely that this is an influencing factor affecting shape of the floral meristem during FMT in Aquilegia.

2) the process of shaping of the carpel primordia after the initiation (where the authors find differences in cell division behaviour). Would it be possible this is a developmental process driven by the internal layers, rather than the epidermis?
Response: We agree that internal layers could be playing a role in shaping carpel primordia,

however this is currently outside the scope of the available imaging capabilities and lineage tracking software packages. We have added discussion of this limitation on to the beginning of the discussion (line 273-290).

2. Since this paper may influence many future works on flower development in other non-model species, I found the current material and method session may better be extended with more details. Particularly, adding a supplementary figure with description showing how the explants are exactly looking, together with the culturing/imaging setup would be very helpful for potential readers to try similar method for different experimental systems. I have noticed and read the detailed protocol provided as an external link of Kramer Lab (they are very detailed and well written!), and it might be more straightforward to grab some key elements like the images of dissected explants to here.
Response: Thank you for this suggestion, we agree that a detailed protocol is needed to accompany this paper, and as you noted, we have a detailed protocol ready to be published. We aim to publish our full protocol as soon as possible, however, several journals advised us that protocol papers are not accepted for publication prior to the publication of the data paper and therefore we are waiting for publication of this paper before we can publish the full protocol. We also attempted to submit the protocol to several pre-print servers, however none of these (biorxiv, Preprints) accept protocol papers. We appreciate your suggestion of some key elements of the protocol should be added to this paper, however, we feel that the full protocol with step-by-step methods will provide other researchers the best chance to successfully replicate these techniques in their systems.

3. This paper focuses on the cellular dynamics, and this 'dynamics' relies on the live-imaging data from an *in vitro* culturing system. This may affect the time scale. In introduction or discussion session, would it be possible to add some comments/descriptions on how long this termination process will take in nature? How would it compare to what happened in real plants? Still I feel this should not be left without mentioning in the paper, an estimate (eg. faster or prolonged?) would be sufficient.
Response: Thank you for this comment, we have added this to the beginning of the discussion (line 273-290).

Minor points:
1. Page 8 - Fig 2: would it be possible to add arrows marking for which primordia they exactly are in the front view panels? It might be rather difficult for a fresh reader to judge the location and identity of emerging primordia, especially for TP1-TP3. Also, some of the MorphographX images in the right two panels were off- centered, and in different magnifications (TP2, TP6 right most panels).
Response: Apologies, this was an oversight. We have corrected the figure.

2. Page 10 - Fig 3: panels A-E/A'-E' lack a scale bar for the heatmap of area expansion rate (A-E).
Response: Apologies, this was an oversight. We have corrected the figure.

3. In Fig 2,3,S1: are these meristem overview 'Top views' instead of 'Front view'?
Response: Thank you for the suggestion. We have made the recommended change to the wording.

Second decision letter

MS ID#: DEVELOP/2021/200256

MS TITLE: Quantitative live-imaging of floral organ initiation and floral meristem termination in *Aquilegia*

AUTHORS: Ya Min, Stephanie J Conway, and Elena M Kramer
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Development, pending our standard ethics checks.

Reviewer 1

Advance summary and potential significance to field

We commend the authors on this beautiful work.

Comments for the author

The authors have thoroughly and thoughtfully addressed our comments.

Reviewer 2

Advance summary and potential significance to field

In the revised manuscript, the authors have adequately addressed the concerns by correcting figures and adding new discussions from the comments of the previous round of review. I feel the paper provides significant technical advances in the field of plant evo-devo, especially for flower development. I would highly recommend it could be published in the Development journal.

Comments for the author

In the revised Figure 2, there were a few scale bars from MorphographX(thin white lines). It may cause a bit confusion as new scale bars have been added for each panel. The author may consider remove it before the final production.