Quantifying Shape Changes and Tissue Deformation in Leaf Development\footnote{This work was supported by the Natural Sciences and Engineering Research Council of Canada (discovery grant to A.-G.R.-L.).} \footnote{Address correspondence to arolland@uottawa.ca.}

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The analysis of biological shapes has applications in many areas of biology, and tools exist to quantify organ shape and detect shape differences between species or among variants. However, such measurements do not provide any information about the mechanisms of shape generation. Quantitative data on growth patterns may provide insights into morphogenetic processes, but since growth is a complex process occurring in four dimensions, growth patterns alone cannot intuitively be linked to shape outcomes. Here, we present computational tools to quantify tissue deformation and surface shape changes over the course of leaf development, applied to the first leaf of Arabidopsis (Arabidopsis thaliana). The results show that the overall leaf shape does not change notably during the developmental stages analyzed, yet there is a clear upward radial deformation of the leaf tissue in early time points. This deformation pattern may provide an explanation for how the Arabidopsis leaf maintains a relatively constant shape despite spatial heterogeneities in growth. These findings highlight the importance of quantifying tissue deformation when investigating the control of leaf shape. More generally, experimental mapping of deformation patterns may help us to better understand the link between growth and shape in organ development.

The analysis of biological shapes is a field of broad interest, with diverse applications ranging from medical imaging to comparative anatomy and botany (Lestrel, 2011). Within plant biology, the wide variation of leaf shapes among species has long intrigued evolutionary biologists, physiologists, and developmental biologists alike. Leaves typically develop into flat structures that maximize photosynthetic surface, with variations that may help to facilitate gas exchange, offset water loss, improve convective cooling, increase mechanical support, or reduce resistance to physical environmental forces, for example (for review, see Tsukaya, 2006; Cronk, 2009). The processes controlling leaf shape development, therefore, are important to plant survival and biomass accumulation and hence have important agricultural implications.

Understanding how leaf shape is controlled typically involves studying shape variation among related species and in shape mutants and requires tools to quantify phenotypic differences. In recent years, several sophisticated semiautomatic methods have been developed to analyze leaf shapes in terms of their two-dimensional (2D) profiles. Analyses range from simple length and width measurements and allometric ratios to statistical analysis of outline coordinates (Langlade et al., 2005; Bylesjö et al., 2008; Weight et al., 2008; Backhaus et al., 2010).

Leaf shape can also be quantified in terms of a three-dimensional (3D) surface, which can range from flat to curved or ruffled, as observed in nature and in many leaf shape mutants. Approaches to 3D shape analysis based on flattened and unflattened leaf dimensions in the proximodistal and mediolateral axes have been presented by Liu et al. (2010) and Wu et al. (2007). Kaminuma et al. (2004) developed a technique for obtaining coordinates of the leaf surface in vivo, from which they measured the angle of the surface across the leaf and fit curves along the leaf proximodistal and mediolateral axes to characterize blade epinasty.

These existing methods for leaf shape analysis are very useful for detecting and quantitatively describing shape differences, but they do not provide information about the underlying mechanisms that give rise to those shape differences. For example, the correspondence between evenly spaced outline points (Langlade et al., 2005; Bylesjö et al., 2008; Weight et al., 2008; Backhaus et al., 2010) is arbitrary. Furthermore, existing work has not provided an analysis of leaf shape in terms of both 2D and 3D phenotypic features together, and with the exception of Kaminuma et al. (2004), the methods are destructive and cannot be used to measure changes in shape in vivo.

Differences in shapes between two organs arise through differences in how the organs grow. It is impossible, however, to ascertain from differences in final organ shape what differences in growth gave rise to them, as the possible combinations of spatial and temporal alterations in growth patterns that could be
responsible for the final shape are endless. By the same token, it can be difficult to conceptualize from growth patterns how the interconnected tissues will deform and, thus, how overall organ shape will change.

To address this issue, Kennaway et al. (2011) recently proposed a simulation modeling framework to investigate how a shape may locally and globally deform under the control of spatially distributed growth- and polarity-regulating substances. This framework was used to hypothesize how growth and shape deformation may be controlled in flowers (Green et al., 2010; Sauret-Güeto et al., 2013) and leaves (Kuchen et al., 2012). However, to date, shape deformation patterns have not been quantified experimentally. Here, we use data collected for a study on leaf growth (Remmler and Rolland-Lagan, 2012) to develop a method for describing 3D surface shape and shape deformations during leaf development. In particular, we generate experimental maps of tissue deformation, which offer a new approach to investigating shape differences and uncovering the link between growth and shape during development.

RESULTS

In this study, we use an existing time-lapse data set of Arabidopsis (Arabidopsis thaliana) first rosette leaf development, which includes 3D leaf surface reconstructions and data on the displacement of particles applied to the 3D leaf surface (Remmler and Rolland-Lagan, 2012). These data, originally collected to track leaf growth patterns, are used here in a different context to develop methodologies for the analysis of shape. Specifically, we extract various measurements of leaf shape and assess local tissue deformations underlying leaf shape changes over the course of development; we measure the age of the plants in days after sowing (DAS).

Global Descriptions of Leaf Shape and Curvature

From 3D reconstructions of leaf surfaces, we compute simple 3D measurements of leaf size (Fig. 1; for details, see “Materials and Methods”) such as length, width, and area (Fig. 2, A and B) along with corresponding measurements of growth (Fig. 2, C and D) and shape (Fig. 2, E and F). The length, width, and area curves dip slightly at DAS16 (i.e. 16 DAS) based on a secondary data set collected a few weeks after the original data set, which was followed from DAS10 to DAS15 and for which plants grew slightly more from DAS14 to DAS15 compared with plants from the first data set (Remmler and Rolland-Lagan, 2012).

Global shape measurements include the leaf index (the ratio between the length and width of a leaf; Tsukaya, 2002) and the curvature index (CI). The leaf index does not fluctuate notably across the time points measured (Fig. 2E), with an overall average of 1.09 (SD 0.07). We calculate CI following the conventions of Wu et al. (2007) and Liu et al. (2010); the CI along the transverse axis ($CI_{\text{trans}}$) is calculated from the ratio between the 3D and 2D widths of the leaf, and the CI along the longitudinal axis ($CI_{\text{long}}$) is calculated from the ratio between the 3D and 2D lengths (see “Materials and Methods”; Fig. 2F). A CI of 0 represents complete flatness, and increasing values represent increasing curvature. On average, $CI_{\text{trans}}$ increases slightly from 0.02 (SD = 0.01) at DAS7, leveling off around 0.03 to 0.04 from DAS11 onward. Mean longitudinal curvature is stronger in the earlier time points, with a $CI_{\text{long}}$ of 0.13 (SD = 0.03) at DAS7, declining over the next few time points and leveling off around 0.05 from DAS11 onward.

Curvature can be visualized for all samples by plotting virtual cross sections (Fig. 3) of the leaf surface across the longitudinal (proximodistal) and transverse (mediolateral) axes (light gray lines in Fig 3). Those representations show that the 3D shape of leaves varies between samples. For instance, at DAS11, we can see that some leaves curve downward while others curve upward.

Quantifying an Average 3D Leaf Shape

In order to quantify the typical 3D shape of a leaf at a given time point, we can average shape data across multiple samples. However, as 3D shapes vary between samples, computing an average 3D leaf shape by simply averaging height values may not accurately represent the data set. Therefore, we also calculate a characteristic leaf shape by averaging the height values of the subset of samples that best represent the data set (for details, see “Materials and Methods”; Supplemental Methods S1; Supplemental Fig. S1). In cases where the shapes of all (DAS7–DAS9) or most (DAS10 and DAS13–DAS19) samples are similar to each other, and in cases where shapes are variable with no predominant shape (DAS12), the characteristic leaf shape will change.
shape is very close to the mean leaf shape (cross sections of characteristic and mean leaf shape overlap are shown in Fig. 3). In cases where samples differ in shape but a majority of samples have similar shapes (DAS11), the characteristic leaf shape matches the data set better than the mean leaf shape (Fig. 3). The 3D characteristic leaf surface changes shape from what looks a bit like a saddle shape at DAS7 to more of a dome shape at DAS19 (Fig. 4A; see Supplemental Video S1 for a 3D animation and Supplemental Fig. S2 for an illustration of all time points from DAS7–DAS19).

Quantifying Spatial Variations in Curvature

Both the 2D and 3D shapes of an organ depend on the organ’s underlying growth patterns. In the case of a thin organ that can be approximated as a surface, excess growth on the perimeter of the organ causes the edges to buckle in 3D to create wavy edges or a saddle shape. Conversely, excess growth in the central part causes that central part to bulge out and make a cup or dome shape (depending on whether it bulges up or down). Therefore, local curvature patterns do not reflect growth patterns in a direct way (ruffled edges do not mean that there is a succession of high and low growth areas along the margin, for instance), but they do result from spatial patterns of growth in a sometimes nonintuitive way (Sharon et al., 2004; Prusinkiewicz and Barbier de Reuille, 2010).

Curvature in the context of growing plant surfaces has been studied using the notion of Gaussian curvature (Sharon et al., 2004; Prusinkiewicz and Barbier de Reuille, 2010), which can be measured at any point on a surface as the product of principal curvatures at that point. A shape with zero Gaussian curvature at all points across its surface can be flattened without any tears or folds, while a shape with negative Gaussian curvature (such as the saddle) will result in excess folds when flattened, and a shape with positive Gaussian curvature (such as the dome) will result in excess bulges when flattened.

Figure 2. Mean ± s leaf size, shape, growth, and curvature measurements over time. A, 3D length and width. B, 3D surface area. C, Relative growth in 3D length and width. D, Relative growth in 3D surface area. E, Leaf index (3D length-to-width ratio). F, C_long and C_trans (3D-to-2D measurement ratio). n = 12 to 34 samples.

Figure 3. Transverse and longitudinal cross sections of all leaf samples at each time point, with corresponding cross sections of mean and characteristic leaf shape. Digital transverse (A) and longitudinal (B) cross sections of the leaf surface were obtained as per the illustration in Figure 1. Each cross section is scaled to the mean 2D leaf width (A) or length (B) of its DAS. Lines representing cross sections of individual samples are plotted with 60% transparency so that more frequent, overlapping curvatures are more visually dominant and outliers are less so. Cross sections of the mean leaf shape and characteristic leaf shape for each DAS are shown in blue and red, respectively, with no transparency. Units on the horizontal axes are in μm, and bars = 1 mm. n = 12 to 34 samples.
curvature (such as the dome or cup) cannot be flattened without causing tears in the tissue.

Once we have computed the characteristic leaf shape for all time points analyzed, we can calculate the Gaussian curvature locally across the characteristic leaf surface for each time point (see “Materials and Methods”; Supplemental Fig. S3). Results show that the sign of the Gaussian curvature varies across the surface of the leaf (so the leaf surface is never perfectly dome/cup or saddle shaped) and that spatial patterns of curvature vary over time (Fig. 4, C and D), which is consistent with the leaf changing 3D shape. However, we note that the absolute value of the Gaussian curvature remains low and decreases over time: values are on the order of $10^{-6} \, \mu m^{-2}$ at DAS7 and $10^{-8} \, \mu m^{-2}$ at DAS19, compared with higher values on the order of $10^{-4} \, \mu m^{-2}$ in the shoot apical meristem (Dumais and Kwiatkowska, 2002). Detailed results on leaf heights are given in Supplemental Figure S2, and details of the spatial patterns of Gaussian curvature for all time points are given in Supplemental Figure S4.

**Description of Leaf Shape Deformations**

Global shape changes from one time point to the next result from local deformations occurring throughout the tissue. However, different patterns of deformation may give rise to the same overall shape changes (Supplemental Fig. S5). In order to understand the mechanisms underlying shape changes, it is necessary to characterize how the tissue deforms locally over time. We characterized local patterns of deformation using data on the displacement of fluorescent particles applied to growing leaf surfaces.

To illustrate the movement of tissue in a way that can be intuitively related to shape changes, we can use changes in particle $(x,y)$ coordinates from one time point to the next to estimate the 2D geometric transformation that would take the set of particle $(x,y)$ positions at time $t$ to the set of particle $(x,y)$ positions at time $t + 1$. The transformation mathematically describes how the particle $(x,y)$ coordinates at one time point can be warped to their corresponding positions at the next time point (Fig. 5, A–C). When applied to the coordinates of a 2D square grid fitted across a leaf sample at a starting time point $t$, this transformation shows how the grid and leaf outline would be deformed over the course of growth to $t + 1$ (Fig. 5, D and E; for details, see “Materials and Methods” and Supplemental Methods S2).

In order to calculate the average deformation from one time point to the next based on multiple samples, we can compute a transformation for each sample and record the grid and leaf outline before and after deformation. Grid positions and leaf outlines can then be averaged across samples at time $t$ and at time $t + 1$ (Supplemental Fig. S6), and the corresponding “average transformation” from one average grid to the next can be computed and recorded (see “Materials and Methods”). The average grid and outline deformations from one time point to the next are shown in Figure 6 for all pairs of time points from DAS7 to DAS8 to DAS18 to DAS19. Deformed outlines at time $t + 1$ closely match the measured outlines for samples at that stage. Although deformation patterns are computed in 2D, we have the $z$ coordinates at each point of the characteristic leaf surface, which we can use to find the $z$ coordinate of each point of the deformation grids. Consequently, deformations can be viewed in 3D (Supplemental Video S2; see “Materials and Methods”).

Having recorded surface deformations in 3D, we can then test whether those deformations reflect growth patterns obtained from the 3D positions of fluorescent particles described by Remmler and Rolland-Lagan (2012). This is done by computing the relative growth of each square of the grid in 3D space from one time point to the next (Supplemental Fig. S7). The resulting grid growth patterns are in good agreement with the growth patterns obtained from particle tracking (Fig. 7, A, B, and D; Remmler and Rolland-Lagan, 2012), validating the approach.

Substantial deformation occurs from DAS7 to DAS8, DAS8 to DAS9, and DAS9 to DAS10 in an upward-curving manner, with tissue stretching outward more strongly at the base and overall tissue growth increasing from tip to base. Both deformation and tissue growth decrease over time: deformation from one time point to the next is hardly detectable after DAS12, and increase in grid size is minimal beyond DAS14 (Fig. 6).

Once average grids have been computed for each $(t,t + 1)$ pair of time points and the corresponding

![Figure 4](image-url). 3D shape and curvature patterns for the characteristic leaf shape at DAS7 and DAS19. Leaf heights in $\mu m$ at DAS7 (A) and DAS19 (B) are represented relative to a plane $z = 0$ fitted through the leaf surface. Local measures of Gaussian curvature were sampled throughout the leaf surface at DAS7 (C) and DAS19 (D). Bar in A and B = 1 mm.
transformation has been recorded (see “Materials and Methods”), we can apply all transformations in succession from DAS7 (i.e. starting with the average square grid at DAS7) through later time points in order to visualize how the different regions of the leaf displace each other through growth (Fig. 7, C and D). By comparing the reference line in Figure 7C at DAS7 and DAS11, we see that tissue that is initially only about one-quarter of the way up the leaf relative to the overall leaf length is displaced to halfway up the leaf relative to the overall leaf length over the course of those 4 d. The accuracy of applying cumulative deformations across multiple time points can be assessed by computing relative growth patterns of the deforming grid: at early time points, grid growth patterns match the patterns computed from single-day deformations, but by DAS11 this is no longer the case (in Fig. 7C, the growth deformation pattern from DAS11–DAS12 is plotted on the DAS11 leaf). This is because any small discrepancy between the computed and the true deformation from one time point to the next is carried over and compounded through later time points. Therefore, we reset a grid on the DAS11 shape to follow deformation from DAS11 to DAS19 (Fig. 7, C and D).

DISCUSSION

The development of organ shape arises through interactions between growth-patterning processes and tissue deformation: on the one hand, shape changes result from underlying growth patterns; on the other hand, tissue deformation itself changes the domain on which growth-patterning processes occur. In order to understand how a shape is generated and why shapes differ among organs, it is necessary to quantify and visualize spatial and temporal variations in both growth and tissue deformation patterns. We previously showed a method for quantifying growth patterns at the surface of leaves in 3D (Remmler and Rolland-Lagan 2012), and here we present an approach to concurrently quantify leaf 3D surface shape and deformation patterns experimentally throughout development.

The Relation between Leaf Growth Patterns and 3D Shape Can Be Studied Quantitatively and Experimentally

Spatial heterogeneity in growth can distort the 3D shape of a planar organ. For example, the Arabidopsis peapod mutant has abnormally dome-shaped leaves, presumed to be the result of excess expansion of the medial regions compared with the margins (White, 2006). By contrast, the snapdragon (Antirrhinum majus) CINNACINATA mutant has abnormally ruffled leaves, argued to be the result of a growth gradient with upward-curving isolines, in which marginal regions of the leaf grow more than the medial regions (Nath et al., 2003). The link between growth patterns and shape deformation in leaves, however, has not been tested experimentally, and the link between any other type of spatial growth patterns and shape deformation is unclear.

The proposed method makes it possible to investigate how growth patterns correlate with 3D shape changes. In our previous work on quantifying growth patterns for this data set, we found that growth was spatially heterogeneous, with an increasing tip-to-base gradient. In particular, in early time points (DAS7–DAS10), the proximodistal growth gradient has distinctively downward-curving isolines (Remmler and
Rolland-Lagan, 2012), such that at any point along the proximodistal axis of the leaf, the sides of the leaf are growing slower than the center (for instance, compare the relative growth values in lateral and central circles drawn on the DAS9 leaf in Fig. 7A). Curvature measurements show that this growth pattern is associated with an increase in $C_{\text{trans}}$ from DAS9 to DAS10 and from DAS10 to DAS11 only. The $C_{\text{long}}$, however, decreases throughout all time points, and so does the magnitude of the Gaussian curvature across the leaf surface. Kennaway et al. (2011) proposed a modeling framework to simulate tissue deformation in 3D based on hypothetical growth-patterning factors. Therefore, it would be interesting to test whether the observed relation between growth patterns and 3D shape can be reproduced in silico.

Understanding How Shape Changes Are Controlled Requires Local Tissue Deformation Data

The overall leaf shape (measured, for instance, from length-to-width ratios) remained relatively constant throughout the developmental stages analyzed. Based on overall shape measurements only, therefore, one might assume that leaf shape is determined at earlier stages and that over the time points analyzed the leaf simply expands homogenously. However, we know that this is not the case, as growth patterns are spatially heterogenous (Remmler and Rolland-Lagan, 2012). Here, we show that the higher growth in proximal regions compared with distal regions and (until DAS11) the lower growth on the sides of the leaf compared with the center are associated with a

Figure 6. Single-day average leaf tissue deformation patterns. The deformation pattern from one time point ($t$) to the next time point ($t + 1$) is displayed as a predeformation and a postdeformation shape displayed side by side for each pair of time points from DAS7 to DAS9 to DAS18 to DAS19. The predeformation shape (on the left of each pair of shapes) shows the average grid and average leaf outline computed from samples at time $t$ that are tracked from $t$ to $t + 1$. The postdeformation shape (on the right of each pair of shapes) shows the average deformed $t + 1$ grid computed from deformed grids for all samples tracked from $t$ to $t + 1$. Two leaf outlines are displayed on the postdeformation shape: the outline shown in gray (blue in the online version) is the computed average deformed outline at $t + 1$, while the outline shown in black is the actual measured average of all sample outlines at $t + 1$ that were tracked from $t$ to $t + 1$. The deformed outline is displayed on top, such that if both outlines match perfectly only the deformed outline will be visible. Grids of $8 \times 8$ regions are used instead of fine mesh grids with more regions in order to improve visibility. Bars = 1 mm. [See online article for color version of this figure.]
distinctly upward-curving deformation of the tissue, as tissue in lateral positions is being pushed to more distal positions. In other words, tissue at the side regions of the leaf not only originates from the expansion of tissue in that same area but also from displaced tissue from areas of faster growth (Supplemental Fig. S8), allowing the leaf to maintain a relatively constant shape. It will be interesting to characterize growth and deformation patterns in other types of leaves (e.g. mutants or other species) to assess under which conditions nonhomogenous types of growth and deformation patterns allow for leaf shape maintenance.

The results of this study highlight the need to measure local tissue deformation patterns in conjunction with growth in order to describe how global shape changes arise. Combining such quantitative analyses with simulation modeling should provide a rigorous framework for uncovering growth-patterning mechanisms.

Figure 7. Mapping growth of grid deformations. A, Maps of relative growth patterns as computed by Remmler and Rolland-Lagan (2012). Black circles on the DAS9 growth map illustrate the fact that at a given position along the proximodistal axis of the leaf, regions in lateral positions grow slower than regions in central positions. B, Relative growth computed from deformation grids, using fine-grid meshes. For each grid (of 40 rows and 40 columns) fitted at time \( t \) over the mean leaf shape of samples that deform from time \( t \) to \( \tau + 1 \), we compute the relative growth of each square within the leaf from \( t \) to \( \tau + 1 \). Growth is computed from grids in 3D space. C, Cumulative grid deformation and associated relative growth of grid squares. Here, we set a grid on the leaf at DAS7 and let it deform by successively applying the day-to-day deformations computed from one time point to the next. Each colored grid at DAS \( t \) shows how much growth occurs in each grid square from DAS \( t \) to DAS \( \tau + 1 \). How the grid deforms over multiple time points is shown. The top row shows deformations from DAS7 to DAS12, and the bottom row shows deformations from DAS11 to DAS19. Only a subset of grid lines are shown for better visualization of growth patterns and tissue deformation. In the top row, the black arrow on the DAS7 grid points to the third grid line (shown as a thicker line) from the base of the leaf. The black arrow on the DAS11 grid points to the corresponding grid line. D, Color code for relative growth, in percentage, used in A to C. Different color scales are used for different time points to better visualize spatial variations in growth. Bars = 1 mm.
A New Use for Grid Transformations

The grid deformations presented here relate to some of the founding work in morphogenesis. In his renowned book *On Growth and Form*, Thompson (1917) showed how a grid of points defining the adult morphology of one species could be warped to produce the adult morphology of a related species through relatively simple affine transformations. This intriguing result, however, was left without a sound biological explanation. It would be more informative to explore the correspondence in shapes throughout development, since the transformations Thompson (1917) discovered actually reflect the accumulation of differences in deformation between species as they grow (Arthur, 2006). In other words, understanding the underlying mechanisms that give rise to shape differences between species requires a comparative analysis of shape transformations throughout the course of development. Here, we showed that tracking microscopic fluorescent particles applied topically to a leaf surface can be used to compute average tissue deformation over the course of development. This could be used to visualize and quantitatively assess how and at what stage of development the shapes of two species (or a mutant and a wild type) begin to diverge, making it possible to better understand the signaling mechanisms that give rise to shape differences.

In the past few years, frameworks for the simulation modeling of developing tissues have been proposed (Kennaway et al., 2011; Merks et al., 2011; for review, see De Vos et al., 2012). In particular, Kennaway et al. (2011) proposed a framework for simulating the development of whole organs over large deformation scales. This framework was used to study growth and shape in flowers (Green et al., 2010; Sauret-Güeto et al., 2013) and leaves (Kuchen et al., 2012). For instance, cell vertices were used as landmarks by Kuchen et al. (2012) to track growth in three leaf samples at early developmental stages, and the resulting growth data were used to build a simulation model of early leaf morphogenesis. Simulation models need to be validated experimentally and quantitatively (De Vos et al., 2012) and refined in an iterative fashion through feedback between modeling and the quantitative analysis of experimental data, following systems biology and computational morphodynamics approaches (Roeder et al., 2011). The method we propose here makes it possible to compute tissue deformations from experimental growth data and, therefore, could be used to build and/or quantitatively validate simulation models of leaf morphogenesis.

Wider Applicability of the Methodology

Here, we used an image data set acquired using a macroscope (Remmler and Rolland-Lagan, 2012), which permits time-lapse imaging over a wide range of sizes but precludes the analysis of very young leaves (for a comparison with the stages analyzed by Kuchen et al. [2012], see Supplemental Table S1, and for more information on the limitations and wider applicability of the experimental setup, see Remmler and Rolland-Lagan, 2012). In particular, at the earliest stage analyzed (DAS7), cell divisions no longer occur throughout the leaf but are restricted to its base (Kazama et al., 2010). However, the computational methodology presented here is not dependent upon sample dimensions or microscope setup, as it solely relies on the availability of surface and landmark data. The same methodology, for instance, could be applied to quantify shape and surface deformation patterns from confocal microscopy data acquired at earlier leaf developmental stages. More broadly, surface deformation patterns could be computed for other developing tissues and organs of any size and/or developmental stage, provided the existence of suitable landmarks and an imaging setup for surface data acquisition.

CONCLUSION

We have presented a method for quantifying 3D surface shape changes during leaf development as well as a novel approach for describing average tissue deformation. The shape and curvature analysis will be interesting to apply to the study of mutants or other species that have more pronounced curvatures. The grid deformations we present are reminiscent of the famous grid transformations of Thompson (1917) between the morphologies of related species, except that we are able to show how these transformations arise over the course of development as a function of growth patterns. In the future, the tools we have presented can be used to study the basis of shape variations between mutants and/or species and/or plants grown in different conditions and will be an important complement to growth analyses, since not only growth-patterning mechanisms but also the interconnectivity and physical properties of the tissue dictate how tissue deforms. With this information, we will be better able to understand the connection between growth patterns and the development of organ shape.

MATERIALS AND METHODS

The custom computational tools described herein were written in Matlab R2012 and R2013a (The Mathworks) for Windows and are available at http://hdl.handle.net/10393/30401 (permanent link). Computational details of the algorithms are given in Supplemental Methods S1 and S2.

Data Set

A time-lapse data set of the first rosette leaf of Arabidopsis (*Arabidopsis thaliana*; Landsberg erecta [glabra-1, seed stock cs04 from the Arabidopsis Biological Resource Center]) obtained for a previous study (Remmler and Rolland-Lagan, 2012) was used for this analysis. The data were originally obtained by applying fluorescent microparticles to the surfaces of multiple leaf samples and imaging each sample every 24 h with a fluorescence macroscope (Leica Z16 APO A MacroFluor motorized fluorescence macroscope; Leica Microsystems). In each leaf imaging session, a multifocused image of the leaf,
a multifocussed image of the fluorescent particles on the leaf, and a depth map of the leaf surface were obtained. These images were then used to digitally reconstruct each leaf surface in 3D as a mesh and to identify and track the coordinates of particles on the leaf surface over time. The data covered a wide range of developmental stages, from DAS7, when the leaf surface is exposed, through DAS19, when growth has nearly stopped. Each sample was monitored for 4 to 7 days to obtain images for 12 to 34 samples per time point. Details on growing conditions, particle application, imaging, 3D leaf reconstruction, particle identification, and particle tracking are given by Remmler and Rolland-Lagan (2012).

Measuring Leaf Dimensions Digitally in 3D

To measure the leaf dimensions from the 3D surface reconstruction, we place a line of points along the midline of the leaf from the base to the tip (longitudinal axis) and another line of points across the broadest part of the leaf from side to side (transverse axis), as depicted in Figure 1, and then interpolate the z coordinate of each point on the leaf surface. We compute the 3D length of each line from the sum of the 3D distances between each pair of points. The length of the line along the proximodistal axis gives the leaf length, and the length of the line along the transverse axis gives the leaf width.

The lengths of the 2D projections of these lines onto a regression plane fitted through the leaf surface give the corresponding 2D measurements.

Measuring Leaf Surface CIs

Leaf curvature can be estimated by CIs as described by Wu et al. (2007) and Liu et al. (2010). In particular, we compute CItrans and CIlong as follows:

\[
\text{CI}_{\text{trans}} = (\text{3D width} - 2 \times \text{2D width})/2 \times \text{2D width}
\]

\[
\text{CI}_{\text{long}} = (\text{3D length} - 2 \times \text{2D length})/2 \times \text{2D length}
\]

Computing Virtual Cross Sections

The longitudinal virtual cross section of a 3D leaf shape is computed by sampling the 3D leaf surface at 100 equally spaced points along the proximodistal axis of the leaf (the leaf is centered on \(x = 0, y = 0\), so that the section is taken at \(x = 0\)). In the same way, the transverse virtual cross section is computed by sampling the 3D leaf surface at 100 equally spaced points along the medio-lateral axis of the leaf (the section is taken at the center of the leaf, i.e. at \(y = 0\)).

Computing a Characteristic Leaf Shape from a Group of Samples

Given a set of leaves, each with \(n\) points equally spaced along their outlines, we can calculate an average 2D outline by averaging the \((x,y)\) positions of the corresponding points. Then, for any given leaf, we can compute the warping transformation that takes the outline points of the leaf to the points of the average outline. For each leaf, we have also recorded the 3D surface as a set of points with \((x,y,z)\) coordinates. Therefore, we can apply the warping transformation to the \((x,y)\) coordinates of the leaf surface so that it is aligned with the average 2D leaf outline. This procedure is repeated for all leaves of the set. We then resample the surface of each leaf using precise \((x,y)\) grid positions that will correspond between all samples and compute the corresponding \(z\) coordinates using linear interpolation (for this we use the scatteredInterpolant class in Matlab). The average 3D leaf shape is computed by calculating the average \(z\) coordinate (from all samples) at each \((x,y)\) position of the grid.

The averaging method detailed above can generate a 3D surface representative of the data set as long as samples have similar \(z\) coordinates at the same \((x,y)\) positions. However, if \(z\) coordinates are variable between samples, with, for instance, 60% of samples being dome shaped (i.e. curving down) and 40% being cup shaped (i.e. curving up), taking the average 3D shape will give a much less curved shape than that of any sample in the data set. In order to compute a 3D shape representative of a data set in which 3D shapes vary, we compute a characteristic leaf shape by averaging the \(z\) positions of the most frequent observations. The pseudocode for calculating the characteristic leaf shape is given in Supplemental Methods S1 and S2 (Supplemental Fig. S1), and here we only give a brief description of the methodology. At each \((x,y)\) position on the leaf surface, we take the \(z\) coordinates of all samples for that position and define four classes of values based on the range of \(z\) coordinates observed (the number of classes could be adjusted depending on the data set). We then assign to each sample its corresponding class (e.g. if the \(z\) coordinate of a sample is 10 and the classes are \([-20,0],[0,20],[20,40],[40,60]\], that sample will be assigned to class \([0,20]\)). The samples that have \(z\) coordinates fall within the class with the most observations are recorded on a list. This procedure is repeated for all grid positions. The samples that appear on the list most often are then used to compute an average leaf shape in 3D as described above to obtain the characteristic leaf shape.

Computing Local Gaussian Curvature across the Leaf Surface

Given a leaf surface recorded as a set of \((x,y,z)\) grid points, at each grid point position we fit a paraboloid through the grid point and its neighboring points (the 24 closest points on the surface; Supplemental Fig. S3). The principal curvatures and Gaussian curvature at that point then can be computed as described elsewhere (Stoker, 1955; Dumais and Kwiatkowska, 2002).

Describing Local Tissue Deformations Using Warping Transformations

In order to compute the 2D geometric transformation that deforms a leaf at time \(t\) to that same leaf at time \(t+1\), we use the Matlab built-in cp2tform function, inputting the \((x,y)\) coordinates of the particles from the leaf at time point \(t\) and the corresponding coordinates of the particles at the next time point, \(t+1\). cp2tform can be used to compute different types of transformations, including linear transformation, polynomial transformation of second or higher order, and local weighted mean transformation (Goshtasby, 1988). We compute a third-order polynomial transformation to mathematically describe the spatial deformation occurring from time \(t\) to \(t+1\). That transformation can be applied to a grid fitted to the leaf outline at time \(t\), and to the leaf outline itself, to obtain a deformed grid and leaf outline at time \(t+1\). Note that if tissue deformation was more heterogeneous across the leaf, it could be more suitable to use a local weighted mean transformation (Goshtasby, 1988). For details, see Supplemental Methods S1 and S2 and Supplemental Figure S7.

Describing Average Local Tissue Deformations in 2D and 3D

Consider a group of samples tracked from time \(t\) to time \(t+1\), for which we have calculated a 2D warping transformation as well as leaf outlines and grids predeformation and postdeformation. We can then compute the average grid predeformation and the average grid postdeformation (Fig. 6; Supplemental Fig. S6), along with the average predeformation and postdeformation leaf outlines. In order to obtain the deformation in 3D, we first find, for each \((x,y)\) position of the grid predeformation, its corresponding \(z\) coordinate on the characteristic leaf shape of samples at time \(t\) that are tracked to time \(t+1\). The \((x,y)\) positions of the grid predeformation are not the same as the \((x,y)\) positions of the characteristic leaf shape. Finding the \(z\) coordinate for each grid position, therefore, is done using the Matlab scatteredInterpolant class with the linear interpolation option. The same procedure is repeated to find the \(z\) coordinate of each grid postdeformation using the characteristic leaf shape of samples at time \(t+1\) that were tracked from time \(t\). We use the linear extrapolation option in scatteredInterpolant to find the \(z\) coordinates of the deformed leaf outline (shown as blue lines in Fig. 6) in cases where it goes slightly beyond the average outline.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Illustration of data storage for calculating the characteristic leaf shape, as explained in Supplemental Methods S1 and S2.

Supplemental Figure S2. Leaf surface height for the characteristic leaf shape from DAS7 to DAS19.
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