ABSTRACT

A major challenge in morphometrics is to analyse complex biological shapes formed by structures at different scales. Leaves exemplify this challenge as they combine differences in their overall shape with smaller shape variations at their margin, leading to lobes or teeth. Current methods based on contour or on landmark analysis are successful in quantifying either overall leaf shape or leaf margin dissection, but fail in combining the two. Here, we present a comprehensive strategy and its associated freely available platform for the quantitative, multiscale analysis of the morphology of leaves with different architectures. For this, biologically relevant landmarks are automatically extracted and hierarchised, and used to guide the reconstruction of accurate average contours that properly represent both global and local features. Using this method, we establish a quantitative framework of the developmental trajectory of Arabidopsis leaves of different ranks and retrace the origin of leaf heteroblasty. When applied to different mutant forms, our method can contribute to a better understanding of gene function, as we show here for the role of CUC2 during Arabidopsis leaf serration. Finally, we illustrate the wider applicability of our tool by analysing hand morphometrics.

KEY WORDS: Multiscale morphometrics, Image analysis, Leaf, Heteroblasty, 2D:4D finger length ratio

INTRODUCTION

Morphometrics, the quantitative analysis of size and shape of forms, is used to quantify the species-to-species variation of complex biological structures, to analyse the effects of mutations or environmental factors, to describe shape ontogeny or to reconstruct the evolution of biological structures from an evo-devo perspective (Adams et al., 2004; Slice, 2007; Klingenberg, 2010).

Leaves are a challenging model for developing novel morphometric methods as they exist in tremendously diverse sizes and shapes (Tsukaya, 2014). The diversity in leaf shape mostly results from variations in their dissection pattern: leaves can be simple when the blade forms a unique unit or compound when it is dissected into multiple leaflets (Blein et al., 2010; Bar and Ori, 2014) (Fig. S1). In addition, the leaf or leaflet margins can be entire, toothed or lobed. Leaf shape is important in plants because it contributes to efficient photosynthesis by affecting not only light interception but also thermoregulation, wind resistance, hydraulic and biomechanical constraints (Nicotra et al., 2011). Accordingly, leaf shape is controlled by both endogenous and environmental factors. As an example, there is a general trend for leaves to be more dissected under colder climates (Royer et al., 2009), which is used to reconstruct the mean annual temperature in paleoclimates (Greenwood, 2005).

Whatever their mature shape, all leaves start their development as small, undissected finger-like primordia that become more complex through differential growth at their margin (Blein et al., 2010). Numerous factors, including transcription factors, miRNAs and hormones control leaf development (Bar and Ori, 2014; Rodriguez et al., 2014; Sluis and Hake, 2015). Transcription factors of the CUP-SHAPED COTYLEDON2 (CUC2) family, for example, have a central role in the dissection of the leaf margin into teeth or leaflets (Nikovics et al., 2006; Blein et al., 2008; Berger et al., 2009; Kawamura et al., 2010; Bilsborough et al., 2011; Hasson et al., 2011; Cheng et al., 2012). Other factors have also been shown to affect the patterns of cell division, growth or differentiation and have been associated with changes in leaf shape and/or size (Vlad et al., 2014; Das Gupta and Nath, 2015; Gonzalez et al., 2015). Despite the important progress made in the past few years, bridging gene activity with the cellular behaviour and fine changes in leaf shape still remains a challenge, notably because of the difficulty in retracing precisely complex changes in leaf shape throughout their development. More generally, understanding how a primordium develops to reach a complex mature shape would benefit from accurate and precise morphometric analyses.

Different morphometric methods have been deployed for leaves. Discretisation of shape based on evenly spaced marks along the contour and averaging these marks over many leaves allows the proper description and quantification of simple entire leaves (Langlade et al., 2005; Bensmihen et al., 2008; Weight et al., 2008; Feng et al., 2009). Likewise, landmark-independent Fourier-based analysis of the contour is useful to quantify the general shape of the leaf (Chitwood et al., 2012, 2013, 2014). However, because these approaches result in smooth, averaged contours, neither of them is appropriate to accurately capture characteristic structures with a variable position such as teeth or lobes (Chitwood et al., 2012, 2013). By contrast, dissection can be analysed using a few landmarks defined by experts, but information about the shape between landmarks is lost (Hasson et al., 2011; Viscosi and Cardini, 2011; Klingenberg et al., 2012; Silva et al., 2012; Chitwood et al., 2014; Wang et al., 2014). Global dissection of the leaves can be
analysed through the use of the bending energy of the leaf outline (Backhaus et al., 2010; Kuwabara et al., 2011). These examples illustrate the progress made in leaf shape analysis, as well as the difficulties and limitations encountered with morphometric studies in general, underlining the need for a strategy that allows an integrated, multiscale quantification of complex and highly variable shapes.

Here, we present a novel comprehensive method that enables us to retain the general shape of objects while preserving proper information for smaller, multiscale structures, together with the MorphoLeaf application that integrates the proposed strategy to analyse and quantify leaf shape. The MorphoLeaf pipeline uses as input a series of leaf images from which landmarks related to its dissection are automatically identified. These landmarks are then used to perform a non-uniform reparametrisation of the leaf outline that enables homologous morphological regions to be defined along the contours of different samples. This is the basis for a biologically meaningful computation of mean shapes. The MorphoLeaf application is available as a plug-in for Free-D software (Andrey and Maurin, 2005). Both the plug-in and software are freely available. The method can be used to analyse the shape of mature leaves of different architectures or to reconstruct developmentally relevant, meaningful trajectories. As a proof of concept of the usefulness of our method, we reconstructed the developmental trajectory of *Arabidopsis* leaves of different ranks from their initiation to their mature stage and showed that the heteroblasty observed in mature *Arabidopsis* leaves results from very early divergent developmental paths. In addition, we performed a fine comparative analysis of early leaf shape between the wild type and the *cuc2* mutant that provides novel insights into the mode of action of *CUC2*, a key regulator of leaf shape (Nikovic et al., 2006; Bilsborough et al., 2011; Hasson et al., 2011). Finally, we showed that MorphoLeaf can have wider application by performing a morphometric analysis of the human hand and in particular calculating the ratio of the length of second and fourth digits (2D:4D), which provides a lifelong signature of prenatal hormonal exposure (Manning et al., 1998; Zheng and Cohn, 2011; Meindl et al., 2012; Sanfilippo et al., 2013).

RESULTS

We developed the MorphoLeaf application (available at: morpholeaf.versailles.inra.fr) to analyse leaf shapes. It involves several steps leading from leaf snapshots to data extraction, quantification, averaging and representation (Fig. 1). At some steps of this pipeline (determination of teeth tip and hierarchy), different alternative methods are proposed; the choice can be made either by visual evaluation of the results or by objectively comparing the results of the proposed methods with expert analysis on a smaller training set of leaf images. To illustrate the pipeline, we analysed the early stages of leaves 11 to 13 (L11-L13) of *Arabidopsis* plants grown under short-day conditions. These leaves show 3-4 conspicuous teeth on each side of the blade and have a similar course of development (Fig. S2), allowing them to be analysed together as a single data set. Thereby, 207 young leaves ranging from 100 μm to 2500 μm were sampled and imaged using red-chlorophyll fluorescence microscopy (Fig. 1A).

Automated detection of biologically relevant landmarks

Extraction of the leaf contour

The leaf outline was automatically extracted using a classical watershed-based method (for a more detailed description as well as for the technical aspects of all computational methods and algorithms, please see the supplementary Methods) and manually corrected if necessary (~30-60 s were required per leaf to do the correction, mostly either for young leaves with unclear borders representing ~20% of the leaves in our analysis or for old leaves with deep sinuses representing ~10% of the leaves). During the next step, two landmarks corresponding to the petiole were set manually, which allowed the automatic identification of the blade (the side with the greatest area) and the petiole (Fig. 1B). We chose to do this manually (~5 s per leaf) as this step is central for further analysis and can not be done automatically for all types of leaves, particularly for young primordia in which the petiole is hardly visible. The leaf tip was then automatically determined as the point of the blade contour furthest away from the midpoint between petiole landmarks. This also defined the base-tip axis separating the blade in two half blades (Fig. 1C).

Identification of sinuses and tips of teeth

In the next step, we automatically identified the teeth, which are defined as portions of the blade contour between two (not necessarily consecutive, see below) sinuses. Sinuses, which correspond to contour points with a high concave curvature, are identified in a two-step procedure (see supplementary Methods and associated figures). First, candidate intervals of the contour are determined as continuous domains where the curvature remains concave and above a user-defined threshold. Second, within each candidate interval, the point with the maximal curvature is selected as a sinus (Fig. 1C). After automatic detection of sinuses, errors may be manually corrected (~2 s per sinus) to ensure that tooth limits are correctly positioned and avoid biases in subsequent analyses (see below for the validation of the automatically detected sinuses).

After sinus identification, MorphoLeaf determines the position of the tooth tip between consecutive sinuses (Fig. 1C). The user can choose one of the two strategies available depending on tooth shape. For rather sharp teeth, the selected tip corresponds to the point with a maximal local curvature. For round teeth like those in young *Arabidopsis* leaves, we developed an alternative strategy based on the observation that towards its tip, the tooth is rather symmetrical (see supplementary Methods and associated figures). Hence, the tooth tip is defined as the contour point that maximises a local symmetry criterion.

Tooth hierarchy determination

Using the methods described above, we could identify the teeth along the leaf blade. However, a close examination of leaves of *Arabidopsis* (Fig. 1C,D and supplementary Methods) and of other species (Fig. S3) showed that teeth sometimes have a hierarchical organisation: a primary tooth is any tooth that developed on the main leaf contour, whereas a secondary tooth is formed on a primary tooth. It is essential to take this tooth hierarchy into account for further analyses as, for instance, the area of a primary tooth includes further analyses as, for instance, the area of a primary tooth includes
The second method is a recursive algorithm adapted to developing leaves that relies upon the assumption that the tooth inclination increases with the rank (supplementary Methods). The orientation threshold that determines whether a higher hierarchy is detected is the sole parameter of the algorithm. This method is adapted to leaves like those in *Arabidopsis* in which the basal leaf contour tends to exhibit a rather regular (convex) curvature. Using this method, eight secondary teeth were detected in our *Arabidopsis* set of images.

The outcome of the two methods can be represented by a hierarchical tree in which a node corresponds to a single tooth, whose rank is given by its level in the tree. In addition, sinuses are labelled with a ‘primary’ or ‘secondary’ tag. The term ‘secondary’ generalises to all teeth with a rank strictly superior to 1 because it is sufficient to identify primary teeth and their associated, higher rank structures.

**Validation of automatic sinus detection and hierarchy**

In order to quantify the performance of our semi-automatic sinus detection, we compared its results with manual marking performed independently by four experts on a test data set of leaf images (see supplementary Methods and associated figures). For this, we implemented an algorithm that matches landmarks corresponding to the same biological features both in expert and algorithm results.
Then, we compared the distances between automatically detected sinuses and those detected by experts and we showed that several combinations of parameters provided an automatic sinus positioning within the limits of the inter-expert variability (e.g. ∼1-3 µm for Arabidopsis leaves from 500 to 2000 µm, supplementary Methods). Similarly, several combinations of parameters generated numbers of false positive and of false negative detections within the range of those generated by the experts. Altogether, this indicates a good performance of our method in terms of success rate and precision. Similarly, we evaluated the two methods of hierarchy detection and found that both showed highly satisfactory sensibility and specificity (see supplementary Methods and associated tables).

**Landmark-based quantification of the shape of Arabidopsis leaves L11, L12 and L13**

Using the automatically detected landmarks, we quantified different parameters associated with either the entire leaf or individual teeth. We plotted measurements against blade length, which is used here as a proxy for the stage of development. Blade growth is globally isotropic, because the ratio between blade width and length remains constant, whereas area increases quadratically (Fig. 2A,B). Teeth appear sequentially along the leaf margin and tend to be synchronous on both sides of the leaf, since leaves with even teeth numbers were more frequent than leaves with odd numbers (Fig. 2C). There was, however, an important variability of the leaf size at which teeth initiated. For instance, the first pair of teeth is formed when the blade is between 110 and 333 µm long. The dynamics of individual tooth development was reconstructed and showed that teeth later arising are more pointy than the first ones (Fig. 2D-G). The evolution of the relative position of the sinuses along the proximodistal leaf axis revealed heterogeneous growth of the blade along this axis, with a more important relative growth in the region where the first pair of teeth develops (Fig. 2H,I).

**Reconstruction of developmental trajectories of teeth and whole leaves**

**Developmental trajectories of teeth**

To analyse tooth shape, we next extracted contours corresponding to teeth 1, 2 and 3, i.e. the part of the blade outline situated between two consecutive primary sinuses. In order to put size effects aside, we rescaled all primary teeth by registering their two sinuses. Then, we performed a principal component analysis (PCA) to analyse...
variations in tooth shape (Fig. 3A,B) as previously described for the whole leaf contours (Langlade et al., 2005; Bensmihen et al., 2008; Feng et al., 2009; Chitwood et al., 2014). The first axis corresponds to the variation in tooth height and confirms the difference in pointedness observed between tooth 1 and teeth 2 and 3. Interestingly, variation along the second axis, which corresponds to the degree of tooth asymmetry, showed that there is an increasing asymmetry from tooth 1 to tooth 3.

Fig. 3. See next page for legend.
Development of mature leaf shape in different species

MorphoLeaf was successfully applied to describe and quantify the shape of Arabidopsis leaves. To see how MorphoLeaf performs in a broader context, we tested it on various plant species (Fig. 4, Fig. S4 and Table S1). For all considered species, the maximal curvature and the iterative method were well suited to determine teeth tips and hierarchy, respectively. Reparametrisation based on landmarks also appeared to be essential to obtain representative contours for the mean leaves. Thus, MorphoLeaf can be effectively used to analyse the shapes and to reconstruct the mean contours of mature leaves with different architectures, such as pinnately and palmately lobed leaves, pinnately lobed leaves with secondary structures and palmately compound leaves (Fig. 4). Current limitations of the MorphoLeaf application include the hierarchisation of several levels of dissection in palmately lobed leaves, an accurate quantification of pinnately compound leaves and the analysis of samples with a strong heterogeneity in structure size and number (Fig. S5).

Reconstruction of the developmental trajectory of Arabidopsis leaves of different ranks

Leaves successively formed during a plant’s life often differ in their mature shape, a phenomenon described as leaf heteroblasty (Poethig, 1997; Tsukaya et al., 2000; Zotz et al., 2011). Using MorphoLeaf, we reconstructed and quantified mean mature leaves L01, L03, L05, L07, L09 and L11 from short-day-grown Arabidopsis to quantify the heteroblasty level (Fig. 5A and Fig. S6). This showed that vegetative leaves of increasing rank become more elongated with more and bigger teeth.

Next, we reconstructed the developmental trajectory of leaves of different ranks. For this, we collected individual leaves from their initiation to their mature stage (between 160 and 312 leaves per rank, average 196) and analysed them using MorphoLeaf. The evolution of the shape of leaves of different ranks could be reconstructed [Fig. 5B-I, Fig. S7 and Movies 1-12 (at morpholeaf.versailles.inra.fr)] and, in parallel, leaf morphological parameters can be quantified (Fig. 5J-O). Careful observation of mean leaf shapes (e.g. L01 in Fig. 5H,I and Fig. S7) and the analysis of the number of teeth during development (Fig. 5K) revealed a feature common to all leaves.
ranks: after an initial increase in the number of teeth, reflecting their successive initiation, the number of teeth decreases. This apparent ‘disappearance’ of teeth is due to a progressive smoothing of the sinuses of the most distal teeth (Fig. S7). As a consequence, the homology between teeth is lost: a tooth 2 becomes a tooth 1 when the most distal tooth 1 disappears. To keep the homology between teeth throughout leaf development, we re-examined the most mature leaves and manually added distal teeth if required in order to follow the dynamics of tooth formation before generating the data describing tooth morphometrics (Fig. 5L-O).

Developmental origin of leaf heteroblasty
We next investigated the developmental trajectory leading to leaf heteroblasty in Arabidopsis. Two extreme mechanisms can be envisaged: primordia of leaves of different ranks may be different from their early stages onwards or, alternatively, they could be similar during early phases of development and diverge during later phases. To answer this question, we used MorphoLeaf to compare the evolution of the shape of leaves from different ranks and the associated quantitative parameters (Fig. 5F-O).

Shortly after initiation (at 200 μm long), leaf primordia show a similar shape, although later on, leaves of lower rank become wider than higher-rank leaves (Fig. 5F-J). More teeth are initiated on smaller primordia of higher rank leaves (Fig. 5F-L,K) and in a more distal position along the primordium (Fig. 5G,H,L). This indicates that primordia of different ranks already show divergent features soon after their initiation.

While the increase in tooth width is similar in all leaves (except L01, Fig. 5M), increase in height is more important in higher-rank leaves (Fig. 5N). However, the evolution of the relative tooth area indicates that the dynamics of tooth growth is similar for all leaves, except L01 (Fig. 5O). Together, this shows that teeth are more pointed in higher rank leaves as a result of a faster increase in height. Altogether, this analysis shows that heteroblasty in Arabidopsis results from divergence in developmental trajectories from the very early stages of leaf formation. These differences are enhanced during later steps of tooth growth. Although the patterning of the margin is mostly established during the initial phases of leaf development, it is rearranged during later stages, as shown by the smoothing of the most distal dissections.

Role of CUC2 during leaf serration
CUC2 is an important regulator of leaf margin serration and has been proposed to either locally repress growth to form the tooth sinuses (Nikovics et al., 2006) to promote tooth outgrowth (Kawamura et al., 2010) or a combination of both (Bilsborough et al., 2011). We used MorphoLeaf to finely compare early stages of leaf development between the wild type and the cuc2-1 mutant (Fig. 6). At 175 μm long, while the cuc2-1 mutant leaf primordium has a smooth, convex outline, two faint creases formed in the wild...
type, at the sites where a CUC2:CUC2:VENUS translational reporter is expressed (Fig. 6A,B). This shows that the first visible effect of CUC2 activity is a local repression of growth. This effect is maintained during later stages (Fig. 6C-F). In primordia above 225 µm long, tooth outgrowth becomes visible in the wild type compared with the cuc2-1 mutant, indicating that during a second phase, tooth formation is associated with a local increase in growth, which may result from increased cell proliferation and/or expansion.
To test the robustness of MorphoLeaf, we tested its applicability to other 2D structures. Hand morphometrics relies on the analysis of a 2D structure with protruding outgrowths (the fingers). In particular, the ratio of the length of the second and fourth digits (2D:4D) provides a signature of prenatal hormonal exposure and has been associated with several adult characteristics, including behaviour, fertility and disease risk (McIntyre, 2006). MorphoLeaf allowed the automatic identification of 10 of the 12 landmarks necessary to measure the length of all fingers (Fig. 7A-C). Using landmark-guided reparametrisation, we could reconstitute average hand shapes (Fig. 7D,E) in which finger length was properly represented (Fig. 7G,H) and calculate the widely used finger length ratio. This shows that MorphoLeaf can be widely used to analyse complex 2D biological objects.

**DISCUSSION**

Morphometrics is usually based on either the analysis of the outline of the biological object or on biological landmarks (Adams et al., 2004; Slice, 2007; Klingenberg, 2010). Here, we propose a new method that combines both approaches. Biologically relevant landmarks are automatically identified and used to reparametrise the outline, which allows the placement of corresponding homologous points before averaging the outline of several objects. This reparametrisation is essential to obtain average shapes that are representative of individual leaves. Adding as few as one landmark (the leaf apex) substantially increases the quality of the averaging. Automation of the landmark detection contributes to the reproducibility of the results by limiting variations due to placement by operators. Using this new approach, we provide quantification (number, size, shape, and position) of multiple structures present in the object and a faithful representation of the mean shape of the object. These two outcomes are complementary because the quantifications allow the analysis of precise features on any structure of the object associated with the landmarks, whereas the mean shape allows better capture of the complexity of the biological object as a whole (including regions not directly associated with a particular landmark). The mean representation is also extremely helpful to follow developmental trajectories. The reparametrised outline can also be analysed by principal component analysis (PCA, Fig. S8). It should be noted that because the coordinates of the landmarks are recorded, geometric morphometric methods such as generalised Procrustes analysis (GPA) can also be applied to the data provided by MorphoLeaf.

Using MorphoLeaf, we generated the developmental sequence of *Arabidopsis* leaves of different ranks. We show that the acquisition of mature leaf shape is a complex process that takes place early on when the teeth are initiated along the leaf margin but it is refined during later stages. Indeed, teeth successively initiated along the leaf margin show different growth dynamics resulting in different shapes (Fig. 2E,F). In addition, the most distal teeth are eroded during the later stages and are no more detectable in the mature leaves. This underlines the importance of reconstructing the entire developmental sequence to fully understand the ontology leading to mature leaf shapes. More generally, any molecular or cellular data collected during the course of leaf development could be mapped on the morphological framework generated here, which is a first step towards the production of a virtual leaf. In addition, our method provides information on the distribution of growth within the leaf. For instance, evolution of the ratio between the area of a tooth and that of the whole leaf provides insight into the dynamics of tooth growth. The evolution of the relative position of the tooth sinuses along the leaf proximo-distal axis provides quantitative information about the growth gradient along this axis. Indeed, our observations of leaves >2 mm in length (Fig. S9) are in agreement with a growth arrest front starting at the leaf tip and progressing proximally (Remmler and Rolland-Lagan, 2012) while the data retrieved from earlier stages show that growth is enhanced in the central part of the primordium (Fig. 2H,J). This was not observed in the first leaves (Kuchen et al., 2012).

By comparing the developmental sequences of leaves of different ranks, we show that leaf heteroblasty results from differences in the early pattern of tooth initiation (position along the leaf primordium and size of the primordium at tooth initiation) and differential outgrowth of the teeth. This analysis also underlines the complementarity between the two types of data produced by MorphoLeaf: the average mean shapes and the quantification of biological structures.

The precise morphological data on tooth development generated by MorphoLeaf can help us to determine how the activity of...
members of previously identified genetic and molecular networks precisely affect leaf morphogenesis, as illustrated here for CUC2. CUC2 has been suggested to lead to Arabidopsis leaf serration by two mechanisms: repression of growth to form the sinuses and/or growth promotion to lead to tooth outgrowth (Nikovics et al., 2006; Kawamura et al., 2010; Bilsborough et al., 2011). Our precise comparison of the early shapes of wild type versus cuc2 mutants indeed showed that the two mechanisms occur but at different stages. The first detectable effect of CUC2 activity is a local repression of growth, whereas only slightly later, an outgrowth becomes observable at distance from the CUC2 expression domain. This indicates that CUC2 may shape the margin of simple leaves by dual mechanisms, similar to those proposed for other NAM/CUC3 genes during compound leaf development (Blein et al., 2008). Several different scenarios can explain this observed sequence of morphological changes. CUC2 expression could repress growth locally while simultaneously producing a signal that could promote growth at distance; the delay between the observation of these two processes resulting from the time necessary for the signal to be produced, migrate through part of the leaf and be transduced in a detectable change in shape. For instance, the generation of CUC2-dependent auxin activity maxima could be such a signal (Bilsborough et al., 2011). Alternatively, changes in the growth pattern at sinuses could indirectly modify growth at distance. Identification of the network acting downstream of CUC2 could possibly help to discriminate between these scenarios.

To illustrate the generality of our approach, we analysed the morphology of simple leaves with different architecture. In the case of complex morphologies, identification of the observed hierarchical structure is central to an accurate shape analysis because it allows selection of the homologous landmarks used for landmark-guided reparametrisation. Our method can be also directly applied to hand-selected individual leaflets. A similar strategy could be used to improve the analysis of any object by providing an accurate description of the outline between different landmarks. Finally, we show that MorphoLeaf can be applied to other morphometric studies, such as the morphometrics of human hands and calculation of the second to fourth digit ratio. Therefore, the MorphoLeaf application not only provides a valuable tool to quantify and accurately represent complex leaf shapes, but the strategy described here could also be used for morphometric studies of other models.
MATERIALS AND METHODS

Plant material and growth conditions

All Arabidopsis plants are in the Columbia-0 background. The cunc2-1 mutant and CUC2-CUC2-VENUS reporter line have been described elsewhere (Hasson et al., 2011; Gonçalves et al., 2015). The mir164a-4 mutant (Nikovics et al., 2006) that shows a higher level of leaf complexity was used to validate the hierarchy procedure. Seeds were stratified for 2 days, in water, at 4°C in the dark prior to sowing. Plants were grown on soil in short-day conditions [1 h dawn (19°C, 65% hygrometry, 80 µmol/m²/s light), 6 h day (21°C, 65% hygrometry, 120 µmol/m²/s light), 1 h dusk (20°C, 65% hygrometry, 80 µmol/m²/s light), 16 h dark (18°C, 65% hygrometry, no light)]. The short-day conditions allowed us to raise plants that stayed longer in the vegetative phase leading to more leaves per plant.

Mature leaves from other species were collected in the park surrounding the French National Institute for Agricultural Research (INRA) building in Versailles and identified using flora classification books or were extracted from the Middle European Woods database (Novotny and Suk, 2013).

Leaf dissection and imaging

Leaves number L01, L03, L05, L07, L09, L11, L12 and L13 were dissected using a medical needle, mounted in mounting medium (10 mM Tris-HCl, pH 8.5, 0.01% Triton X-100) on a slide and imaged with a binocular microscope using chlorophyll fluorescence at early stages and white light at later stages. Mature leaves of Arabidopsis and other species were scanned at a resolution ranging from 1200 to 1600 dpi, depending on leaf complexity.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

E.B., M.C., J.B., A.K., M.O., A.B. and P.L. conceived the MorphoLeaf application that was encoded by E.B. with the help of P.A. M.C., A.M.-C., B.G., B.A. and P.L. performed the experiments and E.B., M.C., J.B., A.K. A.B. and P.L. analysed the data. J.B., E.B. and P.L. wrote the paper with input from all other authors.

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Data availability

Movies 1-12 are available at http://morpholeaf.versailles.inra.fr/video/Arabidopsis.html.

Supplementary information

Supplementary information available online at http://dev.biologists.org/lookup/doi/10.1242/dev.134619.supplemental

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