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Current Biology

Formation and Shaping of the *Antirrhinum* Flower through Modulation of the *CUP* Boundary Gene

Highlights

- Boundary genes play a key role in the evolution of diverse 3D plant shapes
- *CUP* boundary gene needed for formation of the complex lower Snapdragon corolla
- *CUP* can either promote or inhibit growth depending on tissue context
- Petal fusion involves clearing of boundary gene activity at petal junctions

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In Brief

Rebocho et al. shows how the *CUP* boundary gene plays a key role in the shaping of the ornate Snapdragon flower by controlling differential growth. Clearing of *CUP* expression from petal junctions allows the flower tube to form, while activation of *CUP* in the lower petal promotes growth and formation of the convoluted lower palate and lip.

Rebocho et al., 2017, Current Biology *27*, 1–13
September 11, 2017
http://dx.doi.org/10.1016/j.cub.2017.07.064

CellPress

Please cite this article in press as: Rebocho et al., Formation and Shaping of the *Antirrhinum* Flower through Modulation of the *CUP* Boundary Gene, Current Biology (2017), http://dx.doi.org/10.1016/j.cub.2017.07.064

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Formation and Shaping of the *Antirrhinum* Flower through Modulation of the *CUP* Boundary Gene

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http://dx.doi.org/10.1016/j.cub.2017.07.064

SUMMARY

Boundary domain genes, expressed within or around organ primordia, play a key role in the formation, shaping, and subdivision of planar plant organs, such as leaves. However, the role of boundary genes in formation of more elaborate 3D structures, which also derive from organ primordia, remains unclear. Here we analyze the role of the boundary domain gene *CUPULIFORMIS* (*CUP*) in formation of the ornate *Antirrhinum* flower shape. We show that *CUP* expression becomes cleared from boundary subdomains between petal primordia, most likely contributing to formation of congenitally fused petals (sympetally) and modulation of growth at sinuses. At later stages, *CUP* is activated by dorsoventral genes in an intermediary region of the corolla. In contrast to its role at organ boundaries, intermediary *CUP* activity leads to growth promotion rather than repression and formation of the palate, lip, and characteristic folds of the closed *Antirrhinum* flower. Intermediary expression of *CUP* homologs is also observed in related sympetalous species, *Linaria* and *Mimulus*, suggesting that changes in boundary gene activity have played a key role in the development and evolution of diverse 3D plant shapes.

INTRODUCTION

The formation and shaping of plant outgrowths, such as leaves or petals, depends on differential growth at boundary domains. These boundaries are evident at the base of organ primordia or within primordia where serrations form. In cases where organ primordia are organized in whorls, we may distinguish between two types of boundary: *intra-whorl* boundaries, which delimit adjacent organs belonging to the same whorl, and *inter-whorl* boundaries, which delimit adjacent organs belonging to different whorls. Defects in the intra-whorl boundaries can lead to formation of cup-shaped structures, such as congenitally fused cotyledons or sepals (calyx). Defects in inter-whorl boundaries can lead to hybrid structures such as congenitally fused petal-stamens, or stamen-carpels. Loss of boundaries within organs can also lead to reduced dissection (e.g., loss of leaf serrations). All of these phenotypes have been observed for mutants in NAC-domain boundary genes, which include *NO APICAL MERISTEM* (*NAM*, *Petunia*), *CUP-SHAPED COTYLEDON* (*CUC*, *Arabidopsis*), *GOBLET* (tomato), and *CUPULIFORMIS* (*CUP*, *Antirrhinum*) [1–8]. These genes are expressed at the boundaries of organ primordia or serrations, where they are thought to repress growth, though they may also promote growth outside these domains by influencing auxin distribution [9, 10]. Changes in NAC gene expression are thought to contribute to the evolution of planar leaf shape complexity [6, 11]. These findings raise the question of whether NAC boundary genes might also play a role in formation and shaping of non-planar structures, such as the corolla of sympetalous flowers, as these also derive from primordia.

Sympetalous (gamopetalous) flowers, have petals that are congenitally fused (united) or postgenitally fused for all or part of their length. This arrangement is thought to be evolutionary derived from the polypetalous (choripetalous) condition, in which petals grow separately [12, 13]. Sympetalous species might be expected to show diminished activity of genes that promote intra-whorl boundary formation in the second whorl. If reduced NAC gene activity contributes to enhanced petal fusion, mutations that diminish NAC function in sympetalous species should either further increase the degree of petal fusion or have little effect. Contrary to this expectation, inactivation of *NAM* in *Petunia* by virus-induced silencing leads to increased petal separation [14]. This observation suggests that *NAM* activity promotes petal fusion rather than intra-whorl boundary formation. However, this interpretation is called into question by the observation that occasional flowers generated by *nam* mutants have normally united corollas, even though inter-whorl fusions between stamens and petals are observed [1]. This result indicates that removing *NAM* activity has no impact on sympetally. A further difficulty in interpreting these results is that, although *NAM* expression is detected in corollas by qPCR [14], the detailed expression pattern in developing *Petunia* corollas has not been determined by in situ hybridization. Thus, the role of NAC boundary domain genes in the evolution of sympetally remains unclear.

To further explore the role of NAC domain genes in corolla development, we analyze the role of the *NAM* ortholog, *CUP*, in

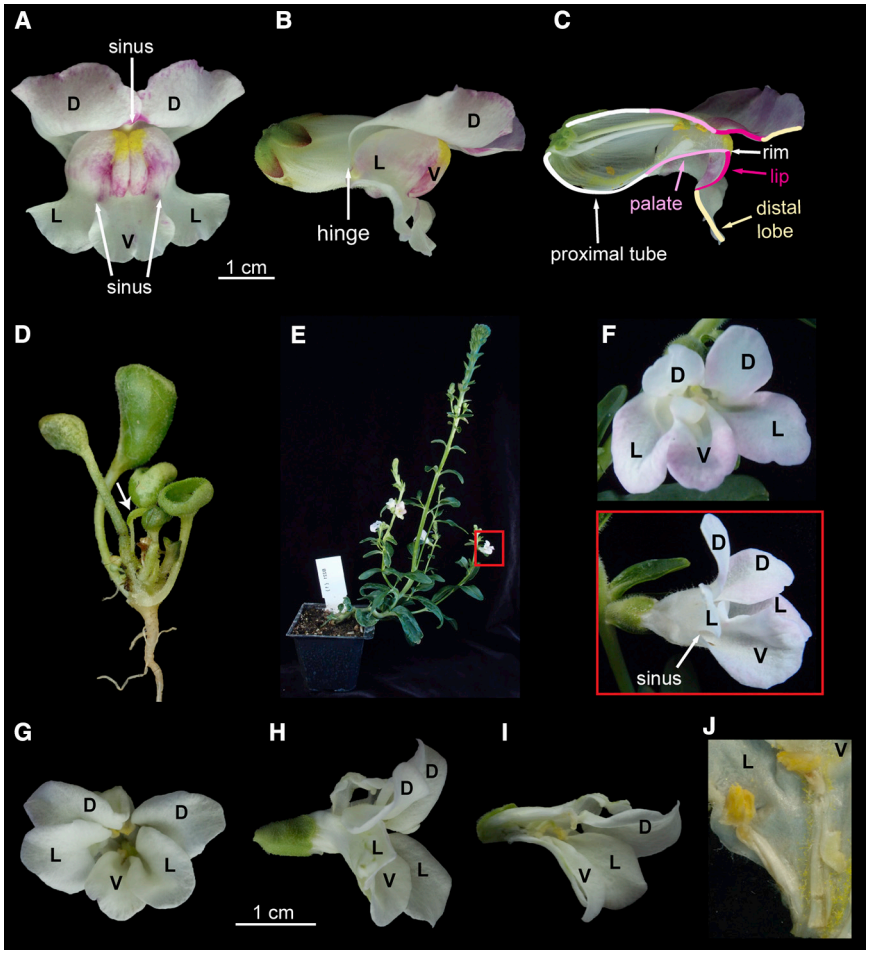

Figure 1. Wild-Type and *cup* Mutant Flower Morphology

(A–C) Wild-type Snapdragon corolla in face view (A), side view (B), and longitudinal section (C). Dorsal (D), lateral (L), and ventral (V) petals are united proximally to form a tube. The petal lobes are located distal to the tube, with a fold at the tube-lobe boundary (rim). Petal fusion extends into the lobes of the upper and lower corolla forming the lip region. The dorsal-lateral junctions have a deep sinus with little or no lip, forming a hinge (B).

(D–F) *cup* mutant. (D) Seedling with cup-shaped leaves and initiation of adventitious shoots (white arrow). (E) Adventitious shoots may grow to form flowering branches. Red box indicates flower shown in (F). (F) Front (top) and side (bottom) views of *cup* flower. The corolla has an open mouth without a clear palate or lip. Dorsal, lateral, and ventral petals can still be identified and are separated by sinuses in the lobe region.

(G–J) Flowers of *cup* mutants show varying degrees of united growth within and between whorls (compare F with G and H) but always fail to make the palate lip 3D deformation observed in wild-type (compare I to C). An example of fusion between petal and stamen whorls is shown in (J). See also Figure S2.

Antirrhinum (Snapdragon). Like *Petunia*, *Antirrhinum* flowers are sympetalous. However, instead of an open trumpet shape, *Antirrhinum* corollas have a closed mouth and exhibit strong dorsoventral asymmetry (zygomorphy). The wild-type *Antirrhinum* corolla comprises five petals: two dorsal (D), two lateral (L), and one ventral (Figures 1A and 1B). The proximal regions of the petals are united (congenitally fused) to form a tube, the distal part of which extends to form the upper and lower palate (Figure 1C). The tube is bounded distally by a fold (rim), beyond which are the petals lobes. The dorsal and lateral lobes are separated by a sinus, forming a hinge, which allows the flower to be opened by pollinators. Growth occurs proximal to the other sinuses allowing two subregions of the lobe to be defined—a proximal united region termed the lip, and a distal lobe region separated by sinuses. The palate and lip of the lower corolla form a wedge-shaped fold, which fits tightly, like a closed lid, over the triangular shaped upper palate [15, 16].

Mutations in *CUP* affect both vegetative and floral development in *Antirrhinum*. The cotyledons of *cup* mutants are united, similar to the situation observed for *nam* mutants of *Petunia* and *cuc1 cuc2* mutants of *Arabidopsis* [1, 2, 4]. Adventitious shoots develop from the hypocotyl of *cup* mutant seedlings and usually terminate with similar cup-shaped leaves (Figure 1D [4]). Eventually, secondary shoots may form with a functional apical meristem, similar to what happens with *nam* mutants in *Petunia*. These escape shoots generate leaves that are often united, and flowers that are deformed and exhibit petal-stamen fusions. As with *NAM* and *CUC*, *CUP* is expressed at the boundaries between organ primordia, where it has been proposed to inhibit growth in combination with other transcription factors [13]. However, the role of *CUP* in corolla development is unclear.

Computational modeling of *Antirrhinum* corolla development shows that the growth patterns and final shape can be accounted for by combinatorial interactions between genes expressed along three axes: dorsoventral, proximodistal, and mediolateral [15, 16]. These genes act in combination to control specified growth rates parallel and perpendicular to local tissue polarity. Tissue polarity is initially oriented proximodistally to form a polarity field, which becomes deformed, together with gene expression patterns, as the tissue grows. *CYCLOIDEA* (*CYC*), *DICHOTOMA* (*DICH*), *RADIALIS* (*RAD*), and *DIVARICATA* (*DIV*) establish different identities along the dorsoventral axis of the corolla [17–19]. *CYC* and *DICH* are dorsally expressed and encode TCP transcription factors that activate expression of the MYB gene *RAD*. *RAD* acts antagonistically to the *DIV* MYB gene, restricting its activity to ventral and lateral regions [19, 20]. The dorsoventral genes are thought to interact with genes expressed in domains along the proximodistal and mediolateral axes to produce the characteristic *Antirrhinum* corolla shape [16]. For example, the wedge-shape form of the lower corolla depends on interactions between *DIV* and inferred gene activities in the primordial palate and lip regions (proximodistal axis), together with genes in the midline and edges of each petal (mediolateral axis) [15]. However, unlike the dorsoventral axis,

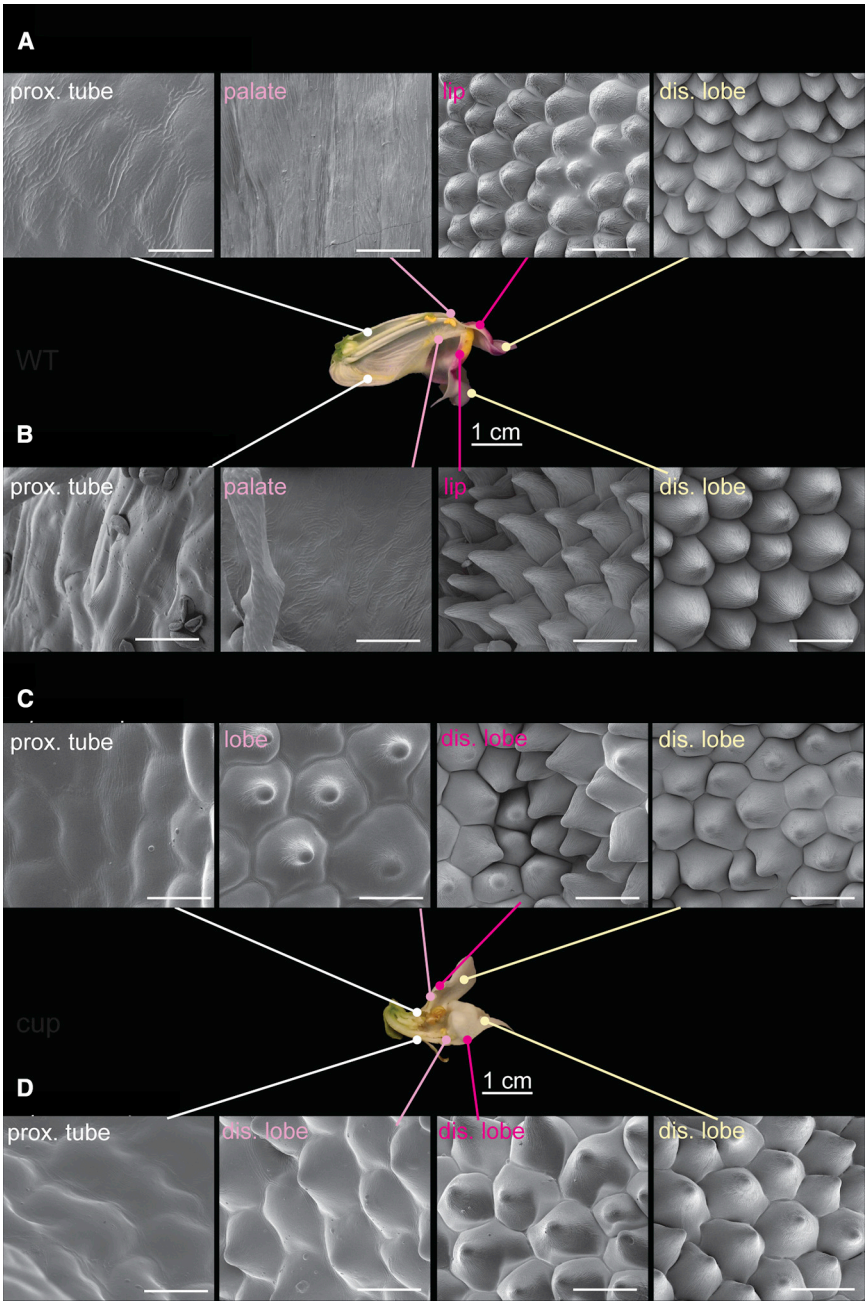

Figure 2. Cell Types in Wild-Type and *cup* Mutant Petals

(A and B) Cell types along proximodistal axis of wild-type dorsal (A) and ventral (B) petals. Four cell types associated with the proximal tube (prox. tube), palate, lip, and distal lobe (dis. lobe) regions. (C and D) Cell types in *cup* mutant for dorsal (C) and ventral (D) petals. Only cell types corresponding to the prox. tube and dis. lobe are observed.

genes underlying the proximodistal and mediolateral domains have yet to be identified. Moreover, the initial starting tissue shape for these computational models is a cylinder with five lobes and thus already exhibits sympetally, raising the question of how this initial form is generated.

Here, we analyze *CUP* expression in *Antirrhinum*, from floral initiation to later stages of development and relate this expression pattern to mutant and wild-type morphogenesis. We reveal a gap in *CUP* expression between proximal and distal regions at boundaries between adjacent petal primordia. Through computational modeling, we show that this gap most likely reflects reduced growth repression needed for formation of the sympetalous corolla. At later stages, distal *CUP* expression is cleared from the lateral-ventral sinuses, where the lip forms, suggesting a role for *CUP* in modulating growth at sinuses. We further show that *CUP* is activated by dorsoventral genes at later developmental stages in an intermediary domain of the lower and upper corolla. This domain corresponds to the presumptive palate and lip regions, and *CUP* is required for their development. Unlike the role of *CUP* at boundaries, expression in intermediary regions leads to promotion rather than inhibition of growth, most likely reflecting interactions with other region-specific factors. Intermediary expression of *CUP* is found in other species with dorsoventrally asymmetric corollas, such as *Mimulus* and *Linaria*. Our findings thus suggest that changes in *CUP* expression have played a key role in the formation and shaping of the sympetalous corolla.

RESULTS

Early Role of *CUP* in *Antirrhinum* Corolla Development

The role of NAC genes in the formation of the *Antirrhinum* corolla was investigated through analysis of flowers on escape shoots generated by the *cup* mutant (white arrow in Figure 1D, red box in Figure 1E). Mutant corollas have an open mouth and narrow tube and lack the complex folds and shaping characteristic of the palate and lip regions of wild-type. Dorsal, lateral, and ventral petals are more similar to each other in shape than those of wild-type (Figure 1F). Sinuses are still present between petal lobes, and the stamen filaments are often fused with the corolla tube (Figures 1G–1J).

To determine the pattern of regional identities in *cup* mutant corollas, we visualized the cell types in wild-type and *cup* petals by scanning electron microscopy. In wild-type dorsal and ventral petals, cell types could be identified that correlated with the proximal tube, palate, lip, and distal lobe regions (Figures 2A and 2B). By contrast, only the proximal tube and distal lobe cell types could be clearly identified in *cup* mutants (Figures

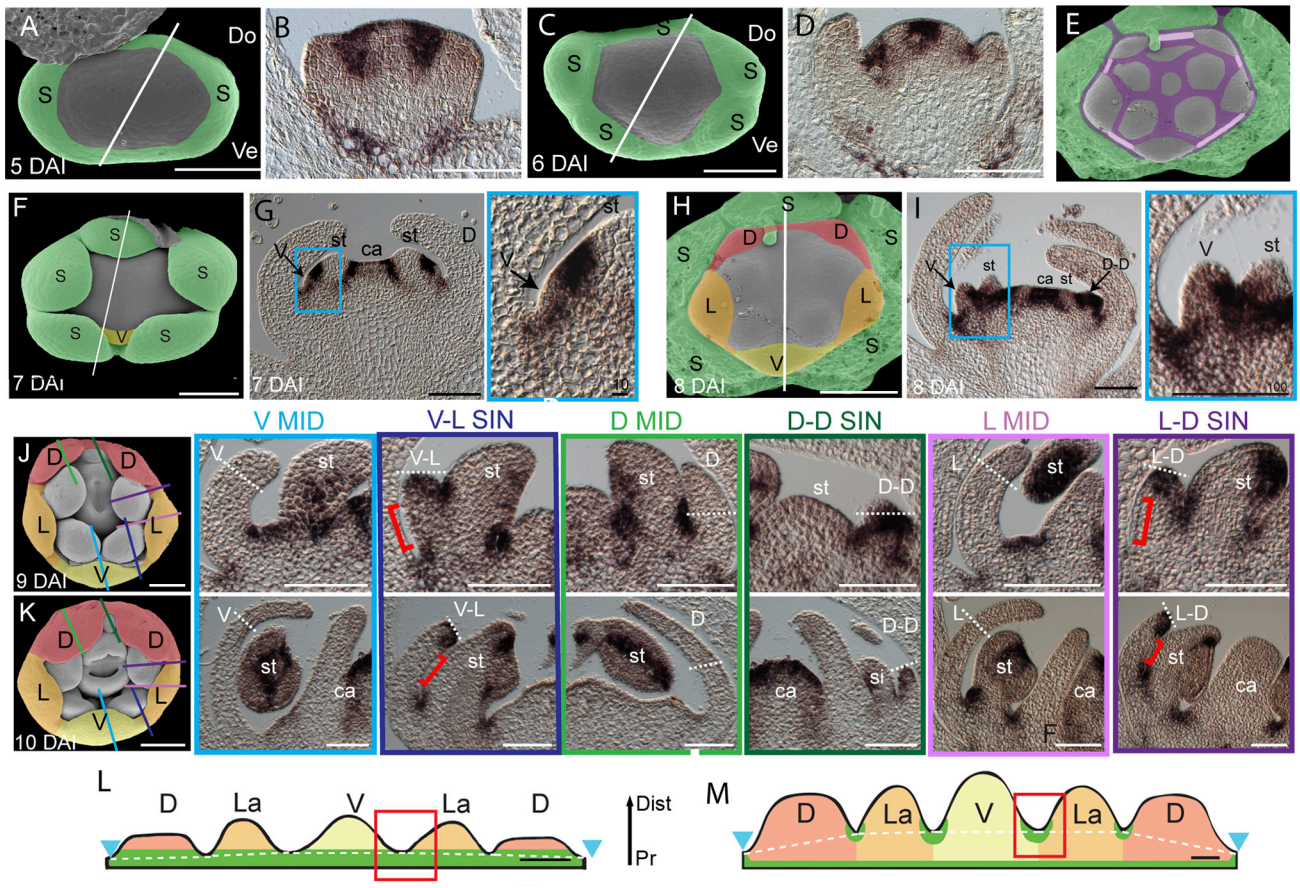

Figure 3. *CUP* Expression during Early Wild-Type Development

(A–D) At 5–6 DAI, *CUP* is expressed in the ring internal (B) to the sepals (green in A and C), and in central regions where carpel and stamen primordia will form (D).

(F and G) At 7 DAI petal primordia are just visible as regions of clearing within the *CUP* domain (ventral petal primordium arrowed in G).

(E, H, and I) At 8 DAI petal primordia emerge (H). Sepals removed to leave only their bases (green). *CUP* expressed as a ring at the base of primordia (I). *CUP* domain color coded in (E), with junctions between petal primordia in light pink, and other regions of expression are in purple.

(J and K) At 9 DAI, gaps (red brackets) are visible in the *CUP* expression domain on the abaxial side of ventral-lateral and lateral-dorsal petal junctions (J). At 10 DAI gaps are also visible on the adaxial side (K). Planes are labeled mid-ventral (V MID, light blue), ventral-lateral sinus (V-L SIN, dark blue), mid-dorsal (D MID, light green), dorsal-dorsal sinus (D-D SIN, dark green), mid-lateral (L MID, pink), and lateral-dorsal sinus (L-D SIN, purple).

(L and M) *CUP* expression pattern (green shading) on flattened petal diagrams, based on measurements of the *CUP* expression pattern on in situ serial sections. Blue triangle indicates where virtual cut was made (dorsal-dorsal junction) to flatten the petal. At 8 DAI, *CUP* is expressed at the base of petal primordia (L), and there is no gap in expression domain at petal junctions (red box). By 9 DAI (M), gaps begin to appear between the proximal and distal domains of *CUP* expression at the dorsal-lateral and lateral-ventral petal junctions (e.g., red box). Arrow indicates orientation of proximal (Pr) to distal (Dist) axis.

Lines on scanning electron micrographs indicate planes of section for in situ hybridizations (color coded in J and K). White dotted lines indicate position of the lip-distal lobe boundary. Rectangular outlines are color coded to indicate relation to lower magnifications or planes of section. D or Do, dorsal; L or La, lateral; V or Ve, ventral; s, sepal primordium; ca, carpel primordium; st, stamen primordium; D-D, dorsal-dorsal junction; V-L, ventral-lateral junction; L-D, lateral dorsal junction. Scale bars represent 100 μm for all panels except for the enlargement in (G), where they represent 10 μm.

2C and 2D), showing that regions displaying palate and lip identities were not evident.

To understand how *CUP* influences corolla development, we analyzed *CUP* expression by RNA in situ hybridization. We first considered expression at early stages (5–10 DAI, days after floral meristem initiation). In early wild-type floral meristems (5–6 DAI), *CUP* was expressed in a meristematic ring internal to whorl 1 sepal primordia (Figures 3A–3D). By 7 DAI, regions lacking *CUP* expression, most likely corresponding to petal primordia, could be detected (Figures 3F and 3G, arrowed in blue inset of Figure 3G). By 8 DAI, petal and stamen primordia were clearly visible, with *CUP* expressed at their boundaries, as previously described (Figures 3H and 3I) [1, 2, 4]. This expression domain included both inter-whorl boundaries (purple, Figure 3E) and intra-whorl boundaries at junctions of petal primordia (pink, Figure 3E). By 9 DAI, *CUP* expression around petal junctions became separated into a proximal and distal domain, with a gap in-between on the abaxial side. The gap was evident for both the ventral-lateral junctions (Figure 3J, V-L SIN, red bracket) and lateral-dorsal junctions (Figure 3J, L-D SIN, red bracket). By 10 DAI, the gap had enlarged and was also evident on the adaxial side (Figure 3K, V-L SIN and L-D SIN, red brackets). Thus, *CUP* is initially expressed in a continuous domain, both between whorls and at petal junctions (Figure 3L). The junction regions later

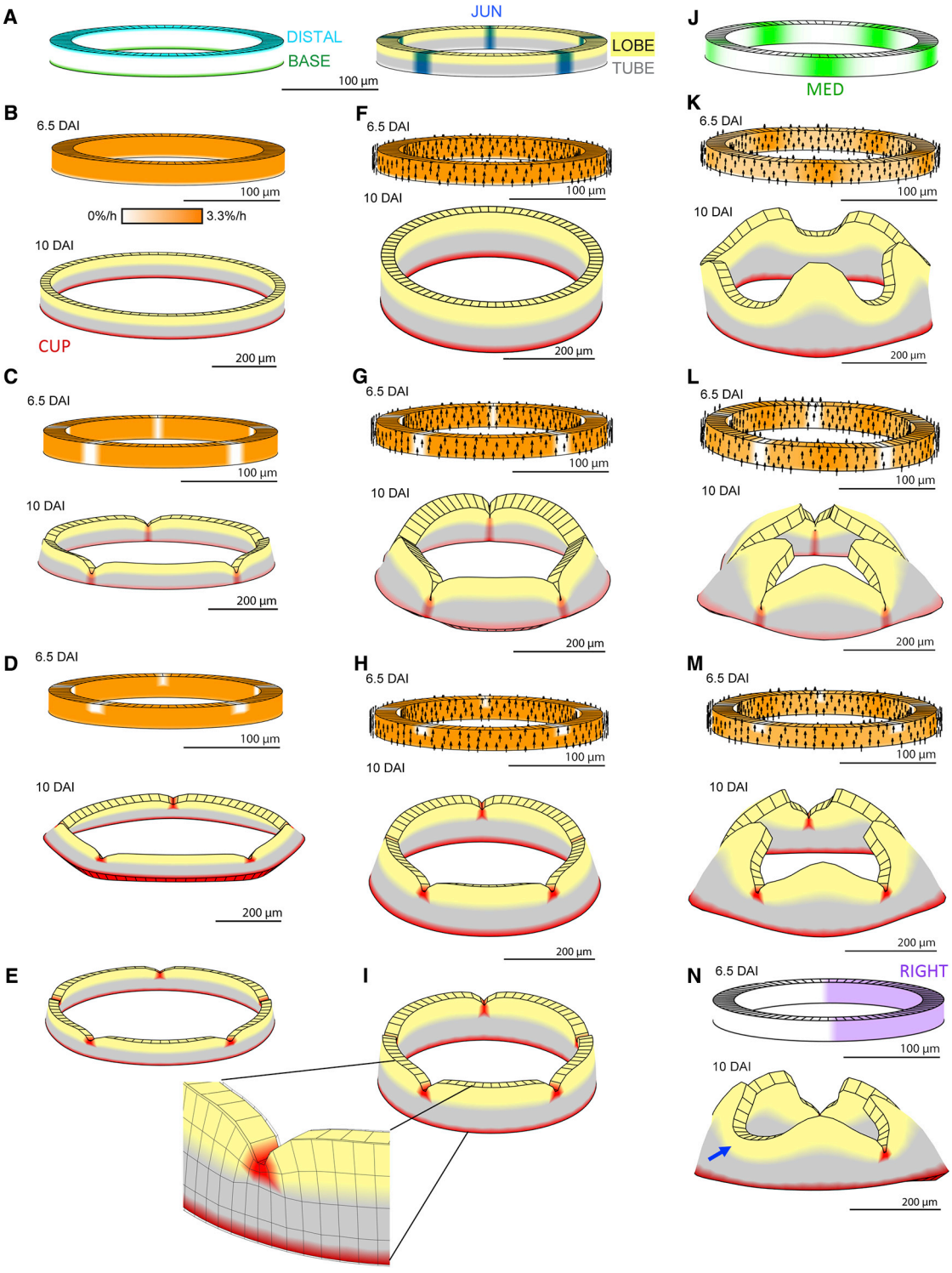

Figure 4. Modeling Sympetalous Corolla Formation

(A) Initial cylindrical canvas representing primordial corolla ring at 6.5 DAI, with domains of expression used to establish growth patterns and polarity. BASE and DISTAL define regions at the proximal and distal ends of the cylinder, respectively. The proximal half of the cylinder expresses TUBE and the distal half LOBE. JUN is expressed at the petal junctions.

(B) Uniform isotropic specified growth. Expression domains of TUBE, LOBE, and inter-whorl CUP (red) are shown at 10 DAI.

(C) Repression of isotropic specified growth rate by CUP at petal junctions leads to formation of deep sinuses between petal primordia. (D) As in (C) except CUP expression inhibited by TUBE, leading to formation of shallow sinuses between petal primordia and a united tube. Cylinder slopes inward because of reduced circumferential growth caused by CUP.

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become separated into a more distal domain, corresponding to the petal sinus (e.g., green domain within red box in Figure 3M), and a proximal domain corresponding to the boundaries of whorl 2 with whorls 1 and 3 (green line at bottom of Figure 3M).

The subdivision of the *CUP* domain at junctions into proximal and distal subdomains may be related to the formation of sympetalous corollas. To explore this possibility, we modeled formation of the corolla lobes and sinuses, using the growing polarized tissue (GPT) framework [21] (see STAR Methods for further details). In this framework, tissue is treated as a connected continuous material, termed the canvas. We used a shallow cylinder to represent the corolla meristematic ring around the floral meristem at 6.5 DAI and subdivided it into four domains along the proximodistal axis: BASE, TUBE, LOBE, and DISTAL (Figure 4A). We also subdivided each petal primordium along its mediolateral axis with JUN, which is expressed at the lateral edges or junctions of each primordium.

We first considered the effects of isotropic specified growth in the plane of the canvas. If areal specified growth rate was uniform (Figure 4B, 6.5 DAI), the cylindrical canvas simply became enlarged (Figure 4B, 10 DAI). Inter-whorl *CUP* expression was indicated at the corolla base (shown in red, Figure 4B, 10 DAI). To account for the development of separate petals, we introduced repression of growth at the petal junctions, mediated by the action of *CUP* (Figure 4C, 6.5 DAI). Running this model led to formation of divisions at the petal junctions (Figure 4C, 10 DAI). The depth of the divisions was reduced if junctional *CUP* was repressed in the tube region, corresponding to a gap in the *CUP* junctional domain (Figure 4D, 6.5 DAI). This led to the formation of an inwardly sloping corolla tube with sinuses between the lobes (Figure 4D, 10 DAI). The inward sloping (a passive effect of growth repression) could be reduced by inhibiting growth of the canvas by BASE (Figure 4E). Thus, if *CUP* acts by repressing growth, the generation of a sympetalous flower may reflect inhibition of *CUP* in a petal junction zone, allowing the non-expressing region to grow and form the tube.

Clonal analysis indicates that early petal growth is anisotropic, being higher parallel to the proximodistal axis than perpendicular to it [16]. To determine how this feature influences shape, we introduced a polarity field, with specified growth rate higher parallel to the polarity (Figure 4F, 6.5 DAI). Running this model gave a taller and narrower cylinder than for the isotropic model (Figure 4F, 10 DAI). Repression of growth at the petal junctions through *CUP* (Figure 4G, 6.5 DAI) gave deep sinuses between the petals and an inward sloping corolla (Figure 4G, 10 DAI). Inhibiting *CUP* expression in the tube (Figure 4H, 6.5 DAI) gave shallower sinuses (Figure 4H, 10 DAI). The inward sloping of the corolla could be reduced by inhibiting growth by BASE (Figure 4I). This shape resembled the starting shape used for modeling corolla morphogenesis (compare Figure 4I with Figure 5I, see also [16]). Although specified growth was only repressed in inter-whorl domains in these models, the deformation of tissue extended to nearby regions, illustrated by the deformation of an initially square grid near the sinus (magnified region in Figure 4I). The grid becomes curved and shows modified growth outside the *CUP* domain. Thus, a simple model for early corolla growth is that *CUP* represses anisotropic growth at petal junctions, except for the tube junctional region where *CUP* is cleared.

Sinuses still form in *cup* mutant corollas, showing that *CUP* is not essential for sinus formation. It is possible that boundary genes other than *CUP* can repress growth at the sinus. Another possibility is that sinus formation depends on not only growth repression at boundaries, but also growth promotion within the body of the primordium. To model this hypothesis, specified growth parallel to the polarity was promoted in and around the midline of each petal primordium, using a mediolateral factor MED (Figure 4J). Inward sloping of the corolla was also reduced by inhibiting growth by BASE as in previous models. Running this model gave five primordia separated by shallow sinuses (Figure 4K). Expression of *CUP* throughout the petal junction, led to further sharpening and deepening of the sinuses (Figure 4L). Repression of *CUP* in the tube region, gave a sympetalous corolla with five lobes (Figure 4M). If *CUP* was maintained in only three junctions, by making its expression depend on factor RIGHT (Figure 4N), an asymmetric corolla was generated with a lip region at sinuses where *CUP* was lacking (blue arrow). Thus, shaping and growth around sinuses may reflect a combination of growth repression at different boundaries and promotion within primordia.

Later Role of *CUP* in Palate and Lip Growth

At 11 DAI, a new *CUP* expression domain was observed. In addition, to the proximal and sinus domains, *CUP* expression was seen in an intermediary region of the ventral and adjoining lateral petals, around the junction of the tube and lobe (Figure 5A, V MID and V-L SIN, blue bracket). Serial sections indicated that this domain formed a crescent shape (Figure 5D, large green domain). There was also a clearing or reduction of *CUP* expression at the lateral-ventral sinuses (Figure 5A, V-L SIN, white arrow) creating two pockets of low expression (Figure 5D, dip in green within red box). The gap between distal and proximal domains of *CUP* was also evident at the dorsal-dorsal junction at this stage (Figure 5A, D-D SIN, red bracket).

The crescent-shaped domain of *CUP* expression in the lower corolla enlarged along the proximodistal axis as the petals

(E) As in (D) but with growth rates repressed by BASE, preventing inward sloping of cylinder (only 10 DAI shown, as same scale as in (D).
(F) Uniform anisotropic specified growth oriented by a proximodistal polarity field (6.5 DAI, arrows).
(G) As in (F) but with growth repressed by CUP at petal junctions, leading to formation of deep sinuses.
(H) As in (G) except CUP expression is inhibited by TUBE. A cylindrical tube forms with five lobes.
(I) As in (H) except that growth inhibited by BASE (only 10 DAI shown, at same scale as for H). Magnified region shows how an initially square grid is deformed by growth repression at the sinus.
(J) Expression domain of MED.
(K) Anisotropic growth enhanced by MED, giving sinuses where MED is low.
(L) As in (K) with growth also repressed by CUP at petal junctions, leading to deeper sinuses.
(M) As in (L) except CUP is inhibited by TUBE.
(N) As in (M) except distal CUP only expressed in the presence of factor RIGHT, allowing growth of a lip region (blue arrow) where CUP is not expressed.
Color coding of growth rates rescaled in (K), (L), and (M) for clarity.

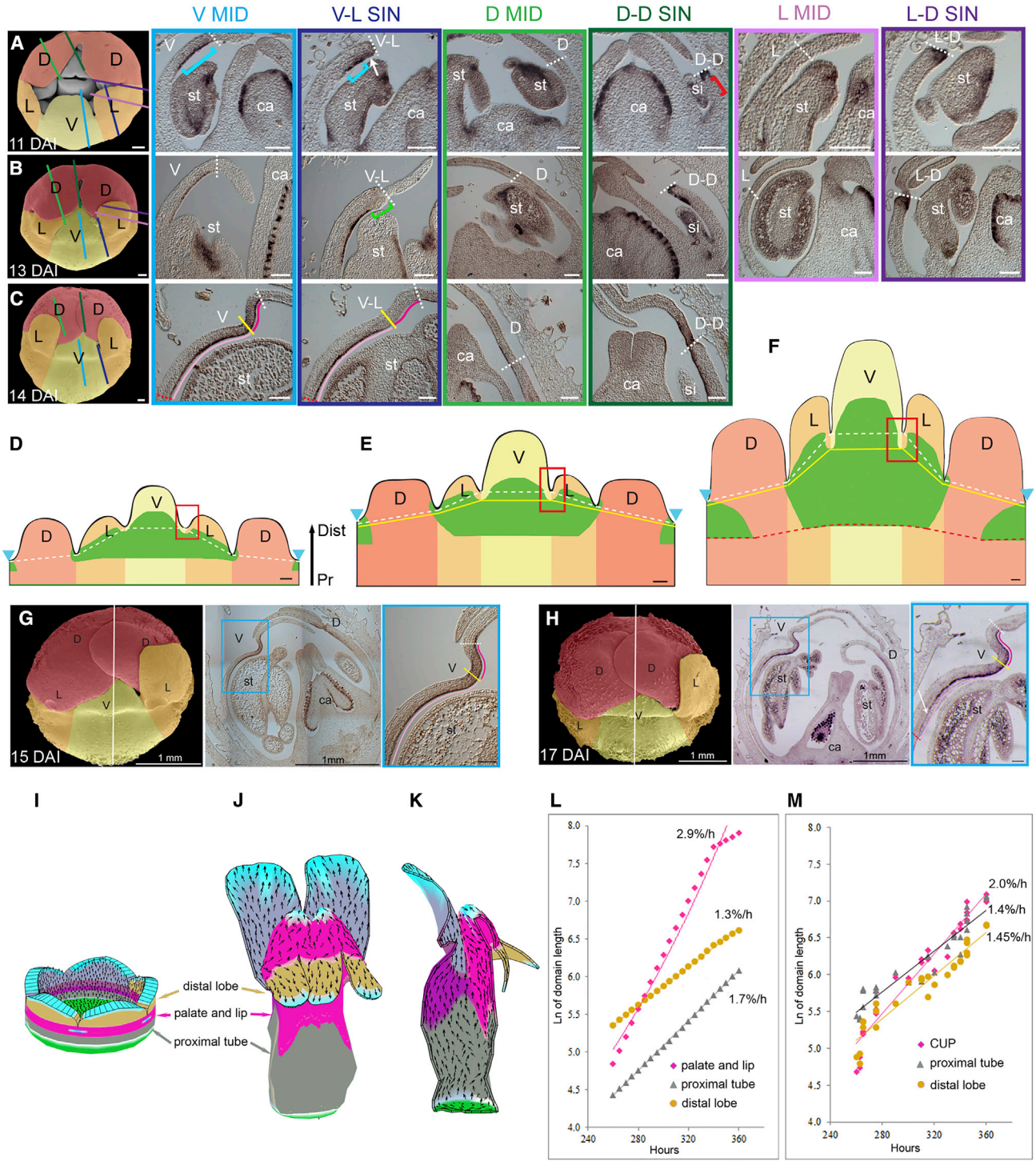

Figure 5. *CUP* Expression Pattern and Growth Analysis at Later Stages of Development

(A–C) Expression of *CUP* from 11–14 DAI. 11 DAI (A) shows intermediary region of *CUP* (blue bracket).A gap in junctional *CUP* expression domain is visible at the dorsal-dorsal petal junctions (red bracket). At 13 DAI (B), the ventral domain has expanded and expression is lost at the distal end of ventral-lateral junctions (green bracket, V-L SIN). By 14 DAI (C), the palate (pink line) and lip (red) regions can be identified by the furrow that lies between them (yellow line). Extended expression around the dorsal-dorsal junction is visible (D-D SIN). Planes of sectioning are mid-ventral (V MID, light blue), ventral-lateral sinus (V-L SIN, dark blue), mid-dorsal (D MID, light green), dorsal-dorsal sinus (D-D SIN, dark green), mid-lateral (L MID, pink), and lateral-dorsal sinus (L-D SIN, purple).

(D–F) Schematic of *CUP* expression (green shading) on flattened petal diagrams from 11–14 DAI. Blue triangle indicates where virtual cut was made to flatten the petal. At 11 DAI (D), a crescent-shaped domain of *CUP* expression is established in the lower corolla with a gap in expression around the ventral-lateral sinus (red

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grew (Figures 5B and 5C and 5E and 5F). In addition, expanded expression was observed near the dorsal-dorsal junction, forming a semi-circular domain (Figure 5C, D-D SIN, Figures 5E and 5F). From 14 DAI, morphological landmarks could be used to map the domains of *CUP* expression. For the lower corolla, the palate-proximal tube boundary (red dashed line, Figure 5C, V MID and V-L SIN) could be identified by a change in trichome densities, the rim (yellow line) by the bend in the petal, and the distal limit of the lip (white dashed line) by tracing a line between petal sinuses (see also white dashed lines in Figures 5D–5F). These assignments showed that the *CUP* crescent domain lies over the palate and lip regions.

As the crescent of *CUP* expression enlarged during growth, so did the pockets of low expression at the lateral-ventral junctions (green bracket in Figure 5B V-L SIN). The pockets of *CUP* low expression were positioned at the lip region of ventral-lateral junctions (Figures 5D–5F). The clearing of *CUP* expression in these regions from 11 DAI onward coincides with growth of the lip at these junctions. By contrast, *CUP* expression was maintained at the sinuses of other junctions, which show little or no lip growth. Thus, growth of the lip at petal junctions correlates with clearing of *CUP* from the sinus domain (Figures 5E and 5F).

Expression of *CUP* in the ventral and dorsal intermediary regions was maintained until 15 DAI (Figure 5G). By 17 DAI, downregulation of *CUP* was observed in the region of palate trichome development (white arrow in enlarged panel of Figure 5H). Taken together, these results suggest that the intermediary CUP domain broadly corresponds to the palate and lip regions of the corolla, consistent with these regions not growing in *cup* mutants.

The increase in length of the *CUP* crescent domain along the proximodistal axis during development might be explained by growth of the *CUP*-expressing region and/or cell-to-cell spread of gene expression. To determine whether growth alone might be sufficient to account for the data, we compared the rate of increase in *CUP* domain length with growth rates estimated from a previously published model of corolla morphogenesis [16]. In this model, the lower palate and lip regions derive from a hypothetical strip of ventral tissue (Figure 5I), which could correspond to the *CUP* crescent domain. The rates of growth of the hypothetical palate-lip and *CUP* crescent domains would not be expected to be exactly the same, as the hypothetical domain was assigned a somewhat arbitrary initial size, and intermediate markers were not available to tune its growth rate. Nevertheless, we would expect these domains to grow similarly in relation to other regions. In particular, the ventral palate-lip domain was proposed to grow faster than regions proximal or distal to it (2.9%/hr compared to 1.3%/hr and 1.7%/hr Figure 5L), leading to formation of the extended ventral palate and lip (Figures 5J and 5K). To see whether this enhanced growth was also observed for the *CUP* crescent, the growth rates of the *CUP* crescent and domains distal and proximal to it were estimated by measuring the length of these regions from in situ hybridizations on longitudinal sections through midline (passing through the middle of the ventral petal) of flower buds at different developmental stages. The *CUP* crescent was initially about 100 μm along the proximodistal axis (11 DAI) and grew to a length of 800 μm by 14 DAI. As in the model, the *CUP* crescent grew faster in the proximodistal orientation than domains proximal or distal to it (2.0% ± 0.11%/hr, compared to 1.4% ± 0.1%/hr and 1.45% ± 0.09%/hr, Figure 5M). This comparison, taken together with the *cup* mutant not forming a palate or lip, suggests that the *CUP* intermediary expression domain most likely maps to the hypothetical palate-lip domain and expands largely through growth, though a further contribution from cell-cell spreading cannot be ruled out.

The *CUP* crescent is a feature of the lower corolla and might thus be expected to be influenced by genes conferring ventral identity. We therefore analyzed expression of *CUP* in the dorsoventral mutant *div* which lacks ventral identity. In contrast to wild-type, the *CUP* crescent domain was not observed in *div* mutants. *CUP* expression was still seen at the sinuses and proximal inter-whorl domain, showing that these domains are not under *DIV* control (Figures 6A–6E; and compare Figures 6P–6R with Figures 6S–6U). Expression at the ventral-lateral sinuses was cleared at later stages (Figure 6R), consistent with growth of the lip in *div* mutants. Mutants with ectopic ventral identity such as *cyc dich*, showed an uninterrupted ring of intermediary *CUP* expression corresponding to ventralization of the flower (Figures 6F–6J). This ectopic activity depended on *DIV*, as in the *cyc dich div* mutant *CUP* expression was again reduced to the petal sinuses and proximal domains (Figures 6K–6O). The extended semicircular domain characteristic of the dorsal petals was also absent in this triple mutant. Thus, *DIV* upregulates *CUP* expression, leading to the crescent of expression in the wild-type lower corolla.

Inter-whorl fusions were not observed in *div* mutants, which had greatly reduced intermediary *CUP* expression. Moreover, inter-whorl fusions were observed at all petal junctions in *cup* mutants, including dorsal-lateral junctions. These observations suggest that organ fusion is most likely due to reduced *CUP* activity in the inter-whorl boundary domain rather than in the intermediary domain.

Both boundary and intermediary *CUP* activity could be integrated within previous models of corolla, in which morphogenesis depends on combinatorial interactions between factors expressed along three axes: dorsoventral, proximodistal, and

box), where the junctional lip region forms. Arrow indicates orientation of proximal (Pr) to distal (Dist) axis. By 13 DAI (E), the ventral crescent has grown and an enlarged domain of *CUP* at the dorsal-dorsal junctions also becomes visible. By 14 DAI (F), the *CUP* domains have further increased in size.

(G and H) From 15 DAI (G), the ventral-lateral palate trichomes start developing, and the *CUP* expression at these region fades by 17 DAI (H, white arrow points to trichomes).

(I–K) Computer model of Snapdragon flower development as described by [16]. The initial canvas corresponds to 10 DAI and comprises a cylinder with five lobes (I). Regions expressing various identities along the proximodistal axis are color coded. The result of running the model (24 DAI, mature corolla) is shown in ventral view (J) or in a longitudinal section (K). Note the greater increase in length of the palate and lip relative to the proximal tube and distal lobe.

(L and M) Growth rates of different regions in the computational model (L) and obtained by measuring lengths of domains from in situ hybridizations (M). In both cases, the magenta region (palate and lip/ CUP domain) grows faster than the proximal tube or distal lobe. The slope of each linear fit gives the growth.

See also Figure S1.

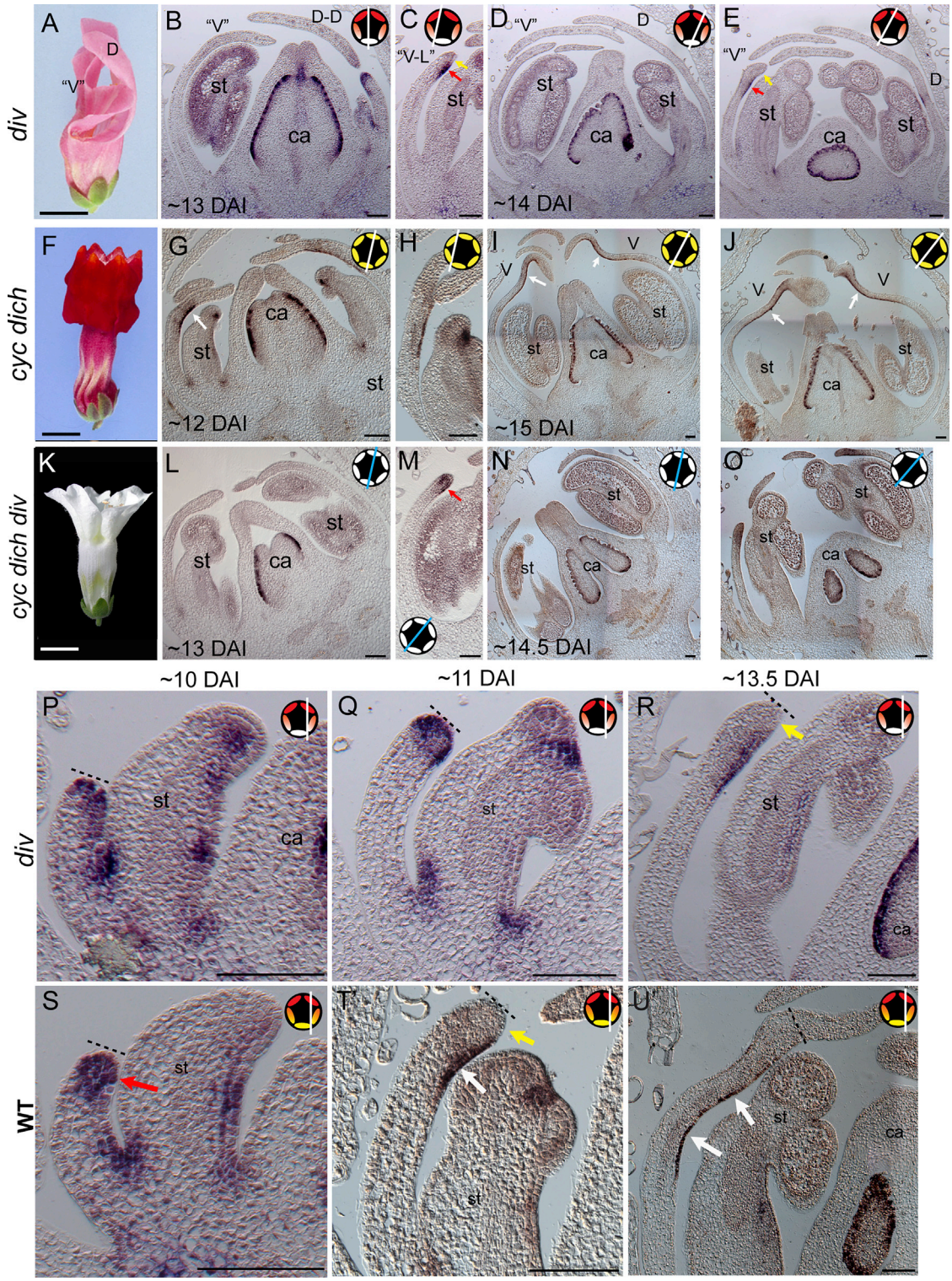

Figure 6. *CUP* Expression in Dorsoventral Mutants

(A–E) The *div* mutant (flower shown in A) lacks a ventral palate region and lacks the crescent-shaped domain of *CUP* expression in the ventral corolla at ~13 DAI (B and C) and ~14 DAI (D and E). Expression of *CUP* still visible around the sinus (red arrows) with a distal gap corresponding to the lip (yellow arrows).

(F–J) The *cyc dich* mutant (flower shown in F) is fully ventralized and expresses *CUP* in an intermediary domain (white arrows) in all petals at ~12 DAI and (G and H) and ~15 DAI (I and J).

(K–O) The *cyc dich div* mutant (flower shown in K) lacks intermediary *CUP* but retains expression at the sinuses (red arrow).

(legend continued on next page)

mediolateral [15, 16]. *CUP* expression at early stages would contribute to shaping the early sympetalous corolla (illustrated in models of Figure 4), which is taken as a starting shape for the previously published model. This would be achieved by *CUP* expression at petal junctions being activated by mediolateral factors, which would act in combination with *CUP* to inhibit growth. Junctional *CUP* expression would be inhibited by a proximodistal factor (TUBE) to allow growth of the corolla tube (united tube). Expression at the distal junction would be inhibited at later stages by mediolateral factors (LIP), modulated by dorsoventral factors (*RAD*), to allow growth of the lateral-ventral lip junction (united lip). Intermediary *CUP* activity would be incorporated by activating *CUP* with a combination of proximodistal (PALATE, LIP) and dorsoventral (*DIV*, *CYC*, and *DICH*) genes. *CUP* in combination with these factors would then promote growth of the palate and lip regions. These interactions would replace roles previously assigned directly to the combined dorsoventral, proximodistal, and mediolateral factors.

Possible downstream targets of *CUP* are *YUCCA* auxin biosynthetic genes. In *Arabidopsis*, *YUCCA1* is expressed in a similar domain to *CUC* and can depend on *CUC* activity [22]. In situ hybridizations with the *Antirrhinum* ortholog of *YUCCA1*, *AmYUCCA1*, showed that it was expressed in a very similar pattern to *CUP*, at the base of primordia, sinuses, and intermediary domain (Figure S1). Moreover, *AmYUCCA1* was cleared at later stages from around the lateral-ventral sinus, similar to *CUP* (Figures S1L and S1M). Expression of *AmYUCCA1* in these domains was not observed in *cup* mutants (Figure S2), suggesting that they are under *CUP* control.

As the *CUP* crescent is involved in formation of the ventral palate and lip, we tested whether a similar intermediary region of expression was present in other sympetalous flowers of the order Lamiales. *Linaria* has a corolla with a closed mouth similar to that of *Antirrhinum* and belongs to the same tribe (Antirrhineae) of the family Plantaginaceae (Figure 7A). Early expression of *Linaria CUP* was in boundary domains, similar to that in *Antirrhinum* (Figures 7C–7E). At later stages, intermediary region of *CUP* expression was observed in the lower petals, similar to that seen in *Antirrhinum*, suggesting that it shares the crescent domain (Figure 7F, blue bracket). *Mimulus* is a more distant genus from *Antirrhinum*, belonging to a different plant family (Phrymaceae). The corolla is not fully hinged like *Antirrhinum* but nevertheless has a bilaterally symmetric shape with palate and lip regions (Figure 7B). Early expression of *Mimulus CUP* was in boundary domains, similar to those in *Antirrhinum* (Figures 7G and 7H). At later stages, expression of *Mimulus CUP* was also detected in an intermediary region of the lower petals (blue bracket Figure 7I). Expression then diminished (Figure 7J). Thus, the intermediary region of *CUP* expression was found in other species with sympetalous corollas exhibiting strong zygomorphy.

DISCUSSION

CUP expression in the developing *Antirrhinum* corolla shows two novel features. First, shortly after petal initiation, expression at the junction between adjacent petal primordia becomes subdivided into a proximal and distal domain, separated by a gap. The proximal domain remains at the inter-whorl boundary, the gap grows to become the junctional region of the corolla tube, and the distal domain forms the sinus. Second, at later stages of development, *CUP* is expressed in an intermediary region, in a crescent-shaped domain in the lower corolla, and in a semi-circular domain in the upper corolla. These intermediary domains correspond to the palate and lip regions that grow to form the characteristic wedge shape of the lower corolla and apposing triangular region of the upper corolla.

Both of these novel features of *CUP* expression may be connected with the formation and shaping of the corolla. A major role of *CUP* and its homologs is to repress growth at the base of primordia, ensuring their separate development [2, 3, 23–25]. This repression may operate at both intra-whorl and inter-whorl boundaries. We show, through computational modeling, that repression of specified growth rates at inter-whorl boundaries leads to relative shortening of the boundary domain and deformation of nearby tissue to generate a sinus. If growth repression is excluded from the observed gap in the *CUP* expression domain, a sympetalous corolla with a united tube is generated. Thus, the sympetalous corolla can be accounted for by repression of *CUP* at petal junctions, creating a gap that allows united growth to occur. Clearing of *CUP* also occurs at later stages from the lateral-ventral sinuses of the corolla, correlated with growth of the lip in these regions. Such clearing is not observed at the dorsal-lateral sinuses where lip growth is greatly reduced, corresponding to the hinge of the corolla. Thus, selective inhibition of *CUP* may play a key role in sympetally and modulating growth proximal to the sinus by allowing subregions of boundary domains to grow. Sinuses still form in *cup* mutants and at lateral-ventral petal junctions, showing that *CUP*-independent mechanisms are involved in sinus formation. These mechanisms may involve action of other boundary genes and/or growth promotion within primordia through the action of auxin or other growth regulators [9, 10].

Similar to orthologous mutants in other sympetalous species, *nam* in Petunia [1] and *gob* in tomato [7], flowers of *cup* mutants show inter-whorl fusion (e.g., petal-stamen fusions), while intra-whorl fusion of petals is not markedly affected. These findings contrast with results of virus-induced silencing of *NAM* in Petunia, which leads to reduced intra-whorl fusion and petal separation [14]. A possible explanation for this discrepancy is that rather than eliminating NAC activity, virus-induced silencing modifies NAC gene regulation, leading to ectopic boundary gene activity.

(P–R) *CUP* expression at the ventral-lateral sinus in *div* mutant. Expression is retained at the sinus (dashed black line) at ~10 DAI (P) and ~11 DAI (Q). By ~13.5 DAI, a gap in expression is seen at the sinus (yellow arrow) corresponding to formation of the lip region (R).

(S–U) *CUP* expression near the ventral-lateral sinus in wild-type. Expression is observed at the sinus at ~10 DAI (S, red arrow), with more extended expression by ~11 DAI (T, white arrow) and gap near the sinus where the lip is forming (yellow arrow). By 13.5 DAI, the ventral crescent (white arrows) is extensive (U).

Planes of sectioning are indicated by white lines in the top right of each in situ image. Only half of the section plane is shown in close-ups (C, H, M, and P–U). "V," mutant ventral petal; "V-L," mutant ventral and lateral petal junction; D, dorsal; ca, carpel; st, stamen. Scale bars represent 1 cm for (A), (F), and (K) and 100 μm for the other panels.

Figure 7. *CUP* Expression in *Linaria* and *Mimulus*

(A and B) Flowers of *Linaria maroccanna* (A) and *Mimulus guttatus* (B) shown in face (left) and side (right) views. Scale bars represent 1 cm.

(C–F) Expression of *Linaria CUP*. At early stages the *LmCUP* is expressed at the flower organs basal junctions (C and D), at the petal junctions (V-L in E). The ventral crescent (blue bracket) is visible at later stages (F). se, sepal primordium; st, stamen primordium; ca, carpel primordium. D, dorsal petal; V, ventral petal; V-L, ventral-lateral junction; se, sepal; st, stamen. Planes of sectioning are indicated by white lines in the top right of each in situ image. Scale bars represent 100 μm.

(G–J) Expression of *Mimulus CUP*. Similar to *LmCUP*, *MgCUP* is expressed at the organ boundaries (G and H) and petal junctions (H). Ventral crescent (blue bracket in I is visible at later stages (I–J). D, dorsal petal; V, vetral petal; se, sepal; st, stamen; ca, carpel. Scale bars represent 100 μm.

In *Antirrhinum*, *CUP* has acquired a further role, in establishing lip and palate domains. This role of *CUP* involves expression in an intermediary domain along the proximodistal axis, at the tube-lobe boundary. For the lower corolla, the intermediary domain grows to form a crescent shape, while for the upper corolla it grows to form a semi-circular shape. These intermediary *CUP* domains depend on activity of the dorsoventral genes. In *cup* mutant flowers, the corolla has a greatly simplified shape without an observable palate or lip. Thus, the evolution of *Antirrhinum* corolla shape most likely involved the *CUP* gene being co-opted and brought under the control of dorsoventral genes to modulate palate and lip growth. This co-option must have occurred prior to the divergence of *Antirrhinum*, *Linaria*, and *Mimulus*, as all three species exhibit intermediary domains of *CUP*. *Linaria* is the closer relative of *Antirrhinum* and has a similar closed mouth with palate and lip. *Mimulus* is a more distant member of the Lamiales and has a bilaterally symmetric sympetalous corolla, with upper and lower palates and lips. However, the *Mimulus* flower does not have a closed mouth, suggesting that the specific shaping of the corolla depends on targets of *CUP* and dorsoventral genes.

NAC-domain genes typically repress growth within their expression domains (e.g., boundaries), though they may promote growth outside these domains, by influencing auxin distribution [9, 10]. By contrast, the zone of intermediary *CUP* expression shows enhanced growth. Growth enhancement by *CUP* may involve generation of auxin as the auxin biosynthetic gene, *AmYUCCA1*, is expressed in the intermediary domain, and this expression is absent in *cup* mutants. *YUCCA* expression is also activated in boundary domains of *CUP* and *CUC* [22], though unlike the intermediary zone, growth is believed to be repressed within these domains [5]. Thus, rather than necessarily being a repressor of growth, the *CUP* transcription factor may acquire different roles, presumably reflecting its combinatorial interactions with other genes. These interactions would then lead to modulation of growth-modulating target genes, such as *AINTEGUMENTA* [26–28], known to be expressed in the ventral palate and lip of Snapdragon petals [29].

Shaping of the Snapdragon flower, with its united corolla, hinged palate, and extended lower lip, depends on the interactions between boundary genes, such as *CUP*, and factors expressed along the different axes of the flower. Similar interactions may underlie the shaping of other structures, such as the grass leaf, in which the ligule and auricle provide a hinge at the sheath-blade boundary, where NAC genes are expressed [30]. Thus, our analysis provides further support for

the notion that modulations in boundary genes and their interactors may play an important role in the evolution of diverse plant morphologies.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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- DATA AND SOFTWARE AVAILABILITY

SUPPLEMENTAL INFORMATION

Supplemental Information includes two figures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2017.07.064.

AUTHOR CONTRIBUTIONS

A.B.R. conceived and designed the study, acquired the data, performed computational modeling, analyzed and interpreted the data, and revised the manuscript. J.R.K. analyzed and interpreted the data and provided GFtbox modeling support. J.A.B. conceived and designed the study. E.C. conceived and designed the study, analyzed and interpreted the data, performed computational modeling, and drafted and revised the manuscript.

ACKNOWLEDGMENTS

We would like to thank all lab members for their input during the execution of this project, particularly Desmond Bradley, Katie Abley, and João Raimundo. A particular thanks to Beatriz Goncalves for reading the paper and making comments and suggestions. We would also like to thank Lucy Copsey and Catherine Taylor for excellent plant care, and Kim Findlay for help with the scanning electron micrographs. We are very grateful to Yongbiao Xue for providing access to the unpublished data on the Snapdragon genome sequence. A.B.R was supported by an EMBO long-term fellowship (ALTF 568-2008) and HFSP long-term fellowship (LT000563). The author's research was supported by the UK Biotechnology and Biological Research Council (J.A.B., J.R.K., and E.C., BB/J004588/1 and BB/F005997/1).

Received: March 22, 2017
Revised: June 15, 2017
Accepted: July 27, 2017
Published: August 31, 2017

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-DIG	Roche	11093274910; RRID: AB_514497
Bacterial and Virus Strains		
One Shot Chemically Competent or Electrocomp *E. coli*	Life Technologies	K4575-01
Experimental Models: Organisms/Strains		
Antirrhinum majus NCBI taxon 4151	John Innes Centre, Norwich, UK	*JIC stock 7, div-13 cyc dich div*
Antirrhinum majus NCBI taxon 4151	Gatersleben seed bank, Germany	*cupuliformis-semifertilis MAM71*
Linaria maroccanna	Jorge Almeida, IGC, Portugal	Commercial variety
Mimulus guttatus	John Willis	Wild species
Oligonucleotides		
CUP-F (ATGGAGAATTACAATTGCTACGA)	This paper	N/A
CUP-R (CATCTAATCAACAACGCCACCTCCG)	This paper	N/A
LmCUP-F (GCAATTGCTGAAGTTGATCTCAAC)	This paper	N/A
LmCUP-R (CCTTGGATTTCTTGACAAGTAGTG)	This paper	N/A
MgCUP-F (ATGGAGAATTATCAAGGGTACGAAA)	This paper	N/A
MgCUP-R (GCCGTACCCAGACATGTGATCACC)	This paper	N/A
CUP-specific F (CATTTCCATCCCCGATGTCAGCTC)	This paper	N/A
LmCUP specific forward (GCAATTGCTGAAGTTGATCTCAAC)	This paper	N/A
MgCUP specific F (ATGGAGAATTATCAAGGGTACGAAA)	This paper	N/A
AmYUCCA1-F (ATGTCCTCCTCTAACAAAGAAAAAGATG)	This paper	N/A
AmYUCCA1-R (TTATCTTGGAACAATATTTGAACCACAAC)	This paper	N/A
Software and Algorithms		
GFt-box and associated models	http://cmpdartsvr1.cmp.uea.ac.uk/downloads/software/OpenSourceDownload_CurrentBiology_Rebocho_2017/GPT_Antirrhinum_CUP.zip	N/A

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Enrico Coen (enrico.coen@jic.ac.uk).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Antirrhinum majus wild-type (JIC stock 7), *div-13* [18], *cyc dich* and *cyc dich div* [16] and *cup*^{sem} [4], were grown in the greenhouse at the John Innes Centre. Each *cup* mutant generates several escape shoots, with 2-3 flowers per escape shoots. We have grown and observed more than 30 *cup* mutants. Seed of *Linaria maroccanna* was obtained from Jorge Almieda [31], and that of *Mimulus guttatus* from John Willis. Staging of flowers in Days After Initiation (DAI) was as described in [32]. Mutants were staged by: 1) measuring dorsal petal width in case of *div-13* as this mutant does not affect the development of the dorsal petals; 2) measuring carpel and stamen width in case of *cyc dich* and *cyc dich div*, as a proxy of the wild-type carpel and stamen width values.

METHOD DETAILS

In situ Hybridization

In situ hybridizations were carried out as described previously [15, 33]. To generate specific digoxigenin-labeled riboprobes probes, the ORF of *CUP*, the ORF of *Linaria CUP* ortholog and the ORF of *Mimulus CUP* ortholog were amplified using TAQ DNA polymerase PCR (201205, QIAGEN) and cloned into pCR4-TOPO TA vector (K4575-01, Life Technologies) following manufacture instructions. The cloned fragments were amplified using a forward specific primers and the M13F primer in the case of CUP and the M13R primer in the case of the other two probes (pCR4-TOPO kit), column purified (28104, Qiaquick PCR purification) followed

by a phenol/chlorophorm extraction. Antisense probe was obtained by RNA transcription using the T7 promoter (10881767001, Roche through Sigma-Aldrich) in the case of CUP, and the T3 promoter (11031163001, Roche through Sigma-Aldrich) in the case of the other probes, and DIG-UTP (11209256910, Roche through Sigma-Aldrich), according to manufacture instructions. The probe was hydrolysed at 60°C using a 200 mM carbonate buffer pH 10.2 solution, for 85 min in the case of CUP and 35 min in the case of the other two probes.

To identify the *YUCCA* orthologs expressed during Snapdragon flower development, a draft genome sequence of Snapdragon was used to isolate nine snapdragon *YUCCA* genes. *AtYUCCA1* and *AtYUCCA4*, in *Arabidopsis*, and *FLOOZY,* in petunia, were shown to be expressed during petal emergence [34, 35] Focusing on the snapdragon *YUCCA1/FLOOZY* ortholog, primers were used to amplify the open reading frame from JIC stock2 cDNA.

Microscopy

Scanning electron microscopy was carried out as described in [32]. In situ hybridized material was imaged using a Leica DM 6000 and Nomarski settings, x10 and x20 dry lens and a DFC420 digital camera were used to photographed the in situ sections. All measurements made from in situ images were calculated using the ImageJ software (http://imagej.nih.gov/ij/).

Computational Modeling

Because of tissue connectivity, repression of growth in one region may cause passive deformations in adjacent regions. Computational modeling is therefore needed to determine the shapes that would be generated by particular patterns of growth repression. Models are based on the Growing Polarized Tissue framework (GPT-framework) [21]. In this framework, tissue is treated as a connected continuous material, termed the canvas. The extent to which a tissue region grows depends not only on its intrinsic growth specification but also on mechanical constraints from neighboring regions. We distinguish between two types of growth: specified and resultant. Specified growth is how a region of tissue would deform if it was free from the mechanical constraints of its neighboring regions. Resultant growth is how a region deforms in the context of neighboring mechanical constraints, and includes anisotropies, rotations, and curvature that emerge passively from such constraints [15].

As the main focus of the modeling was to understand corolla formation, we considered growth of whorl 2 alone. The initial canvas comprised a shallow cylinder to represent a corolla meristematic ring around the floral meristem at 6.5 DAI. The base of the cylinder, which corresponds to the petal inter-whorl boundary, was anchored in the z direction. The canvas has two surfaces (A and B). Identity and signaling factors can be specified throughout the canvas. The distribution of factors is shown in Figures 4A and 4J. A growth regulatory network (KRN) controls the specified growth parallel (K_{par}) and perpendicular (K_{per}) to the local polarity, established by taking the gradient of a diffusible factor POLARIZER (POL). For anisotropic growth models, the polarity field is established by producing POL at the bottom of the canvas (through factor BASE) and fixing it to a low concentration at the top (through factor DISTAL).

Figure 4B: Uniform isotropic growth

To model uniform specified isotropic growth the KRN equations are:

$$K_{par} = 0.0165$$
$$K_{per} = 0.0165$$
$$K_{nor} = 0.005$$

where K_{nor} is specified growth rate in canvas thickness.

Figure 4C: Isotropic growth inhibited by CUP at junction

The KRN equations are:

$$K_{par} = 0.0165 \cdot \mathrm{inh}(100, i_{jcup})$$
$$K_{per} = 0.0165 \cdot \mathrm{inh}(100, i_{jcup})$$
$$K_{nor} = 0.005$$

where i_{jcup} is the CUP expressed at the petal junctions (intra-whorl boundary), and inh (x, i_z) denotes inhibition by factor i_z by an amount x [16].

Figure 4D: Isotropic growth inhibited by CUP when excluded from TUBE

As for Figure 4C except that the expression of i_{jcup} is inhibited by TUBE.

Figure 4E: Isotropic growth as in D with growth inhibited at base

The KRN equations are:

$$K_{par} = 0.0165 \cdot \mathrm{inh}(100, i_{jcup}) \cdot \mathrm{inh}(0.5, i_{base})$$
$$K_{per} = 0.0165 \cdot \mathrm{inh}(100, i_{jcup}) \cdot \mathrm{inh}(0.5, i_{base})$$
$$K_{nor} = 0.005$$

where i_{base} is the BASE identity factor.

Figure 4F: Uniform anisotropic growth

The KRN equations are:

$$K_{par} = 0.022$$
$$K_{per} = 0.011$$
$$K_{nor} = 0.005$$

Figure 4G: Anisotropic growth inhibited by CUP at junction

The KRN equations are:

$$K_{par} = 0.022 \cdot \mathrm{inh}(100, i_{jcup})$$
$$K_{per} = 0.011 \cdot \mathrm{inh}(100, i_{jcup})$$
$$K_{nor} = 0.005$$

Figure 4H: Anisotropic growth inhibited by CUP when excluded from TUBE

As for Figure 4G except that the expression of i_{jcup} is inhibited by TUBE.

Figure 4I: Anisotropic growth as in H with growth inhibited at base

The KRN equations are:

$$K_{par} = 0.022 \cdot \mathrm{inh}(100, i_{jcup}) \cdot \mathrm{inh}(0.5, i_{base})$$
$$K_{per} = 0.011 \cdot \mathrm{inh}(100, i_{jcup}) \cdot \mathrm{inh}(0.5, i_{base})$$
$$K_{nor} = 0.005$$

Figure 4K: Anisotropic growth promoted within primordia

The KRN equations are:

$$K_{par} = 0.022 \cdot \mathrm{pro}(0.3, s_{med}) \cdot \mathrm{inh}(0.5, i_{base})$$
$$K_{per} = 0.011 \cdot \mathrm{inh}(0.5, i_{base})$$
$$K_{nor} = 0.005$$

where s_{med} is the MED factor expressed within primordia (Figure 4J), and pro (x, s_z) denotes promotion by factor s_z by an amount x [16].

Figure 4L: Anisotropic growth promoted within primordia combined with CUP repressing growth at junctions

The KRN equations are:

$$K_{par} = 0.022 \cdot \mathrm{pro}(0.3, s_{med}) \cdot \mathrm{inh}(100, i_{jcup}) \cdot \mathrm{inh}(0.5, i_{base})$$
$$K_{per} = 0.011 \cdot \mathrm{inh}(100, i_{jcup}) \cdot \mathrm{inh}(0.5, i_{base})$$
$$K_{nor} = 0.005$$

Figure 4M: Anisotropic growth promoted within primordia combined with CUP excluded from tube

As for Figure 4L except that the expression of i_{jcup} is inhibited by TUBE.

Figure 4N: CUP expression at a subset of junctions

As for Figure 4M except that i_{jcup} expression depends on factor RIGHT.

QUANTIFICATION AND STATISTICAL ANALYSIS

Growth rates for the *CUP* domain, and domains distal and proximal to it, were determined by measuring of these domains from staged in situs. Data from 27 buds at various stages were obtained and log of length of the *CUP* domain in medial sections through the ventral petal, plotted against estimated age (in DAI). The slope of linear fits gave the estimated relative growth rate, and the standard deviation of the slopes was obtained using the LINEST function in excel. As the measurements came from different buds, the standard deviations relate to both technical variation in length/staging estimates and biological variation between samples. In developing the model used for comparison, growth parameters were adjusted to generate approximately the correct final dimensions and were not tuned to domain lengths at intermediate stages. The parameters chosen, and thus the growth rates, were therefore influenced by the initial size of the domain and simplifying assumptions about intermediate stages.

DATA AND SOFTWARE AVAILABILITY

GFT-box software and programs used for modeling can be downloaded from http://cmpdartsvr1.cmp.uea.ac.uk/downloads/software/OpenSourceDownload_CurrentBiology_Rebocho_2017/GPT_Antirrhinum_CUP.zip.