ON THE NATURE OF THINGS
New Ideas and Directions in Botany

The shape of things to come: From typology to predictive models for leaf diversity[1]

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He who loves practice without theory is like the sailor who boards a ship without a rudder and compass and never knows where he may cast.

—Leonardo da Vinci (1452–1519), quoted by Kline (1972)

As first noted by Theophrastus 2400 yr ago, the striking morphological variation of seed plant leaves largely reflects differences in margin geometry (Theophrastus, 1916). Accordingly, leaf forms are often characterized by the arrangement and shape of protrusions and intervening indentations along the leaf margin (Fig. 1A). This abundant variation, often in very closely related species (Fig. 1B) or even the same plant, presents an iconic opportunity for conceptualizing how biological forms develop and diversify. Nonetheless, the morphogenetic basis for this striking variation remains enigmatic.

Remarkably, recent debates on leaf shape echo classical ones, focusing on whether various outgrowths of the shoot, including serrations, lobes, leaflets, or even organ primordia, are equivalent entities. Central to these debates is the question whether the developmental programs regulating simple and dissected leaf shapes represent fundamentally different morphogenetic strategies or are variations on a single program equivalent to that initiating leaves (e.g., Arber, 1950; Barkoulas et al., 2008; Efroni et al., 2010). As genetic information on different taxa accumulates, it becomes clearer that absolute distinctions are difficult to identify and that there is likely a continuum of forms, arising through flexible articulation of different genetic modules. For example, the genetic processes producing lobed and dissected forms are similar (Hay et al., 2002) despite lobed forms often being classified as simple. Similarly, a progression from serrated to deeply lobed leaves can be produced by modifying the expression of a single gene (e.g., Nikovics et al., 2006; Shani et al., 2009), indicating that relatively subtle differences in gene expression are sufficient to produce pronounced morphological changes.

These issues highlight the difficulties in conceptualizing forms in isolation from their generative processes. How, then, can we obtain a precise mechanistic understanding of the developmental origin of diverse leaf forms? Like all aspects of morphology, shape arises from a cascade of feedback loops integrating genetic regulation, signaling, cell division, and growth. Ultimately, those processes account for how changes in the amount, direction, and duration of growth generate form (Coen et al., 2004), augmented in rare cases by programmed cell death (Gunawardena and Dengler, 2006). Until very recently, the theoretical and conceptual tools to understand how gene activities influence growth and form have been sparse. The complexity of interactions linking genes to form makes their comprehension difficult without formal models to organize biological data into causative mechanistic frameworks (Uyttewaal et al., 2010). Recently, an understanding of how leaf-form emerges from patterning, maturation, growth, and mechanics has been fostered by studies synthesizing modeling and experiments (e.g., Bilsborough et al., 2011; Kuchen et al., 2012; Runions et al., 2017).

This interdisciplinary approach enhances our ability to conceptualize morphogenesis, as illustrated by work investigating serration formation in *Arabidopsis thaliana* (Fig. 1C). When serrations are initiated, the leaf primordium grows predominantly at its base, owing to the gradual tip-to-base progression of differentiation. In this region, serrations are organized by an auxin-transport dependent patterning mechanism that creates interspersed maxima of growth-promoting auxin and growth-repressing CUC2 (CUP-SHAPED COTYLEDON 2) (Fig. 1D; Bilsborough et al., 2011). Thus, in *A. thaliana,* marginal patterning and growth are largely restricted to this zone. Modifying growth and patterning within this basal growth zone yields pronounced changes in organ shape (e.g., Shani
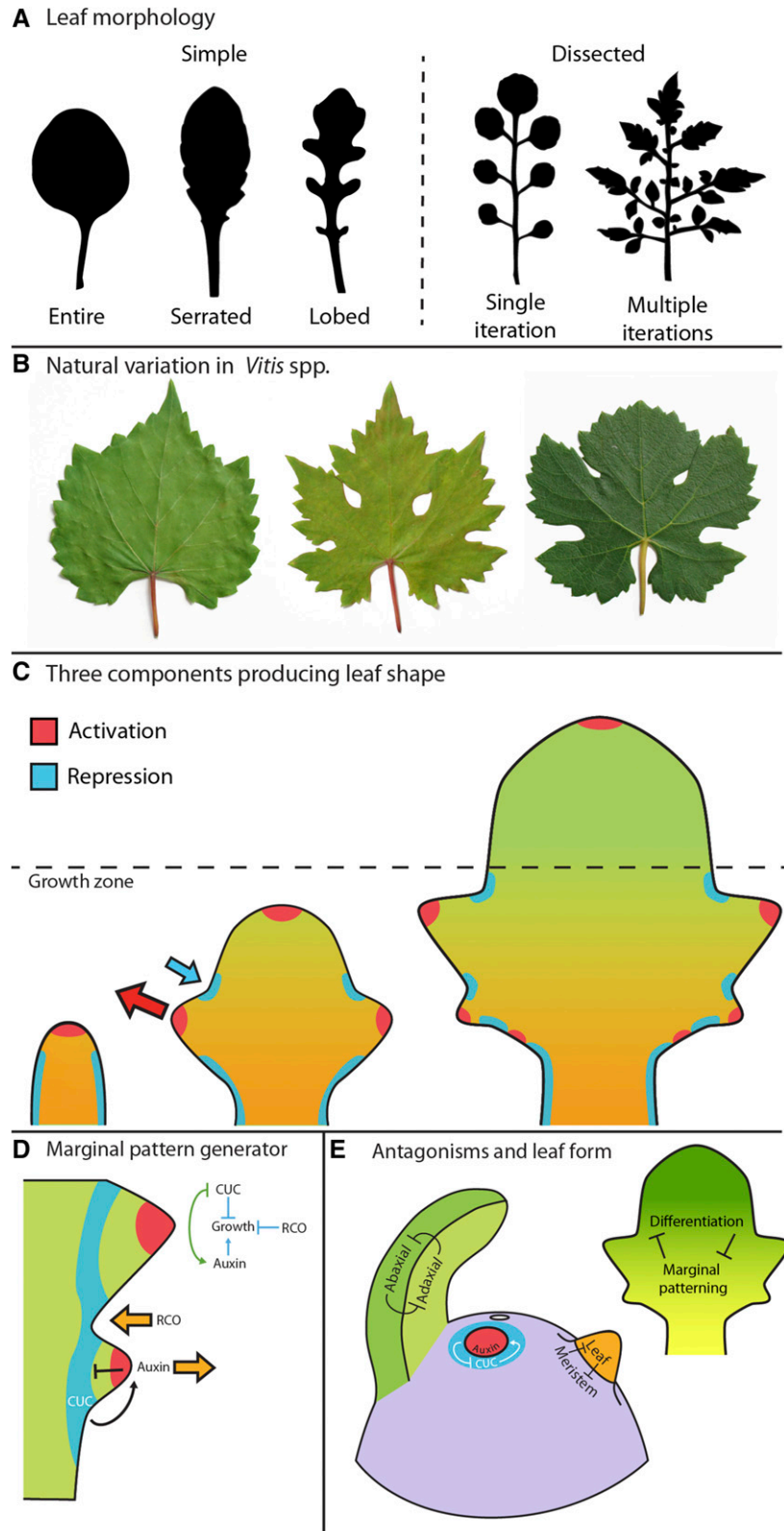
FIGURE 1 Leaf form and processes underlying its generation. (A) Leaf outlines illustrating simple (*Arabidopsis thaliana* and *Arabidopsis lyrata*), and dissected (*Cardamine hirsuta* and *Solanum lycopersicum*) leaf forms. Simple leaves have an undivided blade with a margin (outer edge) that is smooth (entire, first leaf of *A. thaliana*), adorned with protrusions that are small (serrated, adult leaf of *A. thaliana*) or large (lobed, leaf of *A. lyrata*). Dissected leaves (also termed compound) have a divided blade with smaller leaf-like forms (leaflets) arranged either along a single stalk (singly dissected) or hierarchically (iteratively dissected). (B) Diverse leaf forms seen in closely related *Vitis* species, including Ruggeri (*V. berlandieri* × *V. rupestris*, left), and

et al., 2009; Vlad et al., 2014). The broad principle we can derive from this work is that leaf margin shape emerges from three interacting components (Fig. 1C, D): the positioning of the organ growth zone, a marginal pattern generator placing peaks and indentations along the organ flanks within this zone, and local growth repressors and activators sharpening the peaks generated by patterning.

This mechanism helps conceptualize the origin of different leaf shapes and corresponds well to recent biological information in multiple taxa (Bar and Ori, 2014). In crucifers, the gene *RCO* (*REDUCED COMPLEXITY*) promotes leaflet development by repressing growth at the flanks of protrusions generated by CUC-auxin patterning (Vlad et al., 2014; Sicard et al., 2014). This repression enhances the growth differential between protrusion tips and sinuses considerably, as repression occurs within a zone of vigorous growth. Conversely, growth repressors distal to this zone have a reduced effect (Vlad et al., 2014; Vuolo et al., 2016), as decreased growth likely impairs the ability of growth modulating factors to influence shape. We can thus envision variation in leaf shape occurring through differential activity of the three components depicted in Fig. 1C and D, as highlighted by models built on their basis (e.g., Bilsborough et al., 2011; Runions et al., 2017). Such models make certain predictions: rapid recession of growth from tip to base yields a basipetal growth pattern with little intercalation and smaller marginal outgrowths, as there is relatively less space and time for protrusion initiation and growth. Conversely, increasing the length of the growth zone initiates protrusions in an intercalary pattern along the margin (Bilsborough et al., 2011), eventually creating a fractal reiteration of protrusions on protrusions (Runions et al., 2017), as is seen when the basipetal progression of leaf differentiation is delayed or abolished (Alvarez et al., 2016). The overlap between expression of local repressors and growth influences sinus depth and shapes protrusions (Vuolo et al., 2016; Runions et al., 2017). These arguments highlight how the perspective offered by such interdisciplinary work aids in explaining how molecular processes produce diverse leaf forms. In particular, this perspective clarifies how quantitative molecular changes manifest as discrete morphotypes along a notional continuum of leaf margin geometries. This information also empowers efforts to classify leaf shape by helping tease apart the resemblance vs. equivalence of different aspects of leaf form (Runions et al., 2017). Furthermore, it provides a path toward understanding form development in plant species where the genetic inputs shaping marginal protrusions development may require the action of different genes (Hofer et al., 1997).

From this conceptualization, questions also emerge regarding the cellular behavior underpinning this developmental logic.

Answering these requires an understanding of the processes specifying the cellular parameters of growth. In part, this information is derived from regional identities, including leaf identity established at initiation and adaxial–abaxial polarity soon thereafter (Fig. 1E). In both cases, these identities are established by mutual antagonism between the factors promoting them (i.e., leaf vs. meristem or abaxial vs. adaxial, Fig. 1E; Waites et al., 1998; Eshed et al., 2001 and references therein). Mutual antagonism, or conflict, is likewise evident in the positioning of the organ growth zone. Here, the antagonism between factors promoting marginal patterning and differentiation (Fig. 1E) controls the progressive maturation of the leaf (Bar and Ori, 2014), determining where the auxin-CUC-PIN1 module (the pattern generator in Fig. 1D) organizes marginal protrusions. The auxin maxima patterning protrusions emerge from interactions between auxin and its transporter PIN1 producing a competition between neighboring cells for auxin that locally enhances auxin concentrations, while inhibiting nearby accumulation. Notably, common to all these processes is "a balance of self-reinforcing and antagonistic tendencies" (Meinhardt, 2009), which is fundamental to the self-organization of spatial patterns.

Once growth is specified, further antagonisms arise from mismatches between the growth specified by regional identities and growth factors (e.g., auxin, CUC, and RCO), and constraints imposed by tissue geometry and mechanics (Bassel et al., 2014; Coen and Rebocho, 2016). In particular, mechanical constraints introduce discrepancies between specified growth (i.e., how a region would grow if unconstrained by neighboring regions) and resultant growth (i.e., how the region actually grows, Kennaway et al., 2011). Such mechanical conflicts may be a fundamental aspect of tissue growth and can be used to understand the organization of three-dimensional (3D) forms (Coen and Rebocho, 2016), a point underscored by models of leaves with wavy margins and snapdragon flowers (e.g., Sharon et al., 2007; Prusinkiewicz and Barbier de Reuille, 2010; Kennaway et al., 2011). In such cases, only local expansion rates need to be specified—3D changes in form result from mechanical incompatibilities (i.e., stresses), which can only be resolved by buckling (Coen and Rebocho, 2016). In turn, the tissue-level stresses driving growth arise from cellular-level mechanical interactions. At this scale, growth occurs due to stresses induced by turgor pressure that are subsequently reduced by cell-wall yielding. Consequently, cellular growth involves the antagonistic mechanical effects of turgor pressure maintenance and cell-wall yielding. Thus, mechanical conflicts are relevant to growth at the scale of both tissues and cells. Even the purely geometric constraints of space can produce conflicts instructive to morphogenesis

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← the *Vitis vinifera* variants Cabernet Sauvignon (middle) and Pinot Noir (right). (C) The three interacting components generating leaf shape illustrated in a developing *A. thaliana* leaf (developmental age increases from left to right): Component 1, an organ growth zone establishing a basipetal growth gradient (orange indicates growth intensity; dashed line marks the end of the proximal growth zone); component 2, a marginal pattern generator producing an interspersed pattern of (component 3) local growth activators (red) and repressors (cyan). The marginal pattern generator introduces new growth activator foci as space is generated by growth. (D) Marginal pattern generation emerges from a feedback between auxin (red) and CUC (cyan), where CUC enables the accumulation of auxin by the auxin efflux carrier PIN1; auxin in turn represses CUC generating an interspersed pattern of auxin maxima and CUC expression at the leaf margin. Auxin enhances outgrowth of the margin, while CUC represses outgrowth. Additional growth regulators modulate growth to shape the form of protrusions. For instance, RCO inhibits growth in indentations, producing more dissected forms. (E) Conflicts influencing growth during leaf development. Antagonism between factors promoting leaf identity (orange) and meristematic factors (purple) helps establish the leaf primordium, whereas antagonism between adaxial (light green) and abaxial (dark green) factors contributes to outgrowth of the leaf blade. (E, inset) Positioning of the organ growth zone arises through the mutual antagonism of factors promoting differentiation and morphogenesis (i.e., patterning of the leaf margin). The white disk marks the tip of the shoot apical meristem; the pluripotent cell population that gives rise to lateral organs, including leaves.

(Prusinkiewicz and Barbier de Reuille, 2010). For instance, when leaf primordia develop in an enclosing bud, their tight packing induces sustained contact between neighboring leaves and the bud, which may inhibit growth and cause leaf folding, thus shaping their mature forms (Couturier et al., 2011).

These considerations highlight the ubiquity of conflicts arising from opposing molecular states or physical forces (Hay and Tsiantis, 2005; Meinhardt, 2009; Coen and Rebocho, 2016) in morphogenesis. It thus seems that antagonistic interactions provide an engine powering the process of development, producing the ordered mature organism (Hay and Tsiantis, 2005). The notion that antagonistic interactions play a fundamental role in structuring the natural world, by creating and enabling a unity of opposites, was presaged by the ancient Greek philosopher Heraclitus (Kahn, 1981). Now, this notion can be explored algorithmically to understand how it influences the generation and diversification of biological forms.

Ultimately, precisely understanding how geometry is generated requires linking the logic of leaf form development to the cellular specification of growth. Consideration of this link suggests the experimental approaches needed to determine the origin of leaf shape and its diverse manifestations across the plant kingdom. Accurate cell level measurements of growth and gene expression in different species and genetic backgrounds including genetic mosaics of key regulators (Mähönen et al., 2014) will be essential for understanding how genes influence growth parameters (amount, direction, and duration). High-quality cellular-level time-lapse imaging can provide such information and elucidate how growth patterns arise from gene activity during organ ontogeny. In parallel, cell level studies can provide insights into how upstream developmental regulators modify fundamental cellular behavior such as cytoskeletal dynamics, cell wall properties or turgor pressure to influence growth, and clarify how feedbacks between mechanical and geometrical processes modify genetic inputs to growth. These possibilities are becoming technically feasible, highlighting a new standard for developmental biology and comparative genetics. Computational models will be essential in this analysis, and a challenge there is extending existing simulation frameworks (e.g., Bassel et al., 2014; Coen and Rebocho, 2016; and references therein) to construct mechanically faithful physically based frameworks incorporating dividing cells. Together, these approaches will provide the basis for unraveling the morphogenetic origin of organ form in general and the spectacular diversity of natural leaf shapes in particular.

ACKNOWLEDGEMENTS

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