Cells, walls, and endless forms

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A key question in biology is how the endless diversity of forms found in nature evolved. Understanding the cellular basis of this diversity has been aided by advances in non-model experimental systems, quantitative image analysis tools, and modeling approaches. Recent work in plants highlights the importance of cell wall and cuticle modifications for the emergence of diverse forms and functions. For example, explosive seed dispersal in *Cardamine hirsuta* depends on the asymmetric localization of lignified cell wall thickenings in the fruit valve. Similarly, the iridescence of *Hibiscus trionum* petals relies on regular striations formed by cuticular folds. Moreover, NAC transcription factors regulate the differentiation of lignified xylem vessels but also the water-conducting cells of moss that lack a lignified secondary cell wall, pointing to the origin of vascular systems. Other novel forms are associated with modified cell growth patterns, including oriented cell expansion or division, found in the long petal spurs of *Aquilegia* flowers, and the *Sarracenia purpurea* pitcher leaf, respectively. Another good example is the regulation of dissected leaf shape in *C. hirsuta* via local growth repression, controlled by the REDUCED COMPLEXITY HD-ZIP class I transcription factor. These studies in non-model species often reveal as much about fundamental processes of development as they do about the evolution of form.

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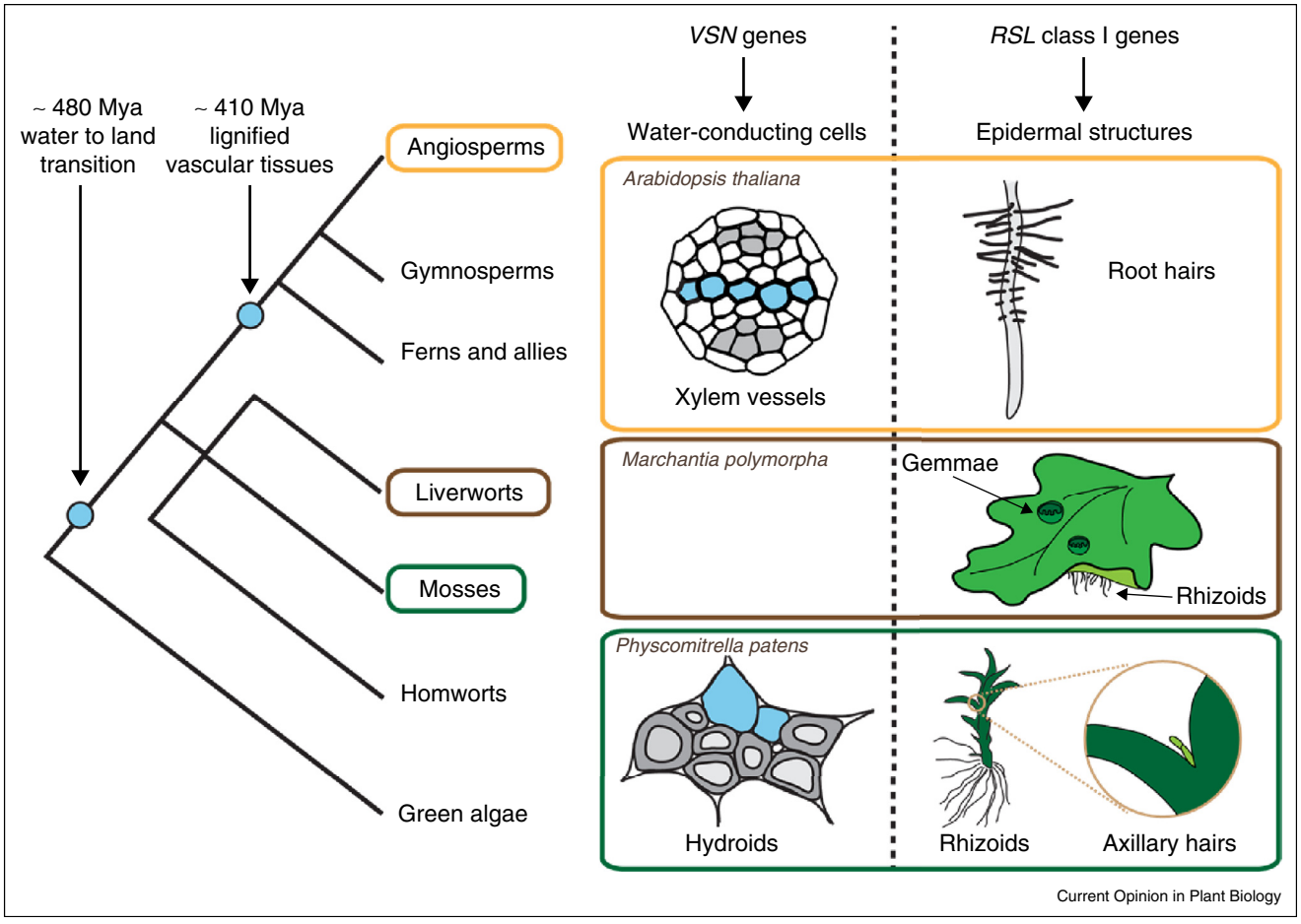
Introduction

The tree of life is filled with an amazing variety of forms, and how and why they appeared at certain times in certain clades during evolution is still mostly unresolved. Understanding morphological variation comes down to decrypting the biological processes that generate pattern and diversity. A useful approach to this problem is to perform developmental studies in a comparative context. Major advances in developmental biology have mostly come from working in model species, but obviously the basis of diversity cannot be comprehended from studying model species alone. A key challenge in recent years has been to establish many of the experimental advantages found in model organisms in other species with divergent morphologies. In particular, the development of genetic tools in non-model species has allowed the rigor of genetics to be applied to ask how diverse morphologies evolved. Moreover, advances in quantitative image analysis have enabled the four-dimensional characterization of growth and morphogenesis and are widely applicable to all species. Computational modeling is another important approach used in recent studies to gain a predictive understanding of the processes underlying development and diversity. In this review, we will discuss work over the past two years that has shed light on the cellular basis of morphological evolution.

Conserved genes control diverse cellular innovations

Model species in early-diverging land plants, such as the moss *Physcomitrella patens* or the liverwort *Marchantia polymorpha*, have proven very useful to assess the degree of conservation versus divergence across large phylogenetic distances (Figure 1) [1,2]. Two recent studies addressed the genetic basis of key adaptations to land: specialized cells for water transport and structural support; and epidermal structures for rooting, reproduction and other functions [3••,4••]. There are striking differences in the morphology of cells specialized for water conduction or support in vascular versus non-vascular plants (Figure 1). For example, xylem vessels and fibers have lignified secondary cell walls in vascular plants, while hydroids and stereids, which function analogously to conduct water and provide structural support in moss, do not. For these reasons, it was surprising that a group of NAC transcription factors (NAM, ATAF1/2, CUC) regulated these different cell types in both *Arabidopsis thaliana* and *P. patens* [3••,5,6]. Knocking-out some of these genes, named *VNS* (*VND, NST/SND, SMB*) in *P. patens*, affected the development of water-conducting hydroid cells and overall desiccation tolerance [3••]. Inducible overexpression of *PpVNS4* caused programmed cell death in both *P. patens* and *A. thaliana*, accompanied by ectopic secondary cell wall deposition in the latter; two features associated with water-conducting cell differentiation [3••]. The transcriptome response of *PpVNS4* overexpression in *P. patens* showed a striking overlap with VNS target genes previously identified in *A. thaliana*, suggesting that the VNS regulatory network in the moss gametophyte and *A. thaliana* sporophyte generations are

Figure 1



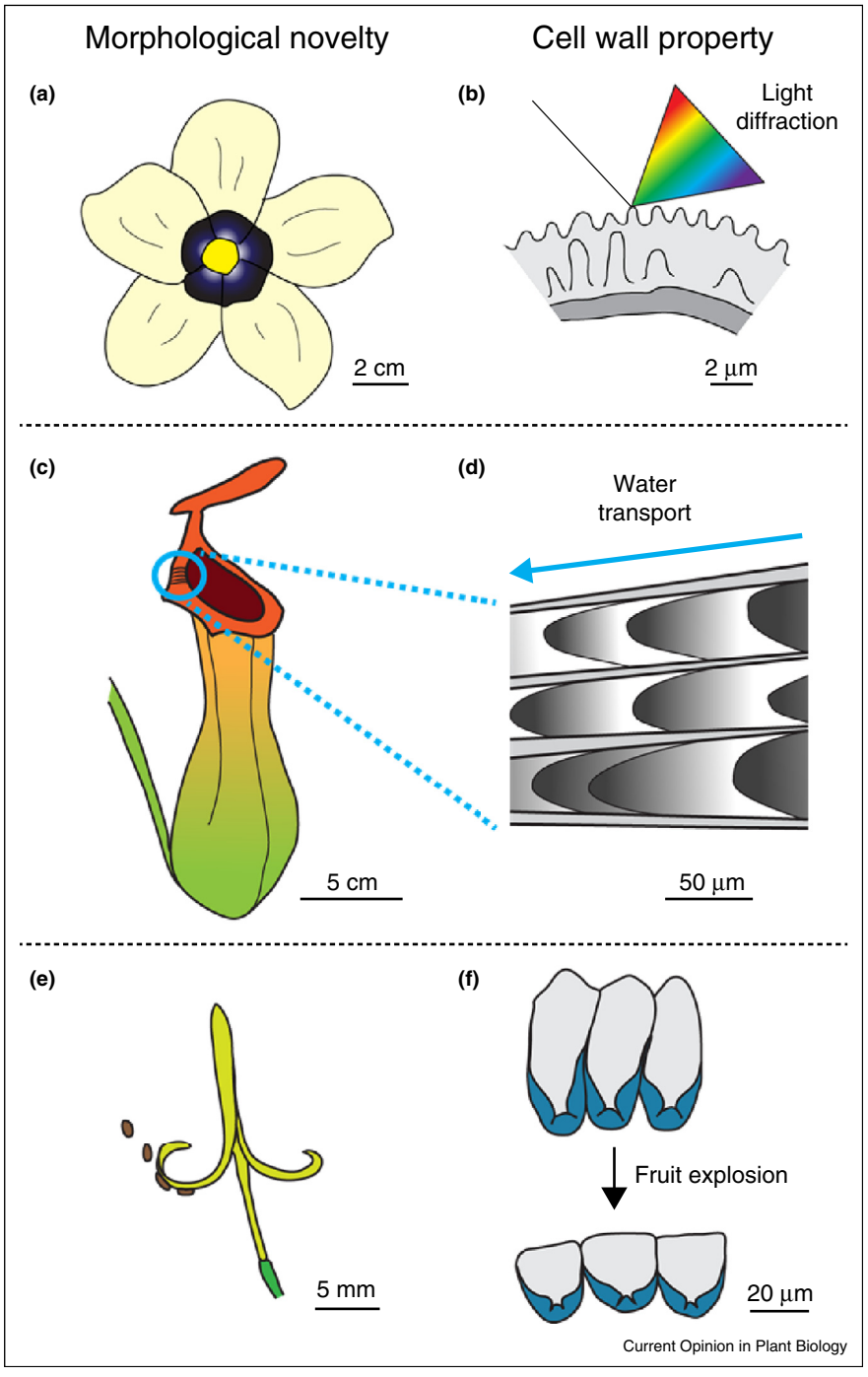
Common genes control diverse cellular adaptations for the evolutionary transition from water to land. The evolution of land plants and vascular plants are indicated on a phylogeny simplified from Ref. [36]. *VSN* genes are involved in the formation of xylem vessels (blue) in *Arabidopsis thaliana*, here depicted on a root cross-section, and in the formation of hydroids (blue) in *Physcomitrella patens*, here depicted in a leaf cross-section. *RSL* class-I genes are involved in the formation of epidermal structures, such as root hairs in *A. thaliana*, rhizoids and gemmae in *Marchantia polymorpha*, and rhizoids and axillary hairs in *P. patens*.

homologous (Figure 1) [3••]. Similarly, *ROOT-HAIR DEFECTIVE SIX-LIKE (RSL)* class I genes are needed for the development of epidermal structures with diverse forms and functions in *M. polymorpha*, *P. patens* and *A. thaliana* (Figure 1) [4••,7,8]. Both loss-of-function and overexpression alleles of *MpRSL1* were identified by screening Marchantia T-DNA populations for mutants that lost or gained rhizoids respectively, and this gene was also found to be necessary, but not sufficient, for the development of gemmae, slime and mucilage papillae [4••]. Additionally, the overexpression of *MpRSL1* restored root hair development in *A. thaliana* mutants lacking *RSL* class I gene function. Therefore, the molecular function of RSL class I proteins to control diverse epidermal structures has been maintained throughout land plant evolution (Figure 1) [4••].

Form and function: secondary cell wall diversity

Differentiated plant cells acquire specific properties related to their function. For example, a secondary cell wall, located between the primary cell wall and the plasma membrane, imparts rigidity; or a cuticle provides hydrophobicity to the epidermal surface, which prevents dehydration. Additional patterning of the cuticle can result in novel functions such as petal iridescence (Figure 2a). This phenomenon has been described in the flower of *Hibiscus trionum* [9–11] but exists in many other genera of flowering plants. The cuticle on *H. trionum* iridescent petals forms regular folds, which diffract sunlight resulting in visible iridescence (Figure 2b). This visual cue is recognized by pollinators; for example, bees can recognize iridescent flowers faster than non-iridescent ones [12•]. These cuticle patterns are characterized by irregularities in the

Figure 2



Cellular basis of morphological diversity: cuticle and secondary cell wall patterning. **(a,b)** Petals of *Hibiscus trionum* display a black iridescent patch in their centre (a). Sunlight is diffracted by regular folding of the petal cuticle, here depicted in a petal cross-section (b). **(c,d)** The pitcher leaf of *Nepenthes alata* displays a striated peristome surface (c), consisting of overlapping microcavities that transport water rapidly in a single direction by enhanced capillary rise (d). This process instantly wets the entire surface of the peristome, rendering it slippery for insects. **(e,f)** The fruit of *Cardamine hirsuta* expels its seeds via rapid coiling of its two valves (e). This explosive mechanism relies on the hinged geometry of lignified cell walls (blue) in the endocarp *b* cell layer (f). Opening these hinges causes the sudden failure of a geometric constraint keeping the valve straight, resulting in explosive coiling.

spacing of the folds, which restricts the range of light diffraction to lower wavelengths, such that petals do not display the full range of colors that could be obtained by total light diffraction [9]. After testing bee preferences for natural versus artificial iridescence patterns, the authors hypothesized that this imperfection might represent a trade-off between having a highly detectable flower that also provides a reproducible visual cue that is mostly invariant to observation angle [12•]. Alternatively, an iridescence that is restricted to a particular color range may enhance the detection of pigments in that range, producing a very strong visual cue for pollinators.

Other striated surfaces are deadly attractive to insects, such as those found on the prey-trapping pitcher leaves of *Nepenthes* (Figure 2c) [13–15]. Regular ridges along the peristome, that is, the edge of the pitcher mouth, had been previously correlated with its wettability. This wet surface causes insects to aquaplane and slip into the pitcher, representing a major strategy for *Nepenthes* to capture prey [14,15]. A recent study investigated the physical basis for this function of the peristome, and found that the surface striations allowed exceptional water conductance that wets the whole peristome surface in a few milliseconds (Figure 2d) [13]. These striations exhibit a complex structure with two-order magnitude microgrooves and periodic arc-shaped microcavities within them (Figure 2d). The authors found that the overlapping of neighbouring microcavities produced unidirectional water transport that was much faster than would be possible by normal capillary rise [13]. As a result, when water evaporates from the pitcher and condenses on the peristome or when rain falls on the peristome, the whole peristome becomes instantly wet and highly slippery for insects that will easily fall into the pitcher. Understanding how these complex structures are regularly formed by epidermal cells on the peristome surface represents an exciting follow-up to this work.

Explosive seed dispersal is another striking innovation found in various flowering plants, including *Cardamine hirsuta*; a close relative of *A. thaliana* [16,17]. This invasive weed uses an explosive mechanism to accelerate its seeds away from the fruit at over 1500 *g*, spreading seeds in a 2 m radius around a single plant (Figure 2e) [18••]. A recent study used a combination of genetics and mathematical modeling to identify cellular properties of the fruit valve that enabled it to rapidly release tension at the right stage of development. The authors discovered that this explosive process was controlled by the geometry of asymmetric secondary cell wall thickenings within endocarp *b* cells [18••]. These stiff, lignified cell walls are shaped like a hinge, which can open (Figure 2f). At maturity, the fruit valve needs to curl along its length to release tension, but its curved cross-section prevents this. Opening the hinge flattens the cross-section of the valve, causing sudden mechanical failure of the structure and rapid coiling. The authors found a strict correlation between explosive seed dispersal and the presence of asymmetric cell wall thickenings in endocarp *b* cells of fruit across the Brassicaceae [18••]. Therefore, the evolutionary novelty of this secondary cell wall pattern was a likely driver of explosive seed dispersal.

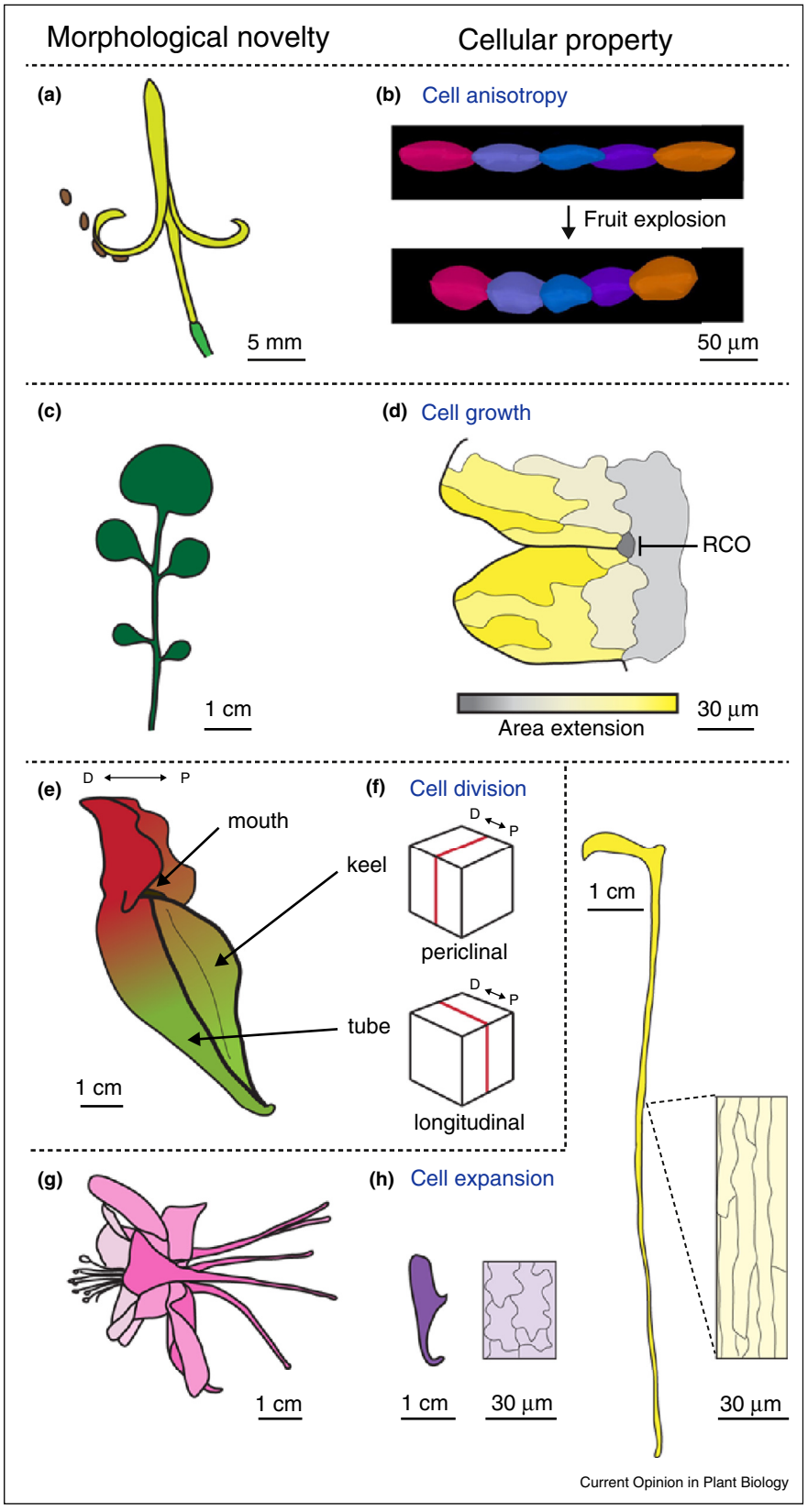
In *C. hirsuta*, the fruit valve generates tension through deformation, in particular a contraction in length, of the exocarp cell layer (Figure 3a,b) [18••]. Meanwhile, the endocarp *b* layer of the valve is stiffened with lignin and cannot contract (Figure 2f). This results in a build-up of tension in the valve that can only be released by the whole valve coiling (Figure 3a). Previous studies had proposed that this contraction occurred as the fruit dried [19,20], but *C. hirsuta* fruit explode while still green and hydrated [21]. Surprisingly, this recent study found that exocarp cells actually used their turgor pressure in order to contract [18••]. By using a finite element model of three-dimensional plant cells, the authors showed that when the cells were pressurized, they contracted in length while expanding in depth [18••]. This anisotropic cellular response relies on the particular geometry of exocarp cells in *C. hirsuta* fruit and the anisotropic properties of their cell walls (Figure 3b).

Cell growth: the dynamics of form

Form and function are also determined at the level of the whole organ. The final geometry of plant organs results from a complex interplay of growth and patterning events during early stages of development [22,23]. Diverse organ geometries can arise from evolutionary 'tinkering' with any of these parameters and the feedbacks between them [22]. This makes it challenging to identify the causal differences underlying divergent forms. Recent advances in computational modeling [22,23], and quantitative imaging have helped address this challenge; for example, MorphoGraphX is an especially useful software platform for the four-dimensional analysis of morphogenesis [24••]. Combined with experimental tools in non-model systems, particularly methods for genetic analyses, these approaches have advanced our understanding of the genetic basis for morphological diversity.

Leaf shape varies tremendously among plants and differs between *A. thaliana* and *C. hirsuta*, which have simple versus dissected leaves respectively [16,25]. Recently, the HD-ZIP class I transcription factor REDUCED COMPLEXITY (RCO), was identified in *C. hirsuta* as a major regulator of leaf shape and diversity (Figure 3c) [26••]. The *rco* mutant was isolated from a genetic screen for mutants that converted *C. hirsuta* leaf shape from dissected to simple, resembling *A. thaliana* [26••,27]. Using time-lapse confocal imaging and lineage tracking, the authors reconstructed the growth parameters of cell lineages at the time of leaflet initiation, in wild-type and *rco* leaves, with MorphoGraphX [26••]. They could visualize the few

Figure 3


Morphological novelty
Cellular property
(a)
5 mm
(b) Cell anisotropy
Fruit explosion
50 µm
(c)
1 cm
(d) Cell growth
RCO
Area extension
30 µm
(e)
D
P
mouth
keel
tube
1 cm
(f) Cell division
periclinal
longitudinal
1 cm
(g)
1 cm
(h) Cell expansion
1 cm
30 µm
30 µm
Current Opinion in Plant Biology

cells located at the boundaries between leaflets, where *RCO* specifically repressed growth, allowing leaflets to emerge from the leaf margin (Figure 3d). Tracing the evolutionary history of *RCO* indicated that this gene was lost from the genome of *A. thaliana*, contributing to its simple leaf shape [26••]. Furthermore, transforming *RCO* into *A. thaliana* was sufficient to reverse this evolutionary transition and make the *A. thaliana* leaf more complex [26••]. Moreover, independent mutations at the *RCO* locus caused repeated evolutionary changes in leaf shape within the Brassicaceae; for example, between the *Capsella* species *C. rubella* and *C. grandiflora* [28]. In this way, *RCO* played a key role in shaping leaf diversity.

The ability to quantify and model growth can also provide insights into the cellular basis of complex organ geometries like pitcher leaves, mentioned previously, and petal spurs [29,30•]. *Sarracenia purpurea* develops pitcher leaves similar to the ones of *Nepenthes*, but organized in two parts called the tube and the keel (Figure 3e). At early stages of pitcher development, the orientation of cell division planes differ between the tube and keel (Figure 3f) [29]. Using a modeling approach, the authors found that they could recapitulate the two parts of *Sarracenia*'s pitcher by forcing cell divisions into the orientation planes that they observed during leaf development. This highlights the potential of modeling to find growth parameters that are necessary and sufficient for morphogenesis. The authors conclude that cell expansion plays little role in generating the form of this modified leaf, which contrasts with recent findings about the cellular basis of petal spur length in *Aquilegia* (Figure 3g) [31].

Variation in the length of petal nectar spurs among *Aquilegia* species is a spectacular example of plant-pollinator co-evolution, since the evolution of longer spurs is associated with shifts to pollinators with longer tongues [32]. This probably contributed to the high speciation rates found in *Aquilegia*, so understanding this variation may offer insights into the relationship between morphological evolution and speciation processes [33,34]. Early petal spur development is similar among different *Aquilegia* species, while the dramatic variation in spur length between species correlates with differences in oriented (anisotropic) cell expansion during later development. For example, *A. vulgaris* and *A. longissima* use a similar number of cells to produce spurs of approximately 1 cm versus 12 cm length, respectively (Figure 3h) [31]. Using a transcriptome study to identify differentially expressed genes during petal spur development in *A. coerulea*, the authors found that an orthologue of the cell proliferation regulator *TCP4* was up-regulated in growing regions of the petal spur [30•]. Knocking-down *TCP4* function by VIGS resulted in short and distorted petal spurs, caused by over-proliferation of cells in the distal part of the petal (Figure 3g). Given the previously described role for *TCP4* in *A. thaliana* petals [35], it appears that regulating the balance of cell proliferation and expansion is an important aspect of petal organogenesis among species with diverse petal morphologies.

Conclusion

Considerable progress has been made in recent years towards understanding the cellular and genetic basis of morphological diversity. Specialized epidermal cells, and cells for water transport and structural support, were key adaptations during plant evolution that enabled the transition from water to land. Surprisingly, a common genetic basis underlies the formation of quite different cell types that perform these functions in vascular versus non-vascular plants [3••,4••]. A key point to emerge from this review is the critical role of such cell differentiation processes in generating evolutionary novelties. Striking trait innovations such as petal iridescence, pitcher leaf carnivory, and explosive seed dispersal, all depend on novel patterning of secondary cell walls or epidermal surfaces [9,13,18••]. The next step will be to identify the causal genetic changes that regulate these processes. *RCO* was identified as an important regulator of leaf shape, underlying repeated evolutionary transitions between dissected and simple leaf forms in the Brassicaceae [26••,28]. Advances in quantitative image analysis were leveraged to understand precisely how differences in *RCO* genotypes were translated into divergent leaf shapes through development [24••,26••]. Computational modeling has been particularly important to unravel the logic that shapes plant form, and to move towards a predictive understanding of development and diversity [22,23]. The fascination with diverse plant forms has motivated the use of non-model plants to investigate how this diversity is produced and how it evolved. Advances in whole genome sequencing, quantitative imaging, and modeling frameworks have done much to level the playing field for research in non-model organisms. But to understand the genetic basis for morphological evolution still requires

(Figure 3 Legend) Cellular basis of morphological diversity: cell growth, anisotropy, division and expansion. **(a,b)** The explosive shatter of *C. hirsuta* fruit relies on differential contraction of tissues in the fruit valve (a). The exocarp tissue contracts in length by an active process that relies on turgor pressure, cell geometry and cell anisotropy (b). **(c,d)** The dissected shape of *C. hirsuta* leaves depends on local growth repression between emerging leaflets (c). The action of RCO to repress growth in this region (dark grey) was determined by quantitative analysis of time-lapse imaging (d). **(e, f)** The pitcher leaf of *Sarracenia purpurea* is composed of two main parts: a hollow tube and a keel, which is a membrane-like protrusion at the front of the tube (e). Differences in cell division plane (shown as red lines oriented to the distal, D, — proximal, P, axis of the leaf) can account for the different morphologies of the tube and keel (f). **(g,h)** Flowers of the genus *Aquilegia* develop long petal spurs (g) that vary in size, depicted here for the spurs of *A. vulgaris* (purple) and *A. longissimi* (yellow) (h). This size variation is caused by differences in oriented cell expansion, depicted here in insets of epidermal regions of identical width for each species.

the advantages of high experimental tractability and good genetic tools in these organisms.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Berger F, Bowman JL, Kohchi T: **Marchantia**. *Curr Biol* 2016, **26**:R186-R187.

2. Rensing SA, Lang D, Zimmer AD, Terry A, Salamov A, Shapiro H, Nishiyama T, Perroud PF, Lindquist EA, Kamisugi Y *et al.*: **The *Physcomitrella* genome reveals evolutionary insights into the conquest of land by plants**. *Science* 2008, **319**:64-69.

3. •• Xu B, Ohtani M, Yamaguchi M, Toyooka K, Wakazaki M, Sato M, Kubo M, Nakano Y, Sano R, Hiwatashi Y *et al.*: **Contribution of NAC transcription factors to plant adaptation to land**. *Science* 2014, **343**:1505-1508.

The function of VSN transcription factors in the differentiation of water-conducting cells, is conserved between *Physcomitrella patens* and *Arabidopsis thaliana*, suggesting that these genes enabled the water to land transition during plant evolution.

4. •• Proust H, Honkanen S, Jones VA, Morieri G, Prescott H, Kelly S, Ishizaki K, Kohchi T, Dolan L: ***RSL* Class I genes controlled the development of epidermal structures in the common ancestor of land plants**. *Curr Biol* 2016, **26**:93-99.

The authors made good use of genetics in *Marchantia polymorpha* to identify a role for *RSL* class I genes in directing growth out of the epidermal plane. This gene function is conserved in *Arabidopsis thaliana*, despite divergence in the type of epidermal structures.

5. Kubo M, Udagawa M, Nishikubo N, Horiguchi G, Yamaguchi M, Ito J, Mimura T, Fukuda H, Demura T: **Transcription switches for protoxylem and metaxylem vessel formation**. *Genes Dev* 2005, **19**:1855-1860.

6. Mitsuda N, Iwase A, Yamamoto H, Yoshida M, Seki M, Shinozaki K, Ohme-Takagi M: **NAC transcription factors, NST1 and NST3, are key regulators of the formation of secondary walls in woody tissues of Arabidopsis**. *Plant Cell* 2007, **19**:270-280.

7. Menand B, Yi K, Jouannic S, Hoffmann L, Ryan E, Linstead P, Schaefer DG, Dolan L: **An ancient mechanism controls the development of cells with a rooting function in land plants**. *Science* 2007, **316**:1477-1480.

8. Jang G, Yi K, Pires ND, Menand B, Dolan L: ***RSL* genes are sufficient for rhizoid system development in early diverging land plants**. *Development* 2011, **138**:2273-2281.

9. Whitney HM, Kolle M, Andrew P, Chittka L, Steiner U, Glover BJ: **Floral iridescence, produced by diffractive optics, acts as a cue for animal pollinators**. *Science* 2009, **323**:130-133.

10. Vignolini S, Moyroud E, Hingant T, Banks H, Rudall PJ, Steiner U, Glover BJ: **The flower of *Hibiscus trionum* is both visibly and measurably iridescent**. *New Phytol* 2015, **205**:97-101.

11. Vignolini S, Moyroud E, Hingant T, Banks H, Rudall PJ, Steiner U, Glover BJ: **Is floral iridescence a biologically relevant cue in plant-pollinator signalling? A response to van der Kooi et al. (2014b)**. *New Phytol* 2015, **205**:21-22.

12. • Whitney HM, Reed A, Rands SA, Chittka L, Glover BJ: **Flower iridescence increases object detection in the insect visual system without compromising object identity**. *Curr Biol* 2016, **26**:802-808.

This report demonstrates that insects detect iridescent flowers better than non-iridescent ones, and that the imperfect light diffraction generated by the petal's cuticle does not compromise petal identity as full diffraction would.

13. Chen H, Zhang P, Zhang L, Liu H, Jiang Y, Zhang D, Han Z, Jiang L: **Continuous directional water transport on the peristome surface of *Nepenthes alata***. *Nature* 2016, **532**:85-89.

14. Bohn HF, Federle W: **Insect aquaplaning: *Nepenthes* pitcher plants capture prey with the peristome, a fully wettable water-lubricated anisotropic surface**. *Proc Natl Acad Sci* 2004, **101**:14138-14143.

15. Bauer U, Bohn HF, Federle W: **Harmless nectar source or deadly trap: *Nepenthes* pitchers are activated by rain, condensation and nectar**. *Proc Roy Soc B-Biol Sci* 2008, **275**:259-265.

16. Hay AS, Pieper B, Cooke E, Mandakova T, Cartolano M, Tattersall AD, dello Ioio R, McGowan SJ, Barkoulas M, Galinha C *et al.*: ***Cardamine hirsuta*: a versatile genetic system for comparative studies**. *Plant J* 2014, **78**:1-15.

17. Hay A, Tsiantis M: ***Cardamine hirsuta*: a comparative view**. *Curr Opin Genet Dev* 2016, **39**:1-7.

18. •• Hofhuis H, Moulton D, Lessinnes T, Routier-Kierzkowska AL, Bomphrey RJ, Mosca G, Reinhardt H, Sarchet P, Gan X, Tsiantis M *et al.*: **Morphomechanical innovation drives explosive seed dispersal**. *Cell* 2016, **166**:1-12.

An interdisciplinary study of explosive seed dispersal in *Cardamine hirsuta* that combines experimental and modeling approaches. Using interactions between cell and tissue-level processes, the authors develop a multi-scale model that reproduces explosive dispersal.

19. Beer T, Swaine MD: **Theory of explosively dispersed seeds**. *New Phytol* 1977, **78**:681-694.

20. Vaughn KC, Bowling AJ, Ruel KJ: **The mechanism for explosive seed dispersal in *Cardamine hirsuta* (Brassicaceae)**. *Am J Bot* 2011, **98**:1276-1285.

21. Schneider S: **Untersuchungen ueber die Samenschleudermechanismen verschiedener Rhoeadales**. In *In Jahrbuecher fuer wissenschaftliche Botanik*, Vol 81. Edited by Fitting H. Verlag von Gebrueder Borntraeger; 1935.

22. Kuchen EE, Fox S, de Reuille PB, Kennaway R, Bensmihen S, Avondo J, Calder GM, Southam P, Robinson S, Bangham A *et al.*: **Generation of leaf shape through early patterns of growth and tissue polarity**. *Science* 2012, **335**:1092-1096.

23. Kennaway R, Coen E, Green A, Bangham A: **Generation of diverse biological forms through combinatorial interactions between tissue polarity and growth**. *PLoS Comput Biol* 2011, **7**:e1002071.

24. •• Barbier de Reuille P, Routier-Kierzkowska AL, Kierzkowski D, Bassel GW, Schupbach T, Tauriello G, Bajpai N, Strauss S, Weber A, Kiss A *et al.*: **MorphoGraphX: a platform for quantifying morphogenesis in 4D**. *Elife* 2015:4.

This is a user-friendly software platform for analysing curved surfaces and quantifying growth parameters including, among many others, cell expansion, cell division, growth anisotropy and local curvature.

25. Hay A, Tsiantis M: **The genetic basis for differences in leaf form between *Arabidopsis thaliana* and its wild relative *Cardamine hirsuta***. *Nat Genet* 2006, **38**:942-947.

26. •• Vlad D, Kierzkowski D, Rast MI, Vuolo F, Dello Ioio R, Galinha C, Gan X, Hajheidari M, Hay A, Smith RS *et al.*: **Leaf shape evolution through duplication, regulatory diversification, and loss of a homeobox gene**. *Science* 2014, **343**:780-783.

RCO is identified as an important regulator of leaf shape variation in the Brassicaceae. Using a combination of genetics and quantitative image analysis, they show that RCO represses growth between emerging leaflets of the *Cardamine hirsuta* leaf.

27. Kougioumoutzi E, Cartolano M, Canales C, Dupre M, Bramsiepe J, Vlad D, Rast M, Dello Ioio R, Tattersall A, Schnittger A *et al.*: ***SIMPLE LEAF3* encodes a ribosome-associated protein required for leaflet development in *Cardamine hirsuta***. *Plant J* 2013, **73**:533-545.

28. Sicard A, Thamm A, Marona C, Lee YW, Wahl V, Stinchcombe JR, Wright SI, Kappel C, Lenhard M: **Repeated evolutionary changes of leaf morphology caused by mutations to a homeobox gene**. *Curr Biol* 2014, **24**:1880-1886.

29. Fukushima K, Fujita H, Yamaguchi T, Kawaguchi M, Tsukaya H, Hasebe M: **Oriented cell division shapes carnivorous pitcher leaves of *Sarracenia purpurea***. *Nat Commun* 2015, **6**:6450.

30. • Yant L, Collani S, Puzey J, Levy C, Kramer EM: **Molecular basis for three-dimensional elaboration of the *Aquilegia* petal spur**. *Proc Roy Soc B-Biol Sci* 2015, **282**:20142778.
This study uses genome-wide expression data and VIGS in a non-model species to investigate petal spur development.

31. Puzey JR, Gerbode SJ, Hodges SA, Kramer EM, Mahadevan L: **Evolution of spur-length diversity in *Aquilegia* petals is achieved solely through cell-shape anisotropy**. *Proc Roy Soc B-Biol Sci* 2012, **279**:1640-1645.

32. Whittall JB, Hodges SA: **Pollinator shifts drive increasingly long nectar spurs in columbine flowers**. *Nature* 2007, **447**:706-709.

33. Fulton M, Hodges SA: **Floral isolation between *Aquilegia formosa* and *Aquilegia pubescens***. *Proc Roy Soc B-Biol Sci* 1999, **266**:2247-2252.

34. Hodges SA, Derieg NJ: **Adaptive radiations: from field to genomic studies**. *Proc Natl Acad Sci* 2009, **106(Suppl. 1)**:9947-9954.

35. Nag A, King S, Jack T: **miR319a targeting of *TCP4* is critical for petal growth and development in Arabidopsis**. *Proc Natl Acad Sci* 2009, **106**:22534-22539.

36. Wickett NJ, Mirarab S, Nguyen N, Warnow T, Carpenter E, Matasci N, Ayyampalayam S, Barker MS, Burleigh JG, Gitzendanner MA *et al.*: **Phylotranscriptomic analysis of the origin and early diversification of land plants**. *Proc Natl Acad Sci* 2014, **111**:E4859-E4868.