Comparative leaf development in angiosperms

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Recent accumulation of our knowledge on basic leaf development mechanisms in model angiosperm species has allowed us to pursue evolutionary development (evo/devo) studies of various kinds of leaf development. As a result, unexpected findings and clues have been unearthed aiding our understanding of the mechanisms involved in the diversity of leaf morphology, although the covered remain limited. In this review, we highlight recent findings of diversified leaf development in angiosperms.

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Introduction

Leaf morphology is highly diversified. Moss leaves, for example, are totally different from ferns leaves and seed plants. Fern and angiosperm (flowering plants) leaves are both megaphylls and develop under a sporophyte program, but are believed to have evolved independently. Angiosperm leaves display great variation in shape and size (Figure 1). The most curious angiosperm leaves are seen in the three-dimensional leaves of carnivorous species, in which dorsoventrality positioning is totally different from that of the more usual leaf types. We do not yet understand the differences in developmental between eudicot-type leaves and monocot-type leaves. For example, different interpretations exist as to what part of the Poaceae (Gramineae) leaves corresponds to the leaf blades and the leaf petioles of eudicot leaves [1]. The divergence in leaf development in angiosperms has already been described in detail several decades ago in classic anatomical studies. However, few developmental genetic and evo/devo studies have been carried out, and diversity of leaf development may be too complex to be understood in the light of slight modifications of processes deduced in the model species *Arabidopsis thaliana* (L.) Heynh. (Arabidopsis). This review discusses recent progress in this field, focusing on angiosperm leaves.

Longitudinal polar diversity in the cell division pattern in leaf primordia

In angiosperms, cells that make the leaf blade come from the base of the primordium; the meristematic zone of the leaf primordia is located at the actual base in monocots, and at the junction between the leaf blade and the leaf petiole in Arabidopsis (Figure 2) [2]. However, the ancestral position of the meristematic zone in leaf primordia is not necessarily basal. In shoot apical meristems (SAMs) it is apical, and in some floral organs (derived from lateral organs), such as petals, it is apical and marginal (Figure 2a) [3]. In fern leaves too, it is apical or marginal. Thus it may be that apical positioning is the 'default' characteristic in angiosperm lateral organs, including leaves. Therefore, if it is assumed, the basal meristem position in the leaf primordia is not an ancient event in angiosperm evolution, leading us to question what genetic changes are involved in changed positioning of the meristematic zone from the apices to the bases? Boyce [4] hypothesized that a change in leaf venation pattern, from an open dichotomous to a reticulate pattern was associated with the reversed positioning of the meristematic zone. Although the mechanism for the reversed positioning remains unknown, Nakayama *et al.* [5••] reported that a phylloclade (cladode: see Glossary) of *Asparagus asparagoides* (Asparagaceae, monocot) has a meristematic zone at its base (Figure 2a). The phylloclade of *A. asparagoides* is a leaflike metamorph of the lateral shoot, ectopically expressing some leaf genes, including the class III homeodomain leucine zipper (HD-ZipIII) gene family, which identifies the adaxial domain of leaves; *miR166*, which identifies the abaxial domain of leaves; and *ASYMMETRIC LEAVES1*, which is involved in the establishment of various axes in leaves [5••]. The primordia of the *A. asparagoides* phylloclade express *AS1* at the base in the early stages, after which expression is confined to the central domain in the later stages of the primordia. The class I *KNOX* genes, which are required for the establishment and maintenance of SAMs, are also expressed in the apex of the phylloclade primordia in the early stages, after which expression is confined to the base [5••]. The mechanism(s) of the shift of expression of the class I KNOX mRNA from the apex to the base of the phylloclade primordia may be a key to understanding the molecular mechanisms behind the

Abaxial: The 'lower' side of a lateral organ that is away from the apical meristem from where the lateral organs initiate.

Adaxial: The 'upper' side of a lateral organ that faces the apical meristem from where the lateral organ initiated.

Bifacial leaf: A dorsoventral (dorsiventral) leaf: a leaf exhibiting both adaxial and abaxial identities. In particular, a leaf with palisade cells in the adaxial parenchyma tissue and spongy cells in the abaxial parenchyma tissue; xylems are arranged in adaxial side and phloems are in abaxial side.

Blade: Two-dimensionally expanded part of the leaf. Lamina.

Blastozone: A specific region at the leaf margin, possessing the organogenic potential. This term is specifically used for primordia of compound leaves.

Cladode: A lateral organ with determinate organogenesis and leaf-like characters that occurs at the site from where a lateral branch is expected to initiate.

Compensation: A phenomenon observed as an abnormally enhanced cell expansion in a lateral organ where cell proliferation is severely defective.

Compound leaf: A leaf bearing several individual leaflets borne on a supporting stalk-like structure (rachis). If the leaf lamina is only dissected but not separated into independent leaflets, it is called as 'dissected leaf'.

Epiphylly: Ectopic occurrence of organs on leaves. Epiphyllous bud formation is often seen in many species and contributes to their asexual propagation.

Heteroblasty: Changes in leaf size and shape observed along with aging of plants.

Lamina: Two-dimensionally expanded part of the leaf. Blade.

Lateral organ: An organ that occurs from the flank of an apical meristem. Leaves and floral organs that initiate from the SAMs are the typical examples.

Megaphyll: A leaf seen in ferns and seed plants, which has a leaf trace associated with a leaf gap.

Metamorph: Change in shape/form of organs, in particular at the organ-species level, such as seen in a change from a leaf to a petal or from a lateral shoot to a cladode.

Microphyll: A leaf seen in Lycopodiophyta, which does not have a leaf trace associated with a leaf gap.

Petiole: A stalk that occurs at the base of a leaf and serves as a physical support.

Phylloclade: A stem with determinate organogenesis and leaf-like characters.

Simple leaf: A leaf without separation into several independent parts. Even if the leaf lamina is deeply dissected, unless the dissected parts are independent from the other part by the presence of stalk-like structure (rachis), it is not considered as a compound leaf but is a simple leaf.

Unifacial leaf: A leaf with a lamina that has only an abaxial identity. Usually the basal, leaf sheath area is bifacial even in unifacial leaves. Although only abaxialized unifacial leaves are known in nature, adaxialized unifacial leaves are also known for mutants or transgenics in laboratory.

reversed positioning of the meristematic zone in leaf primordial from the apex to the base in angiosperms.
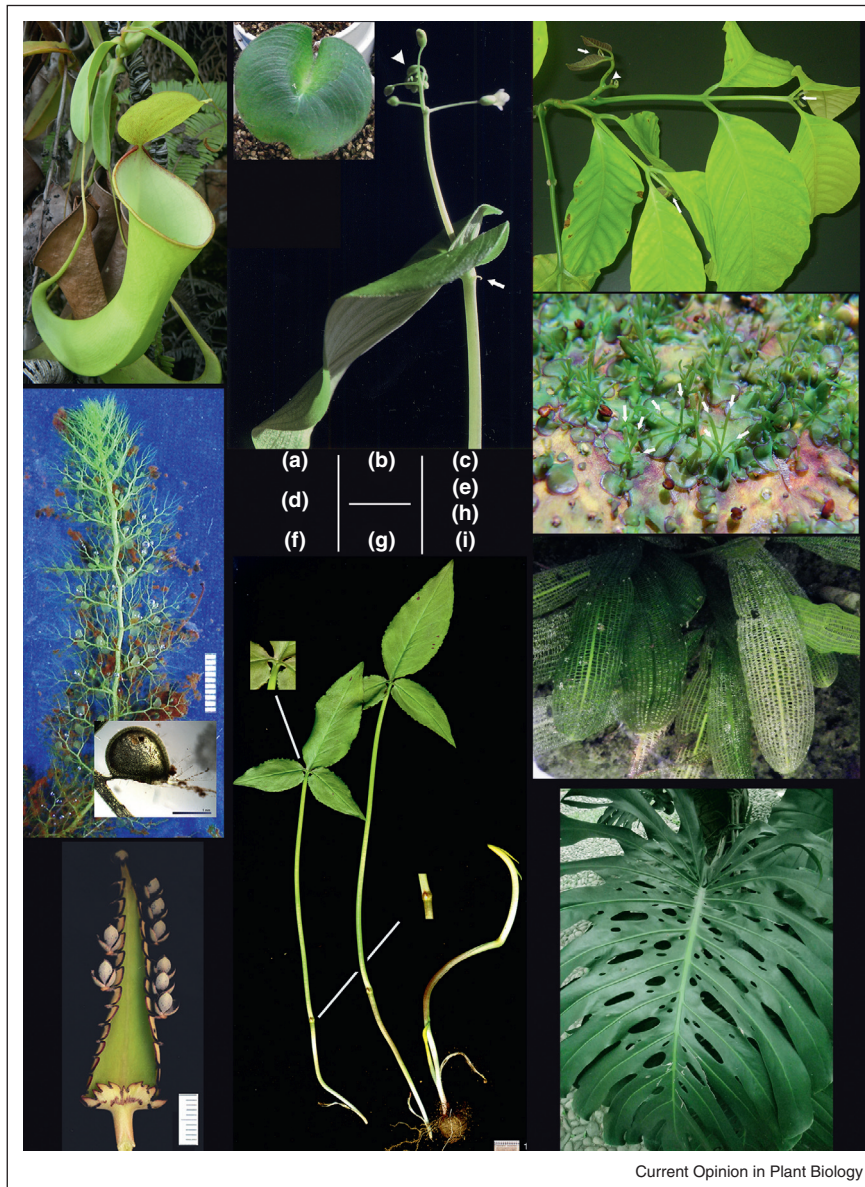
Similarly, how the direction of organogenesis is controlled in compound leaves is also not understood well. Compound leaves have repeated organogenesis of leaflets with simple, leaflike morphologically distinct units (leaflets) on a primordium in a region known as the marginal blastozone [6]. Comparative anatomy among simple leaves, compound leaves, and shoots has been an ongoing debate since Arber's leaf partial shoot theory [1].

Accumulating evidence has suggested that simple leaves, compound leaves, and shoots share common gene regulatory networks (GRNs) [7–9]. Since molecular genetic studies on differences between simple and compound leaves are more advanced than studies on other aspects of leaf diversity [10•,11], we have focused on the variation in the direction of leaflet inception along the longitudinal axis of the leaf primordia. Some species develop their leaflets from the base to the apex along the longitudinal axis of the leaf primordia (acropetal type) whereas other species develop their leaflets from the apex to the base (basipetal type) (Figure 2b). Considering that SAMs always retain meristematic activity in their apices and show an acropetal organogenesis, the divergence of polarity in organogenesis in compound leaves is puzzling. It was once hypothesized that leaflet initiation was triggered by differential perimeter expansion, thus accounting for the difference in polarity among species [12]. Namely, if growth is more active in the basal part of the leaf primordium, new leaflet primordia will develop basipetally; however, if growth is more active in the apical part, new leaflet primordia will develop acropetally. Ikeuchi *et al.* [13] tested this hypothesis by measuring the growth of *Chelidonium majus* subsp. *asiaticum* leaf primordia cultivated *in vitro*. They found that while *C. majus* leaf primordia showed basipetal organogenesis, the gradient of growth along the longitudinal axis was not stable, confounding the above hypothesis. They also found that a putative regulator of tissue maturation in *C. majus*, a homolog of the TCP transcription factor gene, *CINCIN-NATA* (*CIN*), had higher expression levels in the apical parts than in the basal parts during the organogenetic phase. This local expression of the *CIN* homolog might be why leaflet formation is restricted in the base of the *C. majus* leaf primordia. Moreover, this was not applicable to other species, and expression of the *CIN* homolog was not elevated in either the apical or basal parts of the *Eschscholzia californica* leaf primordia, which showed acropetal leaflet formation [13]. Considering that the two species (*C. majus* and *E. californica*) belong to the same family (Papaveraceae), the directionality of leaflet initiation could have diversified by different mechanisms in a case-by-case manner.

Diversity in the longevity of the leaf meristem: determinate or indeterminate

Longevity of meristematic activity is also different among angiosperm leaves. Most leaves are determinate organs, and cell proliferation or meristematic activity is lost after a set number of cells are supplied, which is programmed. In some plants, however, meristematic activity is maintained for months or years, resulting in indeterminate organogenesis [14]. Angiosperms have two types of indeterminate leaves: those with a basal meristem as seen in the genera *Monophyllaea* (Figure 2c) and *Streptocarpus* (Gesneriaceae), and those with an apical meristem as seem in the genera *Chisocheton* (Figure 2c) and *Guarea* (Meliaceae)
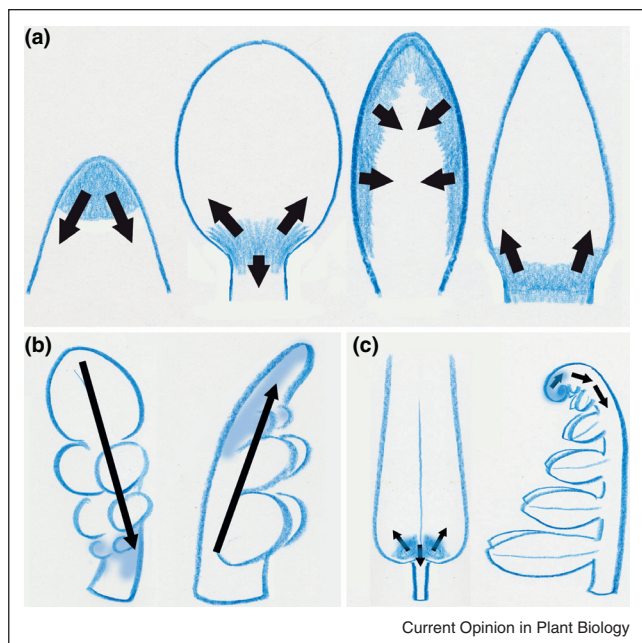
Figure 1

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Unusual leaves. **(a)** A *Nepenthes* sp. (carnivorous pitcher plant, Nepenthaceae) leaf. Note the extensively developed, three-dimensional pitcher part in comparison with a flat, leaf-lamina-like part connected to the pitcher by an elongated structure. **(b)** A flowering individual of *Monophyllaea glabra* (one-leaf plant, Gesneriaceae). The large green part is the phyllomorph and is a shoot–leaf intermediate organ derived from a cotyledon; an arrowhead indicates an inflorescence meristem derived from the basal meristem of the phyllomorph; an arrow denotes an undeveloped cotyledon. An inset shows a young individual in a vegetative stage, bearing only one leaf. **(c)** A young shoot of *Chisocheton macrophyllus* (Meliaceae). An arrowhead indicates the position of the shoot apical meristem (SAM); arrows point to leaf apical meristems of the indeterminate compound leaves. **(d)** A *Utricularia australis* (bladderwort, Utriculariaceae) shoot. A shoot apex is seen above; pale green sacs are traps developed on segmented 'leaves'; inset shows a magnified trap. Scale for the shoot = 1 cm; for the trap = 1 mm. **(e)** *Hydrobryum puncticulatum* (Podostemaceae). Arrows indicate leaves; the flat green organ in this species are modified roots. **(f)** A leaf of *Bryophyllum daigremontianum* (mother of thousands, Crassulaceae) bearing epiphyllous somatic embryos. **(g)** Leaves of *Pinellia ternata* (Araceae). Insets indicate that a bulb is formed among leaflets and on the petiole. The right-hand side shows a young leaf before unfolding. Scale, 1 cm. **(h)** Leaves of *Aponogeton madagascariensis* (lace plant, Aponogetonaceae). The leaves carry holes that have arisen by programmed cell death and **(i)** A leaf of *Monstera deliciosa* (Araceae). Again the holes in the leaves have arisen by programmed cell death.

Photographs were taken in the Maliau Basin Conservation Area, Sabah, Malaysia **(a)**, under cultivation at the University of Tokyo **(b,c,f,h)**, in Okazaki, Aichi, Japan **(d,f,g)**, on Yakushima Island, Japan **(e)**, and in Bogor Botanical Garden, Java, Indonesia **(i)**. A young seedling shown in panel **(c)** is a kind gift from Dr. Dedy Darnaedi, the Indonesian Institute of Sciences (LIPI), Indonesia.

Figure 2

Schematic drawings of various leaf organogenesis. **(a)** Schematic drawings of shoot, leaf and petal primordia showing positioning of the meristematic region. From left to right: a shoot apical meristem (SAM), a leaf primordium of Arabidopsis, a primordium of an Arabidopsis petal, and a primordium of the phylloclade of *Asparagus asparagoides.* Meristematic zones are shaded and arrows indicate the direction of the supply of new cells. **(b)** Two types of direction in the initiation of leaflets in the primorida of compound leaves. On the left: a basipetal type. On the right: an acropetal type. Arrows indicate the temporal order of leaflet initiation. **(c)** Schematic drawings of indeterminate leaves. On the left: a basal type of indeterminate leaf as seen in the genus *Monophyllaea*. The distal tip of the leaf is gradually lost by senescence. On the right: an apical type of indeterminate leaf as seen in the genus *Chisocheton*. A lateral view is shown. Meristematic activity is shown by shading; arrows indicate the direction of cell supply. Also see Figure 1b,c.

[14]. We do yet not know what types of genetic modification are behind the basal-type indeterminate leaves.

The basal-type indeterminate leaves are often associated with abnormal regulation of the SAM, such as loss (*Monophyllaea* spp. [15]) or irregular positioning (some *Streptocarpus* spp. and Gesneriaceae species) [16•], suggesting that the basal meristem of these indeterminate (called 'phyllomorphs') might be derived from the SAM. Indeed, the basal meristem develops inflorescence meristems after the transition to floral evocation (Figure 2c). Neither how the leaves remain indeterminate nor how the meristematic activities in the leaf primordia are halted in determinate leaves such as in Arabidopsis is known [17•]. What is known is that the so-called 'arrest front' is kept in a steady position at a certain distance from the base in the leaf primordia of Arabidopsis and then it suddenly moves to the base, resulting in cessation of cell proliferation in

the primordia [18•]. The only candidate genes that might regulate the determinacy of the leaf meristem belongs to the *BLADE-ON-PETIOLE* (*BOP*) gene family [19,20]. Loss-of-function of *BOP1* and *BOP2* in Arabidopsis results in a defect in positioning and determinacy of the meristematic region of the leaf primordia [3,18•,19]. Expression of the class I *KNOX* gene in leaf primordia is often believed to be directly linked to compound leaf formation [21]. However, while the class I *KNOX* gene is expressed in indeterminate leaves of *Streptocarpus* spp., the leaves are simple and not complex [22]. It is also the case for the indeterminate simple leaves of gymnosperm, *Welwitschia mirabilis* which express class I *KNOX* [23]. Similarly, leaves of *bop* mutants of Arabidopsis ectopically express class I *KNOX* genes but are simple in form, while their morphogenesis continues much longer than wild-type leaves [20]. Thus, repeated or fractal organogenesis must be governed by elements other than class I *KNOX* genes. Since *bop* mutations enhance complexity and prolong morphogenesis of *asymmetric leaves2* mutant leaves, which also fail to suppress the expression of class I *KNOX* in their leaves, this strongly suggests that class I KNOX functions to prolong meristematic activity. Identifying the mechanisms of leaf primordia determinacy, that is, how the meristematic region is localized at the base of the leaf primordial, is essential.

Apical-type meristem systems in compound leaves (Figure 1c) seem to be simpler. A molecular phylogenetic analysis suggested that such indeterminacy has been acquired only once in Meliaceae [24]. Anatomically, this leaf apical meristem is similar to the SAM, except for its dorsoventral structure and the lack of lateral bud formation (although some Meliaceae species such as *Chisocheton pohlianus* exceptionally develop an inflorescence from the rachis) [25–27]. Thus, the indeterminate apical meristems of these leaves are fundamentally the same, with the SAM bearing the stem cells, which may have lost some of the pluripotency by differentiating leaflets and the rachis. Noting which factors are lost in these indeterminate leaf meristems in comparison with SAMs is important. These factors could provide the key for determinacy of the leaf primordia and for differentiation of leaf primordia from SAMs.

Cell number and cell size
Because both the number and size of leaves vary among species and each species has species-specific leaf-size characteristics, some coordinated mechanisms must underlie organ-size regulation in leaves. Studies on 'compensation,' a phenomenon defined by an abnormal enhancement in cell expansion after a certain level of cell proliferation defects has been reached in mutant leaf primordia [17,28], have revealed some important coordinated mechanisms in Arabidopsis [29,30]. Additionally, as reported by Usami *et al.* [31], in Arabidopsis, heteroblasty, or age-dependent leaf-size changes, are associated with
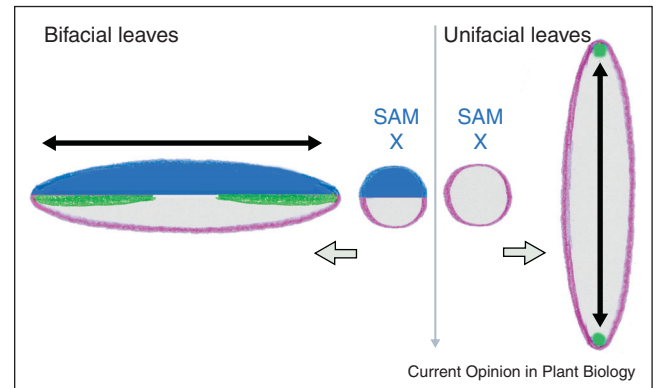
both an increase in the number of cells and a decrease in the size of cells, and is regulated by members of the *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* (*SPL*) gene family. The *SPL* gene family might be linked to a system that coordinates the cell size and cell numbers in the leaf primordia. Heterochronic mutations in the SPL system may have contributed to diversified leaf sizes among closely related species.

Endoreduplication is also involved in establishing the relationship between cell number and cell size in a leaf. Endoreduplication is a modified cell cycle lacking mitosis [32] that results in an increase in nuclear ploidy and enhanced cell enlargement. Since Arabidopsis plants exhibit severe endoreduplication, endoreduplication is often thought to be crucial for differentiation and/or cell expansion, but this is not the case. Many annual and perennial weeds exhibit endoreduplication, whereas other plants, such as trees and rice, mostly do not undergo endoreduplication in their leaves [33]. Therefore, endoreduplication is not a fundamental system for organogenesis of leaves. In Arabidopsis, it was found that the relationship between the level of ploidy and enhanced cell enlargement differed among mutant strains [34], suggesting that no simple direct link exists between the endoreduplication-dependent increase in the ploidy level and enhanced cell enlargement. Considering that endoreduplication is often seen in short-lived annual weeds [33], it is more likely a derivative characteristic evolved to shorten the time and cost of cytokinesis.

Dorsoventrality

In most angiosperm leaves, dorsoventrality is rigidly established and thus they are bifacial. The expansion of bifacial leaf laminae occurs along the border between the adaxial and abaxial domain, or ad/ab junction (Figure 3, left) [35]. The lack of either an adaxial or abaxial identity in the leaf primordia results in lamina-less, stick-like, or cylindrical leaves (Figure 3, right). Unifacial leaves lacking establishment of an adaxial leaf domain are often found in various genera and families of monocots as seen in the 'unifacial leaves' of chives (*Allium schoenoprasum* L.: Amaryllidaceae). Some unifacial leaves are cylindrical (chive-like), as expected if flat lamina formation is dependent on dorsoventrality establishment, although other species such as *Iris* spp. (Iridaceae) develop flat unifacial leaves. In such cases, the polarity of the lamina expansion differs from that of bifacial leaf lamina expansion: leaves are expanded perpendicular to the direction in which bifacial leaves expand their lamina (Figure 3). Since the functional morphology of floral organs for fertility depends on the establishment of dorsoventrality [36], it seems that the genetic framework for the dorsoventral control of leaves is maintained even in unifacial species.

Figure 3

Two types of leaf lamina expansion. On the left: in bifacial leaves leaf lamina expand along the adaxial/abaxial junction by the activity of the plate meristem. On the right: in unifacial leaves leaf lamina expand by extensive thickening without establishment of an adaxial domain or a plate meristem. The adaxial domain is shaded in blue, the abaxially identified surface is shown in purple, *PRS*-expression domains are colored in green, and growth directions are indicated by black arrows. X marks the position of the shoot apical meristem (SAM) from which the leaf primordia arose.

Thus, unifacial leaves are thought to have evolved, not through the loss of adaxial domain genes, but through organ-dependent changes in the manner of adaxial domain establishment. However, this leaves the question as to how unifacial leaves can remain flat when they lack a border between the adaxial and abaxial domains. Yamaguchi *et al.* [37••] analyzed two unifacial leaf species of the monocot genus *Juncus* (Juncaceae), one with flat leaves and another with stick-like cylindrical leaves, and found that the flat leaf lamina development was at least in part sustained by enhanced expression of the *DROOPING LEAF* (*DL*) gene in the midrib. *DL* was identified from rice as an ortholog of *CRABS CLAW* (*CRC*) of Arabidopsis [38]. This finding indicates that the flat structure of *Juncus* unifacial leaves is formed by extensive thickening of the leaf lamina that cannot expand laterally (Figure 3), and that is why the polarity of the flat plane in unifacial leaves is perpendicular to that in bifacial leaves.

Moreover, in the course of the analyses on the *Juncus* unifacial leaves, *PRESSED FLOWER* (*PRS*) was found to be expressed independently of the identification of the ad/ab junction [37••], whereas it was once believed to be expressed along the leaf margin with the ad/ab junction in Arabidopsis [39]. In Arabidopsis, *PRS* and its paralog *WOX3* expresses not only in the margin but also in the plate meristem along the ad/ab junction [40], and in the *Juncus* flat-type unifacial leaves, it expresses along the false margin, which is not the ad/ab junction region, suggesting that expression is not controlled by the dorsoventral polarity but by the shape of the primordia [37••]. In both cases, *PRS* function was required for expansion of

the flat lamina. More detailed comparative analyses will help explain the regulation of *PRS* expression in leaf primordia.

Conclusion: more unsolved leaf diversities

As summarized here, only a few forms of leaf development diversity in angiosperms have been studied to date, while many more unusual leaves exist. One of the most complex leaf shape belongs to the pitcher plants, genera *Nepenthes* (Nepenthaceae: Figure 1a) and *Cephalotus* (Cephalotaceae). A three-dimensional pit is formed on the elongated tip of the leaf-bladelike organ, in a shape of a closed vase without any open hole but with a vacant space inside and fins and other accessory organs outside. It opens after the formation of abscission layer along the lid on the top. How is dorsoventral control modified to make such a complex organ? In contrast, a much simpler metamorphosis of leaves is seen in the spines of cacti (Cactaceae), although even in this case, and we do not know how genetic regulations of the leaf organogenesis are modified to develop spines instead of normal leaves [41]. Species of the genus *Utricularia* (Lentibulariaceae: Figure 1d) have organs intermediate between the shoots and leaves [42]. Species of the subfamily Tristichoideae (Podostemaceae: Figure 1e) have leaves that arise inside of the base of older leaves [43] and show intermediate features between the SAM and the leaves at the primordium stage, in terms of their gene expression patterns [44••]. While some of the molecular mechanisms behind the epiphyllous somatic embryogenesis in the genus *Kalanchoe* (Crassulaceae: Figure 1f) are known [45], genetic control mechanisms, such as how somatic embryogenesis occurs on the sinus of the leaves in this genus, remain unclear. Leaves of many species of Araceae (Figure 1g) develop epiphyllous buds [46] and a stalk-like structure at the distal position, indicating intermediate features between the shoot and the leaf. Some species, such as palms (Arecaceae) [47], the lace plant (*Aponogeton madagascariensis* (Mirbel) Bruggen: Aponogetonaceae) (Figure 1h) [48], and *Monstera* (Araceae: Figure 1i) [49] use programmed cell death or abscission for finalizing their leaf shapes. How these processes are regulated in these leaves is still unclear.

Studies on these and other unique morphologies will be not only useful in our understanding of how they have evolved, but will also supply important clues to understanding the basic mechanisms of organogenesis, as revealed, for example, by the roles of *DL*, and for expression control of *PRS* in unifacial leaves in *Juncus* [37••]. New whole genome sequencing techniques are being developed and we are close to achieving the next breakthrough in plant evo/devo. Unusual leaf types should be the initial targets of such future studies.

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. Arber A: *The Natural Philosophy of Plant Form*. Cambridge, UK: Cambridge University Press; 1950, .

2. Ichihashi Y, Kawade K, Usami T, Horiguchi G, Takahashi T, Tsukaya H: **Key proliferative activity in the junction between the leaf blade and the leaf petiole of *Arabidopsis thaliana***. *Plant Physiol* 2011, **157**:1151-1162.

3. Dinneny JR, Yadegari R, Fischer RL, Yanofsky MF, Weigel D: **The role of *JAGGED* in shaping lateral organs**. *Development* 2004, **131**:1101-1110.

4. Boyce CK: **Mechanisms of laminar growth in morphologically convergent leaves and flower petals**. *Int J Plant Sci* 2007, **168**:1151-1156.

5. Nakayama H, Yamaguchi T, Tsukaya H: **Acquisition and diversification of cladodes: leaf-like organs in the genus *Asparagus***. *Plant Cell* 2012, **24**:929-940.
•• The authors indicated that the cladodes observed in the genus *Asparagus* are modified shoots, expressing both SAM-related and leaf-lamina-related genes. Comparative analyses of flat cladodes of *A. asparagoides* and rod-shaped cladodes of *A. officinalis* suggest that the morphological divergence between these two species may be derived from the altered expression pattern/levels of adaxial identity genes in the cladode primordia.

6. Hagemann W, Gleissberg S: **Organogenetic capacity of leaves: the significance of marginal blastozones in angiosperms**. *Plant Syst Evol* 1997, **199**:121-152.

7. Blein T, Pulido A, Vialette-Guiraud A, Nikovics K, Morin H, Hay A, Johansen IE, Tsiantis M, Laufs P: **A conserved molecular framework for compound leaf development**. *Science* 2008, **322**:1835-1839.

8. Burko Y, Shleizer-Burko S, Yanai O, Shwartz I, Zelnik ID, Jacob-Hirsch J, Kela I, Eshed-Williams L, Ori N: **A role for APETALA1/ FRUITFULL transcription factors in tomato leaf development**. *Plant Cell* 2013, **25**:2070-2083.

9. Pabon-Mora N, Sharma B, Holappa LD, Kramer EM, Litt A: **The *Aquilegia FRUITFULL-like* genes play ley roles in leaf morphogenesis and inflorescence development**. *Plant J* 2013, **74**:197-212.

10. Efroni I, Eshed Y, Lifschitz E: **Morphogenesis of simple and compound leaves: a critical review**. *Plant Cell* 2010, **22**:1019-1032.
• A very informative review article on the mechanisms of leaf organogenesis.

11. Townsley B, Sinha N: **A new development: evolving concepts in leaf ontogeny**. *Annu Rev Plant Biol* 2012, **63**:535-562.

12. Gleissberg S: **Comparative analysis of leaf shape development in Papaveraceae–Papaveroideae**. *Flora* 1998, **193**:269-301.

13. Ikeuchi M, Tatematsu K, Yamaguchi T, Okada K, Tsukaya H: **Precocious progression of tissue maturation instructs basipetal initiation of leaflets in *Chelidonium majus* subsp. *asiaticum* (Papaveraceae)**. *Amer J Bot* 2013, **100**:1116-1126.

14. Tsukaya H: **The role of meristematic activities in the formation of leaf blades**. *J Plant Res* 2000, **113**:119-126.

15. Tsukaya H: **Determination of the unequal fate of cotyledons of a one-leaf plant, Monophyllaea**. *Development* 1997, **124**:1275-1280.

16. Jong K, Burtt BL: **The evolution of morphological novelty**
• **exemplified in the growth patterns of some Gesneriaceae**. *New Phytol* 1975, **75**:297-311.

17. Tsukaya H: **Leaf development**. 2nd ed.. **The arabidopsis book**.
• Rockville, MD: American Society of Plant Biologists; 2013, e0163 http://dx.doi.org/10.1199/tab.0072: A review on our present understanding of genetic control mechanisms of leaf morphogenesis in Arabidopsis.

18. Kazama T, Ichihashi Y, Murata S, Tsukaya H: **The mechanism of**
• **cell cycle arrest front progression explained by a KLUH/ CYP78A5-dependent mobile growth factor in developing leaves of *Arabidopsis thaliana***. *Plant Cell Physiol* 2010, **51**:1046-1054.
The authors suggest that a boundary between the cell-differentiation zone and the cell-proliferative zone is kept at a steady position, a certain distance from the base in the Arabidopsis leaf primordia. Then this boundary suddenly moved to the base to stop the cell division phase of the primordia. This insight into the cell proliferative activity is an important clue to understanding the determinacy control mechanisms of leaf organogenesis.

19. Ha C-H, Jun JH, Nam HG, Fletcher JC: ***BLADE-ON-PETIOLE1*** **encodes a BTB/POZ domain protein required for leaf morphogenesis in *Arabidopsis thaliana***. *Plant Cell Physiol* 2004, **45**:1361-1370.

20. Ha C-H, Kim G-T, Kim B-C, Jun J-H, Soh M-S, Ueno Y, Machida Y, Tsukaya H, Nam H-G: **The *BLADE-ON-PETIOLE* gene controls leaf pattern formation through regulation of meristematic activity**. *Development* 2003, **130**:161-172.

21. Hay A, Tsiantis M: **KNOX genes: versatile regulators of plant development and diversity**. *Development* 2010, **137**:3153-3165.

22. Harrison J, Möller M, Langdale J, Cronk Q, Hudson A: **The role of *KNOX* genes in the evolution of morphological novelty in *Streptocarpus***. *Plant Cell* 2005, **17**:430-443.

23. Pham T, Sinha N: **Role of *KNOX* genes in shoot development of *Welwitschia mirabilis***. *Int J Plant Sci* 2003, **164**:333-343.

24. Fukuda T, Yokoyama J, Tsukaya H: **The evolutionary origin of indeterminate leaves in Meliaceae: phylogenetic relationships among species in the genera *Chisocheton* and *Guarea,* as inferred from sequences of chloroplast DNA**. *Int J Plant Sci* 2003, **164**:13-24.

25. Steingraeber DA, Fisher JB: **Indeterminate growth of leaves in *Guarea* (Meliaceae): a twig analogue**. *Amer J Bot* 1986, **73**:852-862.

26. Fisher JB, Rutishauser R: **Leaves and epiphyllous shoots in *Chisocheton* (Meliaceae): a continuum of woody leaf and stem axes**. *Can J Bot* 1990, **68**:2316-2328.

27. Fisher JB: **Indeterminate leaves of *Chisocheton* (Meliaceae): survey of structure and development**. *Bot J Linn Soc* 2002, **139**:207-221.

28. Tsukaya H: **Interpretation of mutants in leaf morphology: genetic evidence for a compensatory system in leaf morphogenesis that provides a new link between cell and organismal theory**. *Int Rev Cytol* 2002, **217**:1-39.

29. Kawade K, Horiguchi G, Tsukaya H: **Non-cell-autonomously coordinated organ-size regulation in leaf development**. *Development* 2010, **137**:4221-4227.

30. Fujikura U, Horiguchi G, Ponce MR, Micol JL, Tsukaya H: **Coordination of cell proliferation and cell expansion mediated by ribosome-related processes in the leaves of *Arabidopsis thaliana***. *Plant J* 2009, **59**:499-508.

31. Usami T, Horiguchi G, Yano S, Tsukaya H: **The *more and smaller cells* mutants of *Arabidopsis thaliana* identify novel roles for *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* genes in the control of heteroblasty**. *Development* 2009, **136**:955-964.

32. Komaki S, Sugimoto K: **Control of plant cell cycle by developmental and environmental cues**. *Plant Cell Physiol* 2012, **53**:953-964.

33. Barow M, Meister A: **Endopolyploidy in seed plants is differently correlated to systematics, organ, life strategy and genome size**. *Plant Cell Environ* 2003, **26**:571-584.

34. Tsukaya H: **Does ploidy level directly control cell size? Counterevidence from arabidopsis genetics**. *PLoS ONE* 2013, **8**:e83729 http://dx.doi.org/10.1371/journal.pone.0083729.

35. Waites R, Hudson A: **phantastica: a gene required for dorsoventrality of leaves in Antirrhinum majus**. *Development* 1995, **121**:2143-2150.

36. Toriba T, Suzaki T, Yamaguchi T, Ohmori Y, Tsukaya H, Hirano H: **Distinct regulation of adaxial–abaxial polarity in anther patterning in rice**. *Plant Cell* 2010, **22**:1452-1460.

37. Yamaguchi T, Yano S, Tsukaya H: **Genetic framework for**
•• **flattened leaf blade formation in unifacial leaves of *Juncus prismatocarpus***. *Plant Cell* 2010, **22**:2141-2155.
The authors showed that the leaf lamina of unifacial leaves of the genus *Juncus* lack adaxial identity in terms of mRNA markers and describe how the flat structure is formed by extensive thickening of the leaf lamina via the activity of the *DROOPING LEAF* gene.

38. Yamaguchi T, Nagasawa N, Kawasaki S, Matsuoka M, Nagato Y, Hirano HY: **The *YABBY* gene *DROOPING LEAF* regulates carpel specification and midrib development in *Oryza sativa***. *Plant Cell* 2004, **16**:500-509.

39. Matsumoto N, Okada K: **A homeobox gene, PRESSED FLOWER, regulatesregulates lateral axis-dependent development of Arabidopsis flowers**. *Genes Dev* 2001, **15**:3355-3360.

40. Nakata M, Matsumoto N, Tsugeki R, Rikirsch E, Laux T, Okada K: **Roles of the middle domain-specific WUSCHELRELATED HOMEOBOX genes in early development of leaves in Arabidopsis**. *Plant Cell* 2012, **24**:519-535.

41. Mauseth JD: **Structure–function relationships in highly modified shoots of Cactaceae**. *Ann Bot* 2006, **98**:901-926.

42. Sattler R, Rutishauser R: **Structural and dynamic descriptions of the development of *Utricularia foliosa* and *U. australis***. *Can J Bot* 1990, **68**:1989-1990.

43. Koi S, Imaichi R, Kato M: **Endogenous leaf initiation in the apical-meristemless shoot of *Cladopus queenslandicus* (Podostemaceae) and implications for evolution of shoot morphology**. *Int J Plant Sci* 2005, **166**:199-206.

44. Katayama N, Koi S, Kato M: **Expression of *SHOOT***
•• ***MERISTEMLESS*, WUSCHEL, and ASYMMETRIC LEAVES1 homologs in the shoots of Podostemaceae: implications for the evolution of novel shoot organogenesis**. *Plant Cell* 2010, **22**:2131-2140.
The authors show that some species of Podostemaceae have 'leaves' that seem to be an intermediate organ between simple leaves and shoot of the apical meristem (SAM), in terms of expression patterns of leaf-related and/or SAM-related genes. Also see Ref. [43].

45. Garcês HMP, Champagne CEM, Townsley BT, Park S, Malhó R, Pedroso MC, Harada JJ, Sinha NR: **Evolution of asexual reproduction in leaves of the genus Kalanchoe**. *Proc Natl Acad Sci U S A* 2007, **104**:15578-15583.

46. Dickinson TA: **Epiphylly in angiosperms**. *Bot Rev* 1978, **44**:181-232.

47. Dengler NG, Dengler RE, Kaplan DR: **The mechanism of plication in palm leaves: histogenic observations on the pinnate leaf of *Chrysalidocarpus lutescens***. *Can J Bot* 1982, **60**:2976-2980.

48. Arunika HL, Gunawardena AN, Greenwood JS, Dengler NG: **Programmed cell death remodels lace plant leaf shape during leaf development**. *Plant Cell* 2004, **16**:60-73.

49. Arunika HL, Gunawardena AN, Sault K, Donnelly P, Greenwood JS, Dengler NG: **Programmed cell death and leaf morphogenesis in *Monstera obliqua* (Araceae)**. *Planta* 2006, **221**:607-618.