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Forum

The Mechanical Feedback Theory of Leaf Lamina Formation

Yuling Jiao,[1,2,*] Fei Du,[1] and Jan Traas[3,*]

The appearance of leaves with flattened laminae about 400 million years (Myr) ago had broad impacts on the Earth's ecosystem. The influential telome theory presents a model for this evolutionary transition, although it lacks plausible molecular evidence. Recently, microtubule-mediated mechanical feedback was proposed as a parsimonious alternative mechanism to explain leaf blade evolution.

Leaf Evolution

Leaves evolved independently multiple times [1], and the evolution of flat leaves and leaflike organs has played a central role in optimizing photosynthesis, with profound environmental consequences for life on Earth. Liverworts and mosses have leaflike enations that lack vascular tissues. Microphyll and megaphyll leaves (i.e., the leaves of lycophyte and euphyllophyte vascular plants) evolved independently. In fact, megaphyll leaves evolved multiple times among the euphyllophytes, which include ferns, gymnosperms, and angiosperms. Today, megaphyll leaves harvest solar energy to fix ~56 billion tons of carbon or more per year from the atmosphere [1].

In the late Silurian (~420 Myr ago), the situation was dramatically different. The earliest land plants at that time, such as *Cooksonia*, had naked dichotomized branches rather than leaves. It took ~40 Myr for flattened megaphylls to originate and spread from the late Devonian onward. This change is thought to have arisen in response to a ~90% decline in atmospheric CO_2, and it profoundly altered the evolutionary trajectory on Earth [1].

Zimmermann's Telome Theory

Different explanations have been proposed for leaf formation as a critical evolutionary event in Earth's history. Among them, the telome theory proposed over 80 years ago by the German paleobotanist Walter Zimmermann is by far the most influential one and often appears in textbooks. By analyzing fossil records, Zimmermann proposed that telomes, 'undifferentiated elements in the organization of the oldest land plants' [2], are the basic unit of plant morphogenesis. According to the theory, the combination and modification of telomes can explain all plant body plans. To explain leaf evolution, Zimmermann envisaged a series of transformations that would allow the conversion of axisymmetric branches into a megaphyll leaf (Figure 1).

Starting from a dichotomized 3D branching system, as seen in extinct *Rhynia* and *Psilophyton* plants, the first 'overtopping' step separates the stem and the branch. This describes how one branch of a dichotomous pair overgrows another one. In the second 'planation' step, neighboring branches align within the same plane. In the final 'webbing' step, the neighboring branches become connected by photosynthetic tissue to form a laminate blade.

The telome theory provides a plausible explanation for leaf evolution, but in recent years, it has been criticized for lacking an identified developmental mechanism [1,3]. On the basis of fossil history, no single plant lineage has been identified to display all three key steps. Furthermore, it appears difficult to correlate transformational steps in a developmental context. 'Overtopping' requires the initiation of branches and coordination of their relative growth. In angiosperms, branching relies on axillary meristems, which are tightly associated with leaves [4]. The evolution of axillary meristems has not been touched in the telome theory, so the identified mechanisms do not support or refute the telome theory. 'Planation' involves branch initiation point positioning and also coordination of branch growth. Axillary meristem formation is associated with leaf formation, which is under the control of polar auxin transport. Computational simulations indicate that a wide range of leaf arrangements (i.e., phyllotaxis) are possible [5], including telome theory- proposed distichous phyllotaxis, but there is no clear evidence that a change in phyllotaxis was at the basis of a planation-like evolution. For the proposed branch fusion (i.e., 'webbing'), mutations of certain angiosperm genes, such as *CUP-SHAPED COTYLEDON* (*CUC*) genes, potentially promote organ fusions but also inhibit axillary meristem formation [5]. There is again no proof that this type of organ fusion has been at the basis of a webbing process during evolution, and it has been questioned whether simply the lateral outgrowth from a branch is a more plausible mechanism for lamination.
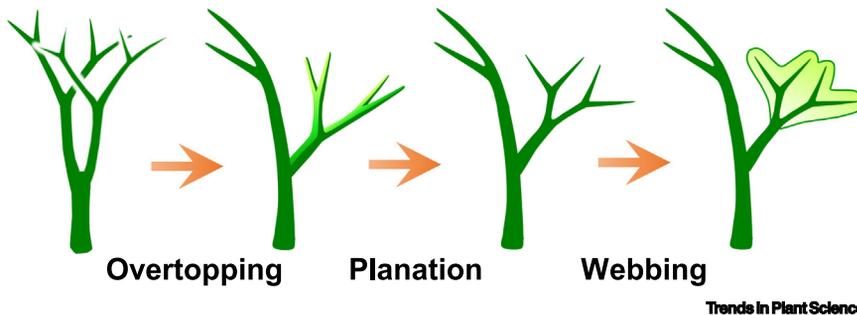
Overtopping   Planation   Webbing

Figure 1. Zimmermann's Telome Theory of Leaf Evolution. According to the telome theory, leaves evolved from 3D shoot branches of early vascular plants. One branch overgrew and formed the main stem, and the other branch became the 3D lateral branch (overtopping). Neighboring lateral branches subsequently aligned within a single plane (planation). Lateral growth and fusion between branches led to the formation of a flattened leaf blade (webbing). Adapted from [1].

Mechanical Feedback

A recent work combined experimental and theoretical analyses to show that leaf flattening is governed by mechanical feedback [6]. Plant cells in tissues experience a tremendous amount of mechanical stress driven by turgor pressure. In a growing cell, the walls slowly yield to the resulting tension, leading to cell expansion at a particular rate and in specific directions. The turgor within each cell, the physical interactions between neighboring cells, and the overall organ shape generate tissue-wide force patterns. There is strong evidence that these patterns provide in turn directional cues for cellular behavior and morphogenesis [7]. In particular, it has been proposed that stress directions are translated into directional growth via a mechanism that relies on the microtubule cytoskeleton. This so-called mechanical feedback hypothesis is based on the observation that cortical microtubules often align along the main predicted force directions. Because the microtubules determine the orientation of the rigid cellulose microfibrils in the walls, the latter are reinforced in such a way that they resist the main tensile stresses operating within the tissue.

This has important implications for leaf shape. Leaf primordia are initiated as flattened bulges at the meristem periphery. This bilaterally symmetric shape is predicted to have a dominant stress direction along the adaxial–abaxial (also called 'dorsoventral') axis. Accordingly, highly anisotropic microtubule and cellulose microfibril alignments are observed along the same axis in internal anticlinal walls. The resulting mechanical anisotropy leads to differential resistance to presumably uniform turgor pressure and strongly reduces growth along the adaxial–abaxial axis. As a result, the structure continues to flatten, whereas the main stress direction is maintained. The feedback mechanism thus contributes to and amplifies organ flatness (Figure 2).

Notably, the mechanical feedback theory provides a parsimonious explanation for leaf evolution and contrasts starkly with the series of transformations proposed in the telome theory. In the mechanical feedback theory, only an initial primordium shape transformation from axisymmetric to bilaterally symmetric is required for flat blade formation. Once bilateral symmetry is established in a primordium, the presumably conserved microtubule-mediated mechanical feedback amplifies the asymmetry to form a planar leaf shape.
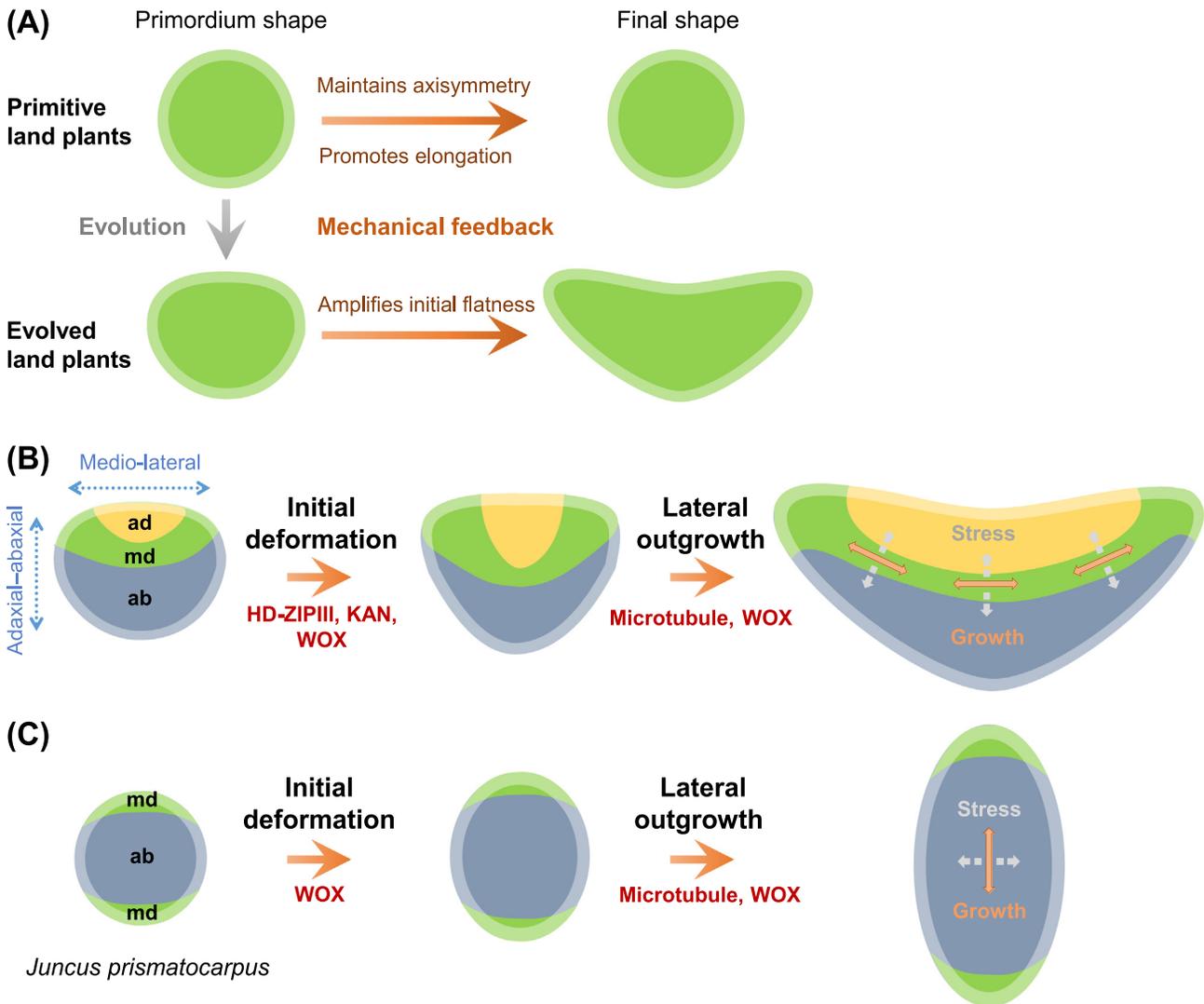
The mechanical feedback model is a unified theory that can also account for radially symmetric, elongated organs, such as stems and roots. In an axisymmetric or close to axisymmetric primordium, flattening either would not or would only very slowly evolve, and mechanical feedback would in that case mainly promote organ elongation along the apical–basal axis [6].

Establishing Bilateral Symmetry

As we have seen, the formation of a bilaterally symmetric, flattened primordium is critical (Figure 2A). It is thought that this results from gene expression patterns at the peripheral zone of the shoot apical meristem, even before the leaves initiate [8]. This prepattern then leads to a polarized leaf primordium expressing specific transcription factors at each of its sides. Subsequently, a middle domain including the leaf margin is established between the adaxial and abaxial domains. This middle domain is characterized by the expression of WUSCHEL-RELATED HOMEOBOX (WOX) genes [9], which promote lateral expansion of the young primordium, thus further contributing to organ flatness [6]. Importantly, when genes involved in leaf polarity are inactivated, leaves tend to become axisymmetric.

Recent evidence shows that, from a mechanical point of view, the initial asymmetry in abaxial/adaxial gene expression is translated into the differential regulation of cell wall mechanics [10]. A transient high elasticity of the abaxial epidermis contrasts with the stiffer adaxial epidermis showing high methyl esterification of cell wall pectins (Figure 2B). Computational modeling indicated that the combination of differences in wall stiffness and the increased growth at the leaf margin is sufficient to promote the bilateral symmetry of the early primordium. Subsequently, microtubule-mediated mechanical feedback amplifies this initial flatness to generate the highly anisotropic growth of the blade [6]. In addition, leaf margin WOX genes act in antagonism to boundary CUC genes [6] and thus cause lateral outgrowth in the margin but not fusion or webbing between primordia.

(A)

Primordium shape   Final shape

**Primitive
land plants**

Maintains axisymmetry

Promotes elongation

Evolution

Mechanical feedback

Amplifies initial flatness

**Evolved
land plants**

(B)

Medio-lateral

Adaxial–abaxial

ad
md
ab

**Initial
deformation**

HD-ZIPIII, KAN,
WOX

**Lateral
outgrowth**

Microtubule, WOX

Stress

Growth

(C)

md
ab
md

**Initial
deformation**

WOX

**Lateral
outgrowth**

Microtubule, WOX

Stress

Growth

Juncus prismatocarpus

Trends in Plant Science

Figure 2. Mechanical Feedback Theory of Leaf Evolution. (A) Conceptual model showing that axisymmetry breaking, once evolved, can be amplified by mechanical feedback to obtain a flat leaf blade. (B) In a typical angiosperm, prepatterned gene expression leads to differential cell growth rates that establish initial bilateral symmetry, which causes strong tensile stress along the adaxial–abaxial axis. Microtubule-mediated mechanical feedback leads to growth perpendicular to the main stress axis and amplifies stress anisotropy. Margin-expressed *WUSCHEL-RELATED HOMEOBOX* (*WOX*) genes further promote blade outgrowth in concert with the microtubules. (C) In *Juncus prismatocarpus*, leaf primordia lack adaxial identity but express middle domain identity genes in altered patterns. The mechanical feedback mechanism may amplify the bilateral symmetry established along the adaxial–abaxial axis to form a leaf blade along this axis. Abbreviations: ab, abaxial identity; ad, adaxial identity; md, middle domain identity; HD-ZIPIII, HD-ZIPIII homeobox transcription factor; KAN, KANNADI transcription factor.

Leaf flattening has evolved multiple times, and the flattened unifacial leaves of *Juncus prismatocarpus* provide additional support for the mechanical feedback theory (Figure 2C). These unifacial leaves develop blades along the adaxial–abaxial axis but not the mediolateral axis. At the molecular level, these leaves lack adaxial identity and have leaf margin genes that are expressed in the margin-like regions along the adaxial–abaxial axis [11]. Even without leaf adaxial identity as in *J. prismatocarpus*, mechanical feedback would be sufficient to amplify the rotated bilateral symmetry along the adaxial–abaxial axis, resulting in a leaf blade along this axis. Similarly, mechanical feedback may also explain flattened stems, which are termed 'phylloclades' and found in phylogenetically distinct plant species.

Concluding Remarks

We propose that a two-step process leads to flattened leaf formation. Early

axisymmetry breaking involving the polarity genes generates a flattened primordium with strong tensile stress in the adaxial–abaxial direction. Microtubules and cellulose microfibrils subsequently align along this orientation, maintaining anisotropic cell growth and further enhancing leaf flattening (Figure 2). The mechanical feedback theory provides an attractive alternative explanation for leaf evolution. It is robust insofar as it can explain both bilaterally symmetric leaves and axisymmetric stems and roots with a minimum of hypotheses. Nevertheless, there are questions that remain to be solved. The initial axisymmetry breaking mechanisms in microphyll leaf species and those that independently evolved in megaphyll leaf species are waiting to be explored. Furthermore, the precise mechanism behind microtubule-mediated mechanical feedback remains elusive [12].

[1]State Key Laboratory of Plant Genomics and National Center for Plant Gene Research (Beijing), Institute of Genetics and Developmental Biology, The Innovative Academy of Seed Design, Chinese Academy of Sciences, Beijing 100101, China
[2]University of Chinese Academy of Sciences, Beijing 100049, China
[3]Laboratoire Reproduction et Développement des Plantes, Université de Lyon, ENS de Lyon, UCB Lyon 1, CNRS, INRAE, Inria, F-69342, Lyon, France

*Correspondence:
yljiao@genetics.ac.cn (Y. Jiao) and jan.traas@ens-lyon.fr (J. Traas).
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