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Commentary

# A touchy subject: $Ca^{2+}$ signaling during leaf movements in Mimosa

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ABSTRACT

*Mimosa pudica*, the sensitive plant, responds to stimuli such as touch and wounding with leaf movements that propagate throughout the plant. The motion is driven by changes in the turgor of specialized cells in a set of motor organs called pulvinae. By imaging cellular $Ca^{2+}$ levels as the wave of movement propagates through the leaf, Hagihara and colleagues now show that $Ca^{2+}$ signals precede and predict the pulvinar movements. These results provide compelling support for a model where Mimosa uses a $Ca^{2+}$-related response system to trigger its leaf movements. These researchers then used CRISPR to delete a critical genetic regulator of pulvinar development, producing plants with immobile leaves. These plants experienced more herbivory than wild type, suggesting that the $Ca^{2+}$-triggered leaf movements are an adaptation to deter herbivory.

Imagine touching a thorn on the stem of a rose. You sense the impending damage, process that information and trigger movements that jerk your hand away from the danger, all in less than a second. You are employing fast, long-distance electrical signals transmitted via nerves that in turn lead to almost instantaneous muscle contraction. At first glance, plants seem to operate on a time scale where similar rapid signaling systems might not be relevant. Watch an insect chewing on a leaf and to our eyes the plant mounts no obvious immediate reaction. Yet, looks can be deceiving. The rapid movements seen when an insect is captured by the trap of a Venus flytrap (*Dionaea muscipula*) or when touch induces waves of movement in the leaflets of the sensitive plant (*Mimosa pudica*; Fig. 1) provide a window into a world where signaling and response in less than a second can be critical to a plant's survival.

How can such rapidly propagating, plant-wide effects occur without the nerve and muscle systems that explain equivalent phenomena in animals? In their recent paper, Hagihara et al. [1] have revealed how $Ca^{2+}$ plays a pivotal role in this fast signaling machinery for Mimosa. The leaves of this plant show rapid movements in reaction to a range of stimuli such as local wounding, temperature stress and mechanical stimulation. Upon touching the leaf tip, the leaflets (pinnules) that make up the Mimosa leaf fold upwards towards each other in a wave of motion that propagates to the leaf base (Fig. 1). These movements occur in a matter of seconds and have been speculated to either startle herbivores or make the plant less conspicuous to a would-be attacker.

These leaflet movements are driven by motor organs called pulvinae. Turgid cells lie above (extensor cells) and below (flexor cells) the pivot point in the pulvinus where the leaflet will fold (Fig. 1). Upon stimulation of the leaf, electrical signals propagate to these cells and trigger the opening of channels that lead to the efflux of ions, such as $K^+$ and $Cl^-$ [2]. These fluxes are accompanied by osmotic loss of water that causes the cells of the extensor to deflate and the bending movement at the pulvinus to occur (Fig. 1A–C; refs [2,3]).

Hagihara et al., [1] have addressed the signals that propagate through the leaf to trigger these waves of movement. They used plants expressing a green fluorescent protein variant named GCaMP6f that has been engineered to dramatically increase its fluorescence intensity upon binding $Ca^{2+}$. This fluorescence then shows where cellular $Ca^{2+}$ signals are occurring. Using this technology, these researchers were able to visualize pulvinus after pulvinus sequentially 'firing' a $Ca^{2+}$ pulse that predicted the wave of leaflet motion moving down the leaf (Fig. 1D-E). These $Ca^{2+}$ signals could be triggered within 0.1 s of stimulation of the leaf and pharmacologically blocking them prevented the wave of movement. These observations provide compelling evidence that Mimosa uses a $Ca^{2+}$-dependent response system to trigger pulvinar activity.

Hagihara et al., [1] then addressed one of the most fundamental questions about Mimosa: Why move at all? They noticed that when insects triggered leaf movements, they tended to move away from the plant, an observation consistent with the often-suggested role for such leaf movements in defense against herbivory. To directly test this hypothesis, these researchers used CRISPR technology to delete the gene *ELONGATED PETIOLULE 1* (*ELP1*) from the Mimosa genome. *ELP1* is critical for pulvinar development and so this manipulation resulted in plants that cannot move. When compared to wild-type Mimosa, plants

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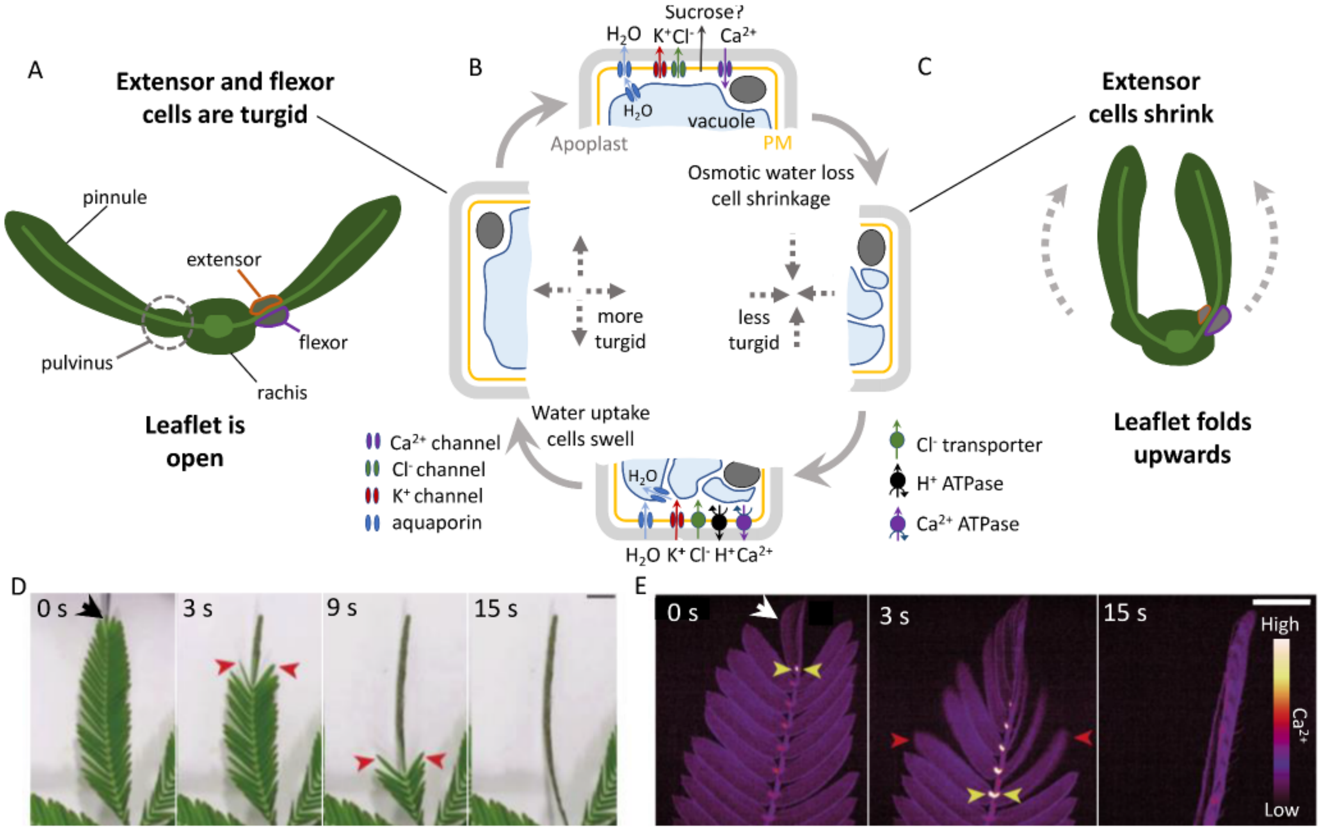


**Fig. 1.** Leaflet movements in *Mimosa pudica*. (A) Cross-section of leaf showing how the fully turgid cells of the flexor and extensor regions of the pulvinus extend the leaflets (pinnules) outwards from the branch (rachis). Pulvinar cells can undergo reversible osmotic water movements facilitated by aquaporins and driven by fluxes of ions such as $K^+$ and $Cl^-$ (and possibly of photoassimilates such as sucrose). The model in (B) proposes roles for putative ion channels and transporters that are triggered by $Ca^{2+}$ influx. These membrane transporters then release $K^+$ and $Cl^-$ (and possibly sucrose) leading to turgor loss and leaflet folding as the extensor shrinks. (C) Upon such turgor loss in the extensor cells, the still turgid pulvinar flexor cells are able to lever the leaflets upwards. (B) These changes are reversible with ion uptake likely being driven by activation of the $H^+$ ATPase. The increase in cell solute content then causes osmotic water uptake and reinstates full turgor in the extensor, levering the leaflets downwards. $Ca^{2+}$ levels are thought to be reset by the action of putative $Ca^{2+}$-pumping ATPases. (D) Wave of leaflet folding triggered by touch (black arrow). (E) Wave of $Ca^{2+}$ increases in the pulvinae in response to touch (white arrow) monitored using plants expressing the $Ca^{2+}$-sensitive fluorescent biosensor GCaMP6f. $Ca^{2+}$ levels are pseudocolor-coded according to the inset scale in (E). Red arrows in (D) and (E) show the next leaflet to fold upwards in the cascade of response. Yellow arrow in (E) show the pulvinus with the most recent $Ca^{2+}$ increase. Scale bars 10 mm. Panels (D) and (E) are modified from [1] under a creative commons attribution 4.0 international license (creativecommons.org/licenses/by/4.0/).

with pinnules that do not respond to touch were subject to more intense herbivory, consistent with an anti-herbivory role for these fast, touch-triggered movements. Such questions about how Mimosa's leaf movements are triggered and what function they serve have been asked for centuries [4] but it took recent advances in technology for Hagihara and his colleagues to directly address them. Neither of the tools essential to their study (GCaMP6f and CRISPR) existed a decade ago [5,6].

In addition to using the latest approaches to answer these long-standing questions about Mimosa's movements, the work of Hagihara et al. [1] also poses a new set of challenges. One key next step will be to define the molecular identities of the channels and pumps involved in generating Mimosa's pulvinar responses. Similar bioimaging technology to the GCaMP6f used with Mimosa has shown $Ca^{2+}$ signals also predict the rapid leaf movements underlying trap closure in the Venus flytrap [7] and the insect feeding responses of the sundew (*Drosera spatulate;* [8]). In the case of the Venus flytrap an array of candidate channels has already been proposed [9]. Similarly, in Mimosa, mechanosensitive $Cl^-$ channels in the pulvinus have been shown to be critical for response [10]. However, for all of these plants, the challenge is now to demonstrate the roles of specific channels and pumps with molecular and genetic precision. There is potentially immense power in such comparative analyses. The Venus flytrap and the sundew are closely related to each other but are phylogenetically quite distant from Mimosa. Any overlap in the molecular details of the systems driving these fast leaf movements therefore offers great potential to define fundamental characteristics of these kinds of rapid plant signaling systems.

## Declarations of Competing Interest

None.

## Data availability

No data was used for the research described in the article.

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