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Commentary

A tail of two horses? Guard cell abscisic acid and carbon dioxide signalling in the Equisetum ferns

Equisetum, or horsetail, is stubborn. This fern is the bane of gardeners and farmers alike and is one of the world's most pernicious agricultural weeds. No wonder it is perhaps the oldest single extant genus of vascular plants. Its mere 16 or so species are the last stout survivors of a mighty lineage rooted deep in the Devonian–Carboniferous coal swamps and forests. Equisetum's story is, in some ways, reminiscent of the reductive evolution of the bryophytes from larger and more complex ancestors lost in the mists of time. Similarly, this convoluted 400-million-year-old backstory has played out in Equisetum's ancient developmental and physiological pathways, not least guard cell signalling and stomatal movement for gas exchange and photosynthesis. By interrogating their mechanisms, we are learning where, when, and how they arose and can infer the suite of adaptations shared by the last common ancestor of horsetails and other vascular plants. This approach, however, is not without controversy, with multiple independent studies over the past few decades coming to different conclusions about the where, the when, and the how.

'The novel ABA responses and insights into CO₂ signalling demonstrated by Meigas et al. fill a crucial knowledge gap and elegantly bolster the mounting evidence that horsetail and other fern stomata are sensitive to CO₂, blue light, red light, low humidity, and shade. . .'

Conditional responses in the early diverging lineages

In an article recently published in *New Phytologist*, Meigas *et al.* (2024; doi: [10.1111/nph.19542](https://doi.org/10.1111/nph.19542)) demonstrate that horsetail stomata respond not only to carbon dioxide and humidity changes but also to the plant hormone abscisic acid (ABA). Abscisic acid induces stomatal closure in many species via specific receptors and a protein kinase-based signalling cascade. Using gas exchange analyses of several Equisetum species collected outside and grown in controlled environments, Meigas *et al.* show that stomatal ABA sensitivities (i.e. whether they respond to ABA or not) are

This article is a Commentary on Meigas *et al.* (2024), doi: [10.1111/nph.19542](https://doi.org/10.1111/nph.19542).

dependent on the species and the growing season. In addition, they reveal that CO₂ signalling in Equisetum species can act independently of ABA signalling and can remain intact even when responses to exogenous ABA are lost. Their growth chamber experiments on juvenile shoots of plant material collected repeatedly across multiple years identifies a critical need for plant science (and particularly funders!) to embrace longer term experiments. Only through repeated measurements of species' environmental responses across multiple years, seasons, and weather patterns can a more complete picture emerge of plant plasticity and adaptation to climate change.

These novel findings in horsetails add to a growing body of evidence of fern stomatal function and guard cell mechanisms, and represent a significant step toward reconciling previous, seemingly contradictory, results in the scientific literature concerning the evolution of stomatal responsiveness. Stomatal evolution has been the subject of a thorough recent review (Clark *et al.*, 2022).

Although ABA-induced stomatal closure in horsetails has not previously been investigated, it has been described as being absent (Brodrigg & McAdam, 2011; Gong *et al.*, 2021; Cândido-Sobrinho *et al.*, 2022) in ferns. Alternatively, Hōrak *et al.* (2017), Grantz *et al.* (2019), and Plackett *et al.* (2021) present data showing that fern stomata do close in response to ABA; however, the response can be conditional, in the sense that it depends on, for example, the growth conditions experienced by the plants. Indeed, this is the situation Meigas *et al.* describe in their paper together with highlighting the technical difficulties associated with conducting experiments of this type.

Intact and active stomatal signalling in the oldest vascular plant lineage

Plant development and age may also play a major role in stomatal activity. In older horsetails, with their characteristic long furrowed and ridged stems (Fig. 1a,b; Cullen & Rudall, 2016), maturational changes in guard cell activity may coincide with the onset of greater rigidity. Equisetum stomata may stiffen with age with a corresponding loss of aperture control (Fig. 1b,d; Cullen & Rudall, 2016), much like moss stomata whose walls often thicken, and pores sometimes fill in, which can be encountered in older sporangia (Merced & Renzaglia, 2014; Caine *et al.*, 2020). Meigas *et al.* made sure to test gas exchange in younger, juvenile plants. All 16 Equisetum species are annuals that, like many ferns, confine themselves to damp ground or wetlands. These life-history traits, their seasonality, and their requirement for water could also lead one to assume that their stomatal control of water loss is lacking. As with moss stomata with occluded apertures under certain growth conditions (Caine *et al.*, 2020), these factors and the local environment arguably contribute both to the conditional responses and conflicting results in the published literature in horsetails and

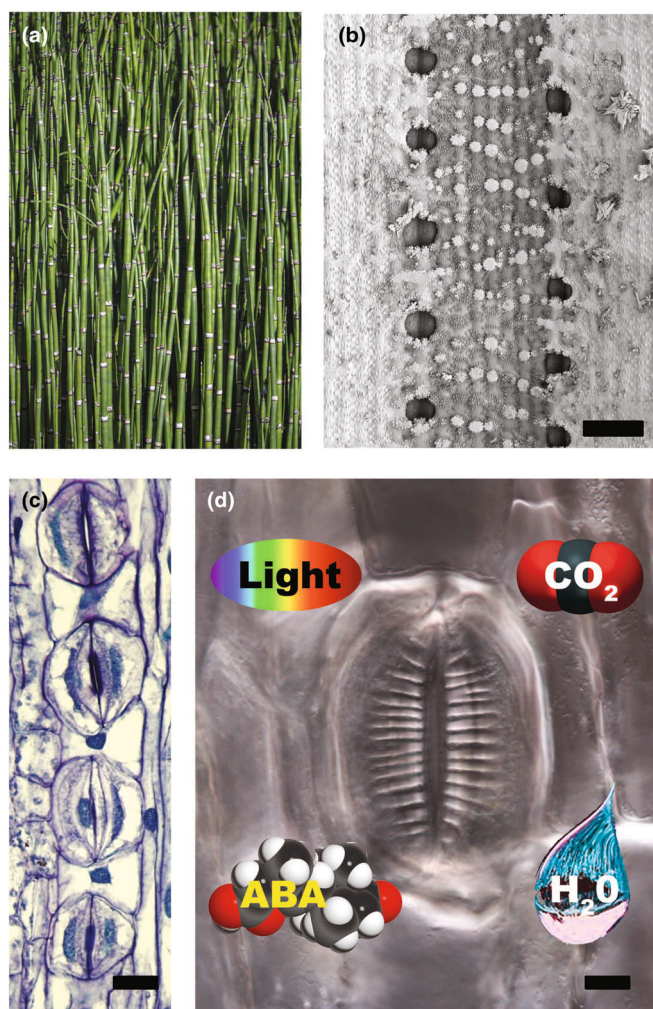


Fig. 1 *Equisetum* stomata. (a) Stand of *Equisetum hyemale*, whose stomata were previously shown to respond to light, humidity, and CO₂ (Doi *et al.*, 2015; Kübarsepp *et al.*, 2020; Cai *et al.*, 2021) (image: Ines Stuart-Davidson, RBG Kew). (b) Scanning electron micrograph of *Equisetum giganteum* surface, showing two files of sunken and closed stomata surrounded by silicified papillae and epicuticular waxes. Bar, 100 μ m. (c) Light micrograph of thin section of a row of four mature stomata in *E. giganteum*, showing guard cells around an open pore (top), each stoma flanked by a pair of lateral subsidiary cells. Bar, 25 μ m. (d) Differential interference contrast image of a single stomata of *E. giganteum*, showing radiating ribs appearing as striated thickenings at maturity. Overlay: The reported sensitivities of horsetail guard cells to light, carbon dioxide, humidity, and now abscisic acid (Doi *et al.*, 2015; Kübarsepp *et al.*, 2020; Cai *et al.*, 2021; Meigas *et al.*, 2024). Bar, 10 μ m. Images in panels b, c, and d courtesy of Paula J. Rudall, methods in Cullen & Rudall (2016).

other earlier diverging plant lineages. The study by Meigas *et al.* is a call to investigate these further.

These new findings in horsetails again challenge the hydropassive *vs* hydroactive dichotomy that has been used to interpret apparent stomatal insensitivities and divergences in stomatal evolution (Brodribb & McAdam, 2011). The novel ABA responses and insights into CO₂ signalling demonstrated by Meigas *et al.* fill a crucial knowledge gap and elegantly bolster the mounting evidence that horsetail and other fern stomata are sensitive to CO₂, blue

light, red light, low humidity, and shade; often equally if not more 'active' than those of angiosperms (Fig. 1d; Doi *et al.*, 2015; Kübarsepp *et al.*, 2020; Cai *et al.*, 2021; Chater, 2021). Meigas *et al.* point out that horsetail stomatal responses are much slower than many angiosperms, however, like many earlier diverging lineages have far lower stomatal conductances, which may be a significant factor in detection and interpretation as well.

Absence of evidence is not evidence of absence

There have been increasing efforts over the past decade or so to unpick the origins of stomatal aperture control. Experimental evidence garnered from diverse species across the land plant phylogeny has increasingly shown stomatal responses to air humidity, light quality and quantity, CO₂ concentrations, and ABA signalling (Doi *et al.*, 2015; Hörak *et al.*, 2017; Grantz *et al.*, 2019; Kübarsepp *et al.*, 2020; Cai *et al.*, 2021; Plackett *et al.*, 2021; Wuyun *et al.*, 2023; Meigas *et al.*, 2024). These findings from whole plant, and guard cell, physiology could be interpreted as convergent properties of gas exchange control that arose independently across land plant evolution in response to universally shared environmental cues. However, the coupling of this physiological evidence with cross-phylum genomic, transcriptomic, and molecular analyses provides support that these active stomatal responses also share the same core genetic machinery (Clark *et al.*, 2022). The discovery of the intracellular signalling networks that operate in angiosperm guard cell movements, largely using insights from the Arabidopsis model, has provided a deeper mechanistic understanding of aperture control from genotype to phenotype. As the Equisetales diverged during the Carboniferous, and Equisetaceae in the Permian, this implies that their ABA signalling networks, and ABA-independent CO₂ and light signalling networks, were intact in the ancestors of horsetails long before *Equisetum*'s Triassic–Jurassic origin (Clark *et al.*, 2019). Any absence of stomatal ABA response that is species-specific, and not conditional, is therefore, a secondary loss (Clark *et al.*, 2022; Meigas *et al.*, 2024). Nevertheless, the diversity of stomatal responses and the oft-conflicting reports mean that many questions still remain. The new work by Meigas *et al.* exemplifies how little we still understand about the effects of seasons, microenvironments, and other conditional influences on plant development and physiology.

Tying up loose ends to the tail

The implications of their work go far beyond *Equisetum* or even fern stomatal signalling. Their findings also add more to the debate over the inter-relationship and cross-talk between stomatal ABA and CO₂ signalling, highlighting a need to address this question, usually studied in the context of angiosperm guard cells, through an evolutionary lens. Similarly, we are left pondering on the crossover between ABA and humidity signalling across land plants, and how very young plants respond to rapid changes in vapour pressure as they grow in an ever-changing environment. Plant plasticity remains a guarded secret and one that makes and breaks experimental biology. Although there is a greater awareness of


epigenetic effects and stomatal ‘stress memory’ among experimental plant scientists, we as a community can do more to understand, acknowledge, and investigate seasonality and environment in our experimental designs. There is a lot more that these ancient lineages can teach us.

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ORCID

Caspar C. C. Chater  <https://orcid.org/0000-0003-2058-2020>

Caspar C. C. Chater 

Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AE, UK;
Plants, Photosynthesis, Soil, School of Bioscience, University of
Sheffield, Western Bank, Sheffield, S10 2TN, UK
(email: c.chater@kew.org)

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