Patchy stomatal conductance: emergent collective behaviour of stomata

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Until recently, most scientists have tacitly assumed that individual stomata respond independently and similarly to stimuli, showing minor random variation in aperture and behaviour. This implies that stomatal behaviour should not depend on the scale of observation. However, it is now clear that these assumptions are often incorrect. Leaves frequently exhibit dramatic spatial and temporal heterogeneity in stomatal behaviour. This phenomenon, in which small ‘patches’ of stomata respond differently from those in adjacent regions of the leaf, is called ‘patchy stomatal conductance’. It appears to represent a hitherto unknown type of emergent collective behaviour that manifests itself in populations of stomata in intact leaves.
Background

Stomata are formed by two specialized epidermal cells, termed guard cells, which are shaped like kidney beans in most dicots and like dumbbells in most monocots. These cells dynamically regulate their concentration of osmotically active solutes to control turgor pressure and volume. As the turgor (and volume) of the guard cells increases, they bow apart to widen the pore. In general, stomatal responses are adapted to control the balance between carbon gain (CO₂ diffusion into the leaf) and water loss (H₂O diffusion out of the leaf), and they can also act to stave off dangerously low water potential in leaves and runaway xylem cavitation by limiting transpiration. To accomplish these tasks, stomata respond to several factors. First, guard cells respond directly to light intensity — increasing their solute concentration to close stomatal apertures as light intensity increases. They also respond to the concentration of CO₂ in the leaf, but in this case the relationship is reversed: high concentrations of CO₂ cause solute efflux and therefore reductions in aperture size. These two responses interact when light intensity is increased.

Guard cells also respond to the rate of water loss from the leaf, closing at high transpiration rates and low humidities. Finally, guard cells close in response to plant hormones, abscisic acid (ABA), which is synthesized in response to water stress and some other environmental stresses. The overall pore aperture under any given set of environmental conditions is a complex function of all these responses, and is made even more complex by circadian rhythms in stomatal sensitivity, delayed metabolism of ABA, and sensitivity to other plant hormones.

Appropriately, there has been substantial research into understanding the biochemical basis for changes in guard-cell solute concentration, and it is clear from these studies that individual guard-cell pairs do not require input from other guard cells to react to most environmental cues. Stomata do respond to some signals that originate in distant tissues; the most obvious example is ABA, which is synthesized in roots and mesophyll cells in response to water stress, and transported to guard cells in the transpiration stream. Other messengers that have also been postulated to coordinate stomatal aperture with photosynthetic capacity. Nevertheless, most stomatal responses seem to originate in guard cells, and require no input or interaction with surrounding epidermal tissue.

Given this focus on the individual guard cell as the functional unit for stomatal responses, the initial reports of ‘patchy stomatal closure’ were surprising. These studies indicated that water stress or applied ABA could cause stomata in discrete areas of the leaf to close substantially, whereas stomata in adjacent areas remained open8,9. Although variation in stomatal aperture over the leaf surface was known to exist before these studies, it was thought to be confined to broad gradients in aperture from one part of the leaf to another or simply small-scale random variation in apertures. Furthermore, all stomata on the leaf appeared to respond similarly to environmental stimuli. Stomatal apertures on a leaf could apparently be treated as a population, and gas-exchange measurements typically averaged stomatal conductance from whole-leaf gas-exchange data. However, microscopic studies of aperture can include only a few stomata at a time, and gas-exchange measurements typically average the responses of 10⁵ stomata or more. Thus, neither of these methods provides spatially explicit information at the intermediate scale where patchy stomatal conductance hides. It is therefore not surprising that patchy stomata were not described in the literature until recently, and trying to interpret measurements made at one scale in terms of processes at the other scale. A paradox emerged when leaf-level gas-exchange studies showed rapid and reversible changes in photosynthetic capacity after water stress or ABA treatments, while biochemical studies showed no effects. This inconsistency was resolved by questioning the familiar assumption that stomatal conductance is relatively uniform and normally distributed, and by recognizing that some patch distributions of stomatal conductance can produce artifacts in gas-exchange calculations8,10,11.

Patchiness makes measurements of stomatal behaviour highly scale-dependent. The summed effects of many patches with potentially different behaviours will determine the dynamics of whole-leaf stomatal conductance. For example, low-amplitude oscillations in whole-leaf stomatal conductance can reflect uniform behaviour (i.e. all stomata oscillating with low amplitude), or it can be caused by a few patches oscillating with large amplitudes or many patches oscillating slightly out of phase12–14. Whole-leaf stomatal conductance can even appear constant, in spite of large amplitude dynamics of some patches, if they are sufficiently out of phase with one another12,15. Patchy stomatal conductance can also affect seemingly leaf carbon–water balance16. These effects are usually detrimental, but they can be essentially neutral or even slightly beneficial under some environmental conditions. Interestingly, patchiness is most often reported under these environmental conditions.

The difficulty in observing stomatal movements at the scale between individual stomata and ABA-deficient mutants has prevented extensive study of stomatal patchiness, and even the most commonly employed techniques for studying the phenomenon have severe limitations. The earliest studies of patchy stomatal conductance used
iodine staining to detect spatial variation in starch concentrations caused by sustained differences in photosynthesis between patches\(^2\), or autoradiography to detect variation in photosynthetic CO\(_2\) uptake\(^3\). However, these methods are destructive, and the former obscures short-term dynamics in patch patterns because starch metabolism is a relatively slow process. Another approach for detecting patchiness involves vacuum infiltrating leaves with water or other liquids\(^4\). This technique works on the principle that surface tension prevents liquid from 'infiltrating' stomata that are nearly closed, but it yields only a binary result (infiltrated or not)\(^5\), and like starch staining and autoradiography, is destructive and static. Furthermore, it has recently been shown that differences in the infiltration of areoles do not necessarily imply differences in their average conductance\(^6\). Most current work on patchy stomatal conductance uses yet another and more technically challenging method. Images of chlorophyll fluorescence imaging; this method is technically difficult, and its use is limited to conditions where stomatal conductance has large and consistent effects on leaf temperature.

**Hydraulic interactions: a possible mechanism?**

Often overlooked in studies of stomatal responses is the effect of epidermal-cell turgor pressure on pore aperture. In contrast to the effect of guard cells, increases in epidermal turgor decrease pore aperture because they exert backpressure, which the guard cells must overcome in order to open the pore. For equal changes in the turgor pressure of both guard and epidermal cells, the epidermal cells have the upper hand; that is, if turgor is increased equally in both epidermal and guard cells, the pore will close slightly\(^7\). This leads to some interesting dynamics in stomatal behaviour. For example, when a leaf is subjected to a sudden decrease in ambient humidity, the transpiration rate increases and rapidly draws water from both the guard and epidermal cells. Turgor pressure drops quickly and roughly equally in both cells, and the pore opens initially. In the longer term (over the course of many minutes), guard cells reduce their turgor through a reduction in osmotic pressure, and this overcomes the initial opening to cause an overall reduction in pore aperture – consistent with the stomatal mandate to conserve water. In some cases, interactions between these two feedback loops can cause damped or sustained oscillations in stomatal apertures\(^2\).
It has been suggested that the initial positive-feedback phase of this loop might serve to coordinate stomatal movements\(^2\). If changes in the aperture of one pore can substantially affect the turgor pressure of epidermal cells that push inwards on adjacent stomata, then an increase in the aperture of one stoma (or several stomata) might trigger a slight increase in the aperture of adjacent stomata (Fig. 3). The feedback loop would thus intensify hydraulic coupling. These results suggest that stomata that experience no such perturbation\(^3\) will draw down the water potential in surrounding veins. This lag period is shorter and less variable under conditions of low air humidity, which should intensify hydraulic coupling. These results support the idea, a computer model of stomatal functioning that incorporated local hydraulic interactions and random variation in stomatal characteristics produced spatial patterns in stomatal conductance that resembled patchy stomatal conductance\(^4\). However, in the model, the temporal behaviour of stomata varied only slightly, and a considerable amount of time was required for the conductance of individual patches to diverge enough to produce a patchy pattern. Thus, other factors must be contributing to the dynamics of patches on a larger scale. It has been hypothesized that hydraulic interactions between areoles on a leaf might provide the missing piece to this puzzle\(^5,6\). These experiments showed that stomata that experienced constant environmental conditions responded to perturbations of adjacent stomata and that the responses were consistent with the hydraulic mechanism described above. More-recent data show that the turgor pressure of epidermal cells around the unperturbed stomata responds in a manner consistent with the hypothesis (K.A. Mott and P.J. Franks, unpublished). Other evidence for hydraulic coupling between stomata has come from studying the response of stomata in a single areole to a change from darkness to high light intensity levels\(^7,8\). After the light is increased it takes a while (up to \(~60\) min) before guard-cell turgor overcomes the epidermal backpressure, creating a lag before the pores open. This lag period is shorter and less variable under conditions of low air humidity, which should intensify hydraulic coupling. These results suggest that stomata with short lag times reduce epidermal turgor within the areole, thus hydraulically coercing their neighbours to open sooner.

Hydraulic interactions might serve to coordinate stomata within an areole or patch, but how does the behaviour of different patches on a leaf become uncoordinated? It is possible that random spatial variation in stomatal characteristics could translate into random differences between areoles in the average or dominant mode of stomatal behaviour. In support of this idea, a computer model of stomatal functioning that incorporated local hydraulic interactions and random variation in stomatal characteristics produced spatial patterns in stomatal conductance that resembled patchy stomatal conductance\(^9\). However, in the model, the temporal behaviour of areoles varied only slightly, and a considerable amount of time was required for the conductance of individual patches to diverge enough to produce a patchy pattern. Thus, other factors must be contributing to the dynamics of patches on a larger scale.

Complexity and predictability

Leaves, and on a larger scale, canopies, should operate like an ideal gas: an enormous assembly of independent units, varying randomly but uniform in basic properties; individually complex and unpredictable but collectively simple and predictable. Yet, to the dismay of scientists who wish to predict biotic responses at these more-complex levels, plants do not meet these requirements—they are not even close. Patchy stomatal conductance shows that the behaviour of things as seemingly elementary as a single leaf can be rather nonlinear and heterogeneous, and can defy dynamic prediction based on the
linear ‘ideal gas’ paradigm. Complex systems theory is a useful perspective for studying patchiness, because complexity is con-
cerned with ‘emergent collective properties’ – properties that result from interactions between small-scale units and cannot be predicted by the analysis of their isolated behaviours. These emergent properties can make observed relationships between variables dependent on the scale of measurement and interest or observation, but which are caused by the interaction of small- and large-scale dynamics. Any system of interacting parts lives on a continuum between two extremes: one where the components are totally independent, and here the system dynamics are independent of scale, and can be understood and predicted by linear mathematics. Any structure at intermediate scales is coincidental. At the other extreme, the com-
ponents interact so strongly that every change in one component is felt by all the others. In this case, the dynamics are also predictable on a large scale, but it is because the system is coordi-
nated rather than random and disjunct. Therefore a system that is too weakly connected will generate only uninteresting, scale-inde-
dependent randomness, whereas one that is too tightly constrained by

References


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