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Plant development

Signals from mature to new leaves

Stomata are microscopic pores on the surfaces of leaves, the number and density of which vary in response to changes in environmental conditions, such as carbon dioxide concentration and light. We show here that mature leaves of *Arabidopsis thaliana* detect and transmit this external information to new leaves of the same plant, producing an appropriate adjustment of stomatal development. As CO_2 concentration controls both stomatal opening[1] and number[2,3], and stomatal numbers also increase with higher light intensity[4], the large gradients of CO_2 and light found within plant communities[5] have the potential to influence stomatal development.

Every year, 40% of the CO_2 in the atmosphere passes through stomata[6]. Stomatal numbers modify both photosynthesis and efficient use of water[1], and so any change in stomatal numbers[7] in response to CO_2 and light can influence photosynthesis and atmospheric CO_2 concentration. The response mechanism described here may exert global effects that are not currently included in canopy and vegetation models of increasing atmospheric CO_2 levels.

A genetic component in the response of stomatal development to increasing atmospheric CO_2 concentration has been identified[8] and the control of stomatal development by CO_2 concentration is known to occur during early leaf development[9,10], when ambient CO_2 concentrations may not be accurately detectable by a new leaf sheathed by antecedent leaf primordia[11]. We therefore tested whether CO_2 concentration can be detected by mature leaves in open ambient conditions, which might then transmit a signal to induce an appropriate developmental response by the stomata of new leaves. Our experimental design using the model plant *Arabidopsis thaliana* is shown in Fig. 1a.

Expanding leaves outside the cuvette (ambient CO_2, 360 p.p.m.), with mature leaves exposed to a high concentration of CO_2 inside the cuvette (720 p.p.m.; Fig. 1a), developed with a reduced stomatal index and density (Fig. 1b, left) compared with control plants grown entirely at ambient CO_2. Reversing the cuvette arrangement so that the mature leaves were exposed to 360 p.p.m. and the expanding leaves to 720 p.p.m. CO_2 resulted in a complete reversal (increase) of the stomatal index and density of the new leaves (Fig. 1b, right).

In these experiments, both abaxial (upper) and adaxial (lower) leaf surfaces responded in a similar manner, indicating that CO_2 concentration is detected by mature leaves which signal to expanding leaves to induce an appropriate developmental response. To our knowledge, this is the first demonstration that mature leaves both detect CO_2 concentration and transmit a long-distance signal that controls stomatal development in young leaves. Expanding leaves appeared to have no capacity to detect ambient CO_2 concentration or to respond to it directly by altering stomatal initiation; no cuvette effect was found to contribute to the responses.

This mechanism of CO_2 detection and signalling could enhance or optimize performance in plant communities. For example, the reduction in stomatal index and density with CO_2 enrichment enhances water-use efficiency[3,12], but such a response will be much less effective[13] when leaves develop in the shade of other plants. We therefore tested whether the mechanisms controlling the response of stomatal initiation to CO_2 might also be accompanied by another that responds to light, reducing the initiation of stomata with increasing shade.

We have previously shown that there is a positive relation in *Arabidopsis* between irradiance and both stomatal index and density. Fully grown leaves were now placed in shaded light, with the expanding leaves under full light. The stomatal index (Fig. 1c) and density (results not shown) of new leaves were reduced, as if they had developed under shade conditions. We conclude that long-distance signalling must also be involved in controlling the response of stomatal development to light, which supports the idea that there is an ecologically important link between the responses evoked by light and by carbon dioxide.

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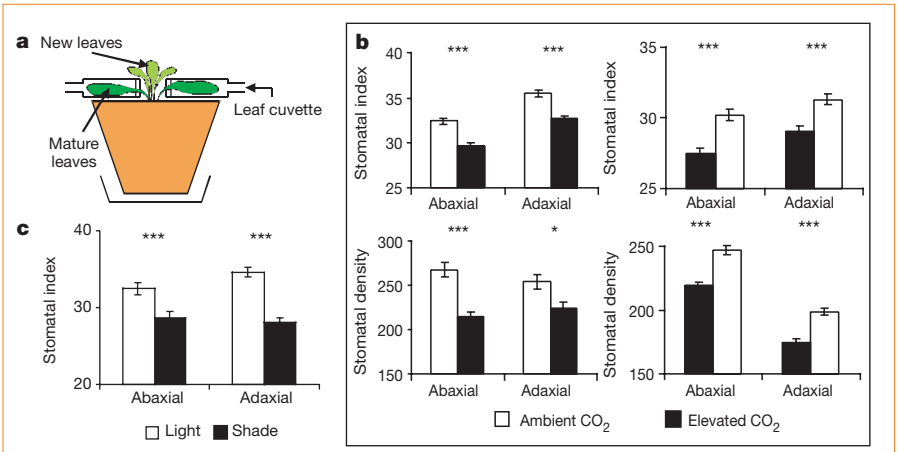

Figure 1 Mature leaves detect changes in CO_2 concentration and elicit a stomatal response in developing leaves. **a,** Leaf-cuvette experiment. Plants of *Arabidopsis* (Columbia, Col-0) were grown for 4 weeks under ambient CO_2 (360 p.p.m.) until leaf insertions 5 to 13 had developed. These mature leaves were enclosed in transparent airtight cuvettes under CO_2 concentrations of either 720 or 360 p.p.m. Subsequent leaf insertions developed outside the cuvette under ambient CO_2. Plants were maintained in cuvettes for 7 to 9 days until the next five leaf insertions had matured, the last three of which were investigated for stomatal density (no. of stomata per mm^2) and index ((no. of stomata/no. of stomata + no.of epidermal cells) × 100). **b,** (left) Stomatal index and density for new leaves (insertions 16 to 19) under ambient CO_2 when mature leaves (insertions 5 to 13) inside cuvettes are supplied with increased CO_2 (720 p.p.m.). Both stomatal density and index are reduced in new leaves if the supply of CO_2 is increased to the mature leaves. Right, reverse experiment: mature leaves inside cuvettes are under CO_2 at 360 p.p.m.; external CO_2 is 720 p.p.m. Stomatal density and index increase in response to the decreased CO_2 around the mature leaves. **c,** Effect on stomatal index of new leaves of reducing light incident on mature leaves by using neutral density filters (shade) or transparent filters (full light). Stomatal index of new leaves is reduced when mature leaves are shaded. ***$P < 0.0005$; *$P < 0.05$; bars, s.e.m.; $n = 150$.

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