

Carbon isotope abundance and discrimination in plant studies: an idiosyncratic explanation of how discrimination works, and how it illuminates plant performance, and some novel simple formulae for plant responses to the environment

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CO₂ enters a leaf through the stomatal pores and then is fixed biochemically into sugar phosphates. In the first step, diffusion discriminates against the heavier and therefore more slowly moving heavy CO₂ containing ¹³C. The carboxylation enzyme also discriminates against heavy CO₂. (There are also some discrimination steps in CO₂ dissolving in cell water, etc., but these are small in net effect; O'Leary, 1981; Farquhar *et al.*, 1989.) The degree of discrimination depends on the balance of diffusive (stomatal) vs. biochemical limitation, and it therefore informs us about the physiological operating point of the leaf. Farquhar and coworkers (Francey and Farquhar, 1982; Farquhar *et al.*, 1982; Farquhar and Richards, 1984; Farquhar *et al.*, 1989) derived a widely-used formula for the discrimination against ¹³C in photosynthesis by terrestrial plants with stomata:

$$\Delta = 4.4\text{‰} + 22.6\text{‰} \frac{C_i}{C_a} \quad (1)$$

This formula applies to plants with the C₃ photosynthetic pathway; we'll discuss C₄ plants later. In the formula, C_a is the CO₂ partial pressure outside the leaf, in ambient air, and C_i is the CO₂ partial pressure inside the leaf, in the stomatal chamber. CO₂ passes through the diffusional resistance of the stomata, declining to a partial pressure equal to C_i. Diffusion discriminates against the slower-moving CO₂ containing ¹³C, with the magnitude of 4.4 parts per thousand, or 4.4‰; that is, diffusion of ¹³CO₂ is slower by a factor 1-0.0044 than is diffusion of ¹²CO₂. That explains the first part of Eq. (1)'s right-hand side. Then, CO₂ reacts with RuBP at the Rubisco enzyme, which discriminates against ¹³C with a magnitude of 27‰. One may ask several questions about Eq. (1): 1) Why is the coefficient of the last term 22.6‰, not 27‰?; 2) Why does discrimination decrease when diffusion is a more pronounced limitation, that is, when C_i/C_a is smaller?; 3) Where does the discrimination against ¹³C originate in the Rubisco enzyme? It is a different kind of effect than the diffusional discrimination, which is more intuitive to us; 4) This equation is used to interpret stomatal limitation to photosynthesis and, more significantly, water-use efficiency, WUE. How is discrimination related to WUE?

Part 1: Monster derivation of Eq. (1) from the separate discrimination factors of diffusion and biochemical reaction, plus the ratio of C_i/C_a

Let's derive Eq. (1) from consideration of the separate fluxes of ¹³CO₂ into the leaf via photosynthesis (J₁₃) and of ¹²CO₂ (J₁₂). We will express each flux two ways: first, as the diffusive flux through the stomatal resistance, and, second, as a rate of incorporation into sugar phosphates by Rubisco and the rest of the biochemical machinery. The flux of either compound through the stomatal resistance, r, is just the difference in partial pressure of that compound from outside air

to inside air, divided by the resistance. For $^{13}\text{CO}_2$, the partial pressure in outside air is the partial pressure of all CO_2 , multiplied by the fraction of ^{13}C in that CO_2 , which is usually denoted as R_a . (Recall that the isotope ratio is also described by δ , which is the ratio in air, divided by the ratio in a standard material, minus 1: $\delta = R/R_s - 1$, and that this is commonly expressed in parts per thousand, or "mil.") Let's denote this fraction simply as R_a , with "a" denoting "air." We'll use R_i for the fraction of CO_2 with ^{13}C in the air inside of the leaf. Recall that the diffusive resistance for the heavy CO_2 with ^{13}C is bigger by a small amount, 4.4 parts per thousand, than it is for light CO_2 . Call this small amount ε , so that the resistance for $^{13}\text{CO}_2$ is $r(1+\varepsilon)$. Then, we have

$$J_{13} = \frac{(R_a C_a - R_i C_i)}{r(1 + \varepsilon)} \quad (2)$$

and

$$J_{12} = \frac{([1 - R_a]C_a - [1 - R_i]C_i)}{r} \quad (3)$$

Let's now express the rates in terms of the biochemical (carboxylation) reaction. This has a slightly complex form, but we can approximate it closely by saying that the rate of reaction for each type of CO_2 is a rate constant multiplied by the simple concentration (partial pressure) of that kind of CO_2 . The rate constant for the heavy CO_2 will be lower, by a factor we will denote as $(1-\gamma)$; the value of γ is 0.027, or 27‰. We can then write

$$J_{13} = (1 - \gamma)k_c R_i C_i \quad (4)$$

and

$$J_{12} = k_c(1 - R_i)C_i \quad (5)$$

Now let's combine equations so we can solve for R_i in terms of R_a , as well as the partial pressures and the discrimination factors ε and γ .

$$J_{13} = \frac{(R_a C_a - R_i C_i)}{r(1 + \varepsilon)} = (1 - \gamma)k_c R_i C_i \quad (6)$$

and

$$J_{12} = \frac{([1 - R_a]C_a - [1 - R_i]C_i)}{r} = k_c(1 - R_i)C_i \quad (7)$$

We'll equate the middle and right-hand sides of Eq. (6), at the same time moving $r(1 + \varepsilon)$ to the right-hand side:

$$R_a C_a - R_i C_i = r(1 + \varepsilon)(1 - \gamma)k_c R_i C_i \quad (8)$$

and now express $k_c C_i$ in terms of Eq. (7):

$$k_c C_i = \frac{([1 - R_a]C_a - [1 - R_i]C_i)}{r(1 - R_i)} \quad (9)$$

Combining Eqs. (8) and (9), we get

$$R_a C_a - R_i C_i = \frac{(1 + \varepsilon)(1 - \gamma)}{(1 - R_i)} ([1 - R_a]C_a - [1 - R_i]C_i) \quad (10)$$

What an algebraic mess! One more little manipulation: let's bring the factor $(1 - R_i)$ over to the

left-hand side, so there are no denominators:

$$(1 - R_i)(R_a C_a - R_i C_i) = (1 + \varepsilon)(1 - \gamma)([1 - R_a]C_a - [1 - R_i]C_i) \quad (11)$$

What's the use of all this? Now we separate the terms by order of smallness. We can multiply out all these terms and keep only those terms that are proportional to R_a or R_i . On the left-hand side, there are only two terms, $R_a C_a - R_i C_i$; the terms coming from multiplying these by $-R_i$ are second-order in the R 's, so we ignore them for now. On the right-hand side, we have a factor of R_i already multiplying everything, so we can only keep the highest-order terms in everything else - no ε , no γ , no other R 's. This leaves us with $R_i(C_a - C_i)$. For the part from the left-hand side to equal the part on the right-hand side, we have

$$R_a(C_a - C_i) = R_i(C_a - C_i) \quad (12)$$

with the obvious solution that $R_i = R_a$...there is no discrimination to first order.

Now let's also keep all the terms that are second order, involving products of R 's with each other or R with either ε or γ . You can verify the algebra that gives us this:

$$\begin{aligned} R_a C_a - R_i C_i - R_i R_a C_a + R_i^2 C_i \\ = (1 + \varepsilon - \gamma)(R_i(C_a - C_i) - R_i R_a(C_a - C_i)) \end{aligned} \quad (13)$$

All the other products are third-order and higher. Several terms cancel because they appear on both sides of the equation, and we are left with

$$R_a C_a - R_i C_i = (\varepsilon - \gamma)(C_a - C_i) \quad (14)$$

or, moving the $R_i C_i$ to the right side and seeing that R_i is a factor in everything, we get

$$R_a C_a = R_i(C_a(1 + \varepsilon - \gamma) - (\varepsilon - \gamma)C_i) \quad (15)$$

Let's divide out C_a , and then bring R_i to the left side:

$$R_i = \frac{R_a}{[1 + \varepsilon - \gamma] - (\varepsilon - \gamma) \frac{C_i}{C_a}} \quad (16)$$

This looks like R_a divided by 1 plus a small quantity. To a close approximation, $1/(1+x)$, where x is small, is just $1-x$, so we can write

$$R_i = R_a[1 - (\varepsilon - \gamma) + (\varepsilon - \gamma) \frac{C_i}{C_a}] \quad (17)$$

This has the right limit. If the stomata posed no resistance, so that $C_i = C_a$, we would have $R_i = R_a$ - that is, no discrimination through the stomata.

We're not directly interested in R_i , which is essentially impossible to measure. We want to know the isotopic composition (and discrimination) in the final sugars that are made, therefore, in plant tissue. This we'll call R_f . Now, R_f and $(1 - R_f)$ are in the same proportion as the uptake (carboxylation) rates of heavy and light CO_2 , so we can write this ratio $R_f / (1 - R_f)$ as the ratio of carboxylation rates expressed in Eqs. (6) and (7). This will let us relate R_f to R_i that appears in those two equations:

$$\frac{R_f}{1 - R_f} = \frac{(1 - \gamma)k_c C_i}{k_c(1 - R_i)C_i} \quad (18)$$

We can divide out the common factor of $k_c C_i$ on the right to get

$$\frac{R_f}{1 - R_f} = \frac{(1 - \gamma)R_i}{1 - R_i} \quad (19)$$

How do we solve this without getting a quadratic mess? Again noting that $1/(1-x)$ looks like $1+x$, we can closely approximate Eq. (19) as

$$R_f(1 + R_f) = (1 - \gamma)R_i(1 + R_i) \quad (20)$$

Keeping only the terms to lowest order in R and γ , we get

$$R_f \approx (1 - \gamma)R_i \quad (21)$$

Now we can rewrite R_i in this equation, using Eq. (17), to get

$$R_f = (1 - \gamma)R_a(1 + (\varepsilon - \gamma) + (\varepsilon - \gamma) \frac{C_i}{C_a}) \quad (22)$$

and keeping only the lowest-order terms, we get

$$R_f = R_a(1 - \varepsilon + (\varepsilon - \gamma) \frac{C_i}{C_a}) \quad (23)$$

We like to take this to the net discrimination,

$$\Delta^{13}C = \frac{\delta_a - \delta_f}{1 - \delta_f} \quad (24)$$

so that it expresses only the plant's part in isotopic composition, factoring out the variable isotopic composition of the air, δ_a ; that is, it expresses plant control of CO_2 uptake and all the rest. First, we convert from isotopic fraction, R_f , to the delta value, using $\delta_f = R_f/R_s - 1$, with R_s the isotopic fraction in the global standard material. We do similarly for air, to get δ_a . We then get

$$\begin{aligned} \delta_f &= \frac{R_f}{R_s} - 1 = \frac{R_a}{R_s} (1 - \varepsilon + (\varepsilon - \gamma) \frac{C_i}{C_a}) - 1 \\ &= (\delta_a + 1)(1 - \varepsilon - (\gamma - \varepsilon) \frac{C_i}{C_a}) - 1 \end{aligned} \quad (25)$$

Then, the discrimination by the plant is very closely just $\delta_f - \delta_a$, or

$$\begin{aligned} \Delta^{13}C &= \delta_a - [\delta_a - \varepsilon + (\varepsilon - \gamma) \frac{C_i}{C_a}] \\ &= \varepsilon + (\gamma - \varepsilon) \frac{C_i}{C_a} \end{aligned} \quad (26)$$

We know (from theory and measurement) that ε is 4.4‰ and γ is 27‰, so we recover Eq. (1), with the coefficient of C_i/C_a being 27‰ - 4.4‰ = 22.6‰. Whew! Now we've answered question number 1.

Part 2. Why does discrimination decrease when stomata close down?

One might expect that the discrimination from diffusion always is present, giving us the base value of 4.4‰ in δ_i , and this is true, as the formulae of Eqs. (1) and (26) indicate. Why, however, does the discrimination by the carboxylation enzyme decrease when stomata close down and cause C_a/C_a to decrease? True, diffusion through stomata may look like the most-limiting process of transport, but don't the processes of diffusion and carboxylation occur in series, so that the discrimination should be compounded from both processes? For some insight into this phenomenon, one may inspect Eq. (17). Plugging in the numerical values of ε and γ , one obtains

$$R_i = R_a \left[1 + 22.6 \text{‰} \left(1 - \frac{C_i}{C_a} \right) \right] \quad (27)$$

Note that, as C_i declines, R_i tends to a limiting value that is a factor of $(1 + 22.6 \text{‰})$ greater than R_a ; the CO_2 in the chamber interior to the stomata is actually enriched in ^{13}C ! What is happening is that the ^{13}C is being rejected by the carboxylating enzyme and is building up.

A way to visualize this is to consider the simple resistance model of CO_2 flow from the outside air through the stomata and then "through" the biochemistry.

C_a -----> C_i -----> 0
 Rate $(C_a - C_i)/r$ $k_c C_i$
 We can solve for C_i by equating the two rates:

$$\begin{aligned} (C_a - C_i) \frac{1}{r} &= k_c C_i & (28) \\ \text{---} > C_a \frac{1}{r} &= C_i \left(\frac{1}{r} + k_c \right) \\ \text{---} > C_i &= \left(\frac{\frac{1}{r}}{\frac{1}{r} + k_c} \right) \end{aligned}$$

When the stomatal resistance becomes large, the term $1/r$ in the denominator becomes negligible and we have

$$C_i \approx \frac{1}{rk_c} C_a \quad (29)$$

Now, each of the forms of CO_2 , light and heavy, reaches its value of C_i independently. We can use the form above for light CO_2 . For $^{13}\text{CO}_2$, we have to modify both r and k_c : r is increased by the factor $(1 + \varepsilon)$ and k_c is decreased by the factor $(1 - \gamma)$, so we have

$$^{13}C_i = \frac{1}{(1 + \varepsilon)(1 - \gamma)rk_c} C_a \quad (30)$$

The ratio of the two C_i values is the new mole fraction of $^{13}\text{CO}_2$ in the interior space, R_i :

$$\begin{aligned}
R_i &= \frac{{}^{13}C_i}{{}^{12}C_i} = \frac{C_a/[(1 + \varepsilon)(1 - \gamma)rk_c]}{C_a/(rk_c)} & (31) \\
&= \frac{1}{(1 + \varepsilon)(1 - \gamma)} \\
&\approx (1 - \varepsilon)(1 + \gamma) \approx 1 + \gamma - \varepsilon \approx 1 + 22.6\text{‰}
\end{aligned}$$

Yes, it is enriched.

Part 3. Why is there discrimination against heavy CO₂ by the carboxylating enzyme, Rubisco?

The answer lies in quantum mechanics, of how light vs. heavy molecules vibrate. For small displacements of the atoms (the nuclei), the atoms act as if bound by ideal springs. The vibrations are those of a harmonic oscillator, like a clock pendulum. The frequency of vibration, ν , is proportional to 1 over the square root of the mass, so that the ¹³C in ¹³CO₂ vibrates more slowly, by a factor of about 4%. Now, quantum mechanics states that vibrations are quantized, only occurring at discrete amplitudes and, thus, at discrete energies, closely equal to $(n + 1/2)$ ($n = 1, 2, 3, \dots$) times $h\nu$. Here, h is Planck's constant, $h = 6.62 \times 10^{-34}$ J s. Thus, the heavy CO₂ molecule, vibrating at a lower frequency, is at a lower energy in its ground state. It's not much lower, but compared with typical thermal energies, it is mildly significant. If the vibrational energy of the heavy molecule is lower by about 3% of typical thermal energies, kT , then the occurrence of sufficient thermal energies is lower by a factor $\exp(-0.03 kT / kT) = 0.97 = 1 - 0.03$. This is about the factor by which reaction of heavy CO₂ is reduced.

Part 4. How is isotope discrimination related to water-use efficiency?

Water-use efficiency, WUE, is the ratio of carbon assimilation to water loss, and it can be defined on many levels: the instantaneous exchange of CO₂ for water vapor by a leaf, or the final carbon accumulation of the plant relative to total water loss by transpiration *and* evaporation from soil, etc. Let's look at the first case, which is the simplest:

$$WUE_i = A/E \quad (32)$$

Consider CO₂ entering a leaf and water vapor leaving with only the stomatal resistance occurring between the outside air and the interior of the leaf. That is, we will ignore the (often, but not always) small resistance of the boundary layer, which arises from the slowing of airflow by air "sticking" to the leaf along the surface. In this case, we can use our formula for photosynthetic carbon gain (call it A) above Eq. (28):

$$A = g_s'(C_a - C_i) \quad (33)$$

where I've changed from using resistance to using conductance, g_s' . The prime indicates that this conductance is for CO₂; usually, the unprimed symbol, g_s , is reserved for conductance for water vapor, which is simply 1.6 times larger.

The rate of water loss, or transpiration, E , is again a conductance times a difference in partial pressures, this time being those for water vapor. Let's call the water-vapor partial pressure in

the external air e_a and that inside the leaf e_i . Then we have

$$E = g_s(e_i - e_a) \quad (34)$$

The order, inside \rightarrow outside, is reversed from that for CO_2 , of course. We can then write

$$\text{WUE}_i = \frac{g_s'(C_a - C_i)}{g_s(e_i - e_a)} \quad (35)$$

We can use the fact that $g_s'/g_s = 1/1.6 = 0.62$ to write

$$\text{WUE}_i = \frac{0.62C_a(1 - C_i/C_a)}{(e_i - e_a)} \quad (36)$$

This form is very convenient, especially for comparing leaves that differ in their physiology (which controls the value of C_i) but share the same environment - that is, the same C_a , the same water vapor content of the air e_a , and (perhaps) the same internal water vapor content e_i . In this case, the differences in C_i/C_a directly cause differences in WUE. Example: a typical unstressed plant with the common C_3 photosynthetic pathway has C_i/C_a near 0.7. The factor $1 - C_i/C_a$ equals 0.3. A stressed plant may have $C_i/C_a = 0.5$, so that the factor $1 - C_i/C_a$ is now 0.5. The increase in WUE is by the factor $0.5/0.3 = 1.67$, a 67% gain in WUE.

Why don't we just measure A and E for various plants and compute their respective magnitudes of WUE? To get C_i/C_a , we have to sample plant tissue and use a mass spec. Well, the problem is that A and E change all the time, over the day, over the season, and between leaves of the same plant. A number of researchers (Farquhar *et al.*, 1989), including us, found this out, to our consternation after making hundreds or thousands of direct gas-exchange measurements. It is better, to get a picture of long-term WUE relevant to crop yield or wild plant fitness, to measure C_i/C_a with the mass spec, inferring it from Eq. (1). This relation of isotope discrimination to WUE has been used for many purposes - crop breeding, inferring stress history in long-lived trees (and therefore inferring dry times in climate history; e.g., Leavitt and Long, 1989), etc. We'll discuss as many examples as is practical in class.

The picture is not quite so neat as this. We can be assured that e_a is a given in the environment. On the other hand, e_i is purely a function of the state of the leaf. For high leaf water potentials, it is simply the saturated vapor pressure, e_{sat} , at the temperature of the leaf, a pressure that rises nearly exponentially between freezing and boiling points (at boiling, of course, it equals total ambient air pressure). There are good approximations such as

$$e_{\text{sat}} \approx 610.8 \text{ Pa} \exp\left(\frac{17.269 T}{(237.2 + T)}\right) \quad (37)$$

with T in degrees Celsius. If the leaf has a significantly low water potential, ψ , this decreases by a factor $\exp(\psi V_w/RT)$, where V_w is the molar volume of water, $18 \times 10^{-6} \text{ m}^3$ per mol. Example: if the water potential is -1 MPa (-10 bars, high stress for a crop plant and "just coasting" for a desert shrub), this factor is $\exp(-18/2500)$, or about 0.993 when the absolute temperature, T, is about 300 K (27°C). This is not a big factor, then, in most cases.

The temperature of the leaf depends upon the balance of energy inputs and outputs -

interception of solar shortwave radiation and terrestrial thermal radiation as inputs; emission of thermal radiation, cooling by transpiration, and cooling by transfer of heat from leaf to air as outputs. Commonly, we want to compare WUE between two leaves (of two genotypes, say) in the same environment. Suppose the leaves have the same photosynthetic capacity but differ in stomatal conductance. We will shortly see how this difference in g_s changes transpiration relatively much more than it changes photosynthesis - thus, it can change WUE (if the relative changes in A and E were the same, then WUE would not change). To go back to the thread of the argument: two plants can differ in C_i because they have different g_s and thus they cool to different amounts - they have different T 's, and different e_i , so we can't immediately say how they differ in WUE.

Wait a minute: didn't g_s cancel out in getting to Eq. (36)? Yes, but g_s still enters implicitly in Eq. (36), in helping to determine C_i . Go back to Eq. (28) and rewrite it in terms of g_s :

$$\begin{aligned} C_i &= \frac{g_s'}{(g_s' + k_c)} C_a \\ &= \frac{1}{(1 + k_c/g_s')} C_a \end{aligned} \quad (38)$$

It is the ratio of photosynthetic (carboxylation) capacity, as measured by k_c , to g_s that determines C_i/C_a . Decreasing g_s , as when a plant responds to water stress, decreases C_i/C_a and increases WUE. (It also decreases A, but not nearly as much. If k_c/g_s' started out at 1/3, so that $C_i/C_a = 1/(1+1/3) = 3/4$, then a 20% decrease in g_s changes C_i/C_a to $1/(1 + 1/[3/0.8]) = 0.706$. The factor $1 - C_i/C_a$ increases from 0.25 to 0.294, a gain of 18%. In contrast, A changes by the same relative factor as does C_i - a drop from 0.75 to 0.706, which is a decrease of only 6%.) Similarly, increasing photosynthetic capacity at constant g_s increases WUE.

Changes in C_i/C_a in unstressed conditions are actually not very pronounced. It is commonly observed (starting with classic experiments of Wong *et al.*, 1985a,b,c) that maximal g_s and k_c pace each other. Plants with greater mineral nutrition (related to photosynthetic capacity) typically develop more and/or larger stomata, so that k_c/g_s stays about constant. The same trends occur during development of a leaf or between different leaves on the same plant, or with growth in high or low light, etc. There are good evolutionary reasons, in that the values of photosynthesis and WUE are balanced about the same in many different conditions of growth and competition.

These regularities are not absolute. There are differences between genotypes within a species in the unstressed values of C_i/C_a and thus in both photosynthesis and WUE. These are exploited in crop breeding, for field crops (e.g., Condon and Richards, 1990; Hall *et al.*, 1990; Wright *et al.*, 1993; Gutschick and Currier, 1992) or even range grasses (Read *et al.*, 1991). Some of these studies demonstrate nicely that raw photosynthetic capacity (high ^{13}C discrimination) contributes best to yield when water is in ample supply but WUE (low discrimination) contributes best when water is very limiting (Condon and Richards, 1993). ^{13}C discrimination has even been used to track quantitative trait loci (QTLs) contributing to water-use efficiency, in beautiful marriages of genetics and biophysics (Martin and Thorstenson, 1988; Brendel *et al.*, 2002). It might be noted that there is less attention in recent times to WUE in crop breeding (or

so is my impression). One reason that yield / performance traits actually take a back seat - a distant back seat - to breeding for pest and disease resistance; the latter efforts are estimated to take about 95% of crop breeders' time (Gutschick, 1987, p. 173).

Variations in discrimination and thus in WUE have also been observed in wild populations and have been seen on occasion to have major ecological consequences (Ehleringer, 1993; Sandquist and Ehleringer, 2003). We'll discuss these in a review framework, below.

Now, aren't you glad you went through all the math, at least once? No one can pull any fast ones on you, in interpreting isotopic measurements. However, there will be a short quiz; please get out your exam books and pencils.

Sequelae: other uses of ^{13}C discrimination studies in plant physiology, ecology, climatology, etc.

This will be a brief survey.

A. Determining photosynthetic pathways, and contributions of various photosynthetic activities, from leaf to ecosystems.

Plants with the C_4 pathway have a biochemical pump; CO_2 is taken up first by PEP carboxylase, as the start of a shuttle to cells inside a sheath around leaf veins, where the CO_2 is released in other enzymatic reactions. With this pump, CO_2 can be drawn down to very low levels in the leaf (C_i is very low) while the flux of CO_2 into photosynthesis is very high (PEP carboxylase has a high affinity and high catalytic rate compared to Rubisco; also, the CO_2 release inside the bundle sheath cells builds up CO_2 concentrations to levels much higher than in outside air, so that the Rubisco in these cells, which do the final photosynthesis into sugars, operates near saturation). Because C_i is so low, WUE is high. Because the CO_2 partial pressure near Rubisco is so high, less Rubisco is needed for the same photosynthetic rate: the efficiency of using nitrogen is high.

The low value of C_i/C_a translates to a low discrimination against ^{13}C . Thus, the isotopic ratio in plant tissue is a very strong indicator of photosynthetic pathway (see, *e.g.*, Griffiths, 1991), and, consequently, of ecological status. One can also find C_3 - C_4 intermediate plants, with isotopic signature being a first indicator (von Caemmerer, 1989); this helps track evolution in progress. One needs a model of the biochemistry of C_4 photosynthesis (with variable perfection, hence, variable leakage of CO_2 back out of the bundle sheath cells) to do this (*ibid.*). The leakage also varies with stress, such as salinity, also detectable by the discrimination vs. ^{13}C (Bowman *et al.*, 1989).

The isotopic signal is usable on larger scales of space and time. The $\delta^{13}\text{C}$ value of soil carbon can tell us the fraction of primary production by C_4 vs. C_3 plants (*e.g.*, McPherson *et al.*, 1993; Wedin *et al.*, 1995; Monger *et al.*, 1998), and the history of that contribution. It can help

trace the evolution of C₄ photosynthesis, a large and fascinating topic in itself (Ehleringer *et al.*, 1991, 1997). The fraction of C₄ plants in the diet of animals can often be discerned, from the isotopic signal of mixed carbonates in tooth material (*e.g.*, Smith *et al.*, 2002). Besides illuminating modern diets, this information can lead to interesting inferences about large-scale evolution. It has been proposed that horses native to North America died off when C₄ grasses evolved. Being more abrasive because of their silica bodies, they wore down the teeth of horses prematurely and notably reduced their Darwinian fitness (Wang *et al.*, 1994; MacFadden *et al.*, 1999).

Because the discrimination in C₄ plants varies relatively little, their isotopic signature is often used to estimate air $\delta^{13}\text{C}$ (Pedicino *et al.*, 2002). The $\delta^{13}\text{C}$ of air has in the past century undergone a continuous and accelerating shift because of additions of CO₂ from fossil fuels (*ibid.*). Consequently, air composition at the time that a plant sample is taken is needed in order to interpret $\delta^{13}\text{C}$ of C₃ plants as indicators of stress and other conditions (see B, below). On global scales, air trapped in ice cores can be used to estimate air $\delta^{13}\text{C}$ (Francey *et al.*, 1999), but there can be local variations in air composition, as in understories where respired CO₂ mixes in strongly; then, the plant samples are useful. Similarly, when one cannot date the other (C₃) material and thus cannot use the ice-core air samples, the co-occurring C₄ material is useful.

Back to the scale of the single plant, one can use isotopic composition changes to help determine the sources of carbon for new growth. New leaves import carbon from photosynthesis done by older leaves, or even now-dead leaves. One can distinguish current from old sources if one can measure sugars separately. Such methods have been developed (Brugnoli *et al.*, 1988).

One can measure carbon (and other) isotopes in free air above an ecosystem. This can inform us about the isotopic composition of respired air. At any one time, we don't know how much CO₂ was respired, so we can't estimate its isotopic composition - we would see the same shift in $\delta^{13}\text{C}$ from an amount x of respired CO₂ that is, say, 4‰ different from the air and from an amount $2x$ that is only 2‰ different. The way to get the information is to plot the value of $\delta^{13}\text{C}$ against $1/C_a$. The intercept (that is, when all the CO₂ comes from respiration) gives us the $\delta^{13}\text{C}$ of respired CO₂. This, in turn, can tell us if the respired carbon had been photosynthesized by C₃ or C₄ plants, or a mixture. Pataki *et al.* (2003) give a lot of theory and experimental methodology.

B. Back to C₃ plants: discrimination as an indicator of the environment: water stress, humidity, salinity, etc.

I've already mentioned that water stress commonly induces a decrease in stomatal conductance to increase WUE, and that this response is reflected in a lowering of discrimination against ¹³C, markedly so in C₃ species. This response can distinguish phreatophytes that can experience a lesser stress than other plants growing in the same area (Anderson *et al.*, 1996). The response can also indicate salt stress in C₃ plants (Flanagan and Jeffries, 1989; Guy *et al.*, 1989). It can also indicate long-term acclimation (or even genetic adaptation) to water stress (Hamerlynck *et al.*, 2004). The latter point is interesting: in short-term stress, one expects that stomatal responses are fast while photosynthetic capacity changes only slowly, if at all. One then expects

a decrease in ^{13}C , an increase in WUE, and a decrease in $\Delta^{13}\text{C}$. In the long term, however, a stressed plant likely will decrease its photosynthetic capacity per leaf area, returning the ratio of k_c / γ toward that for other plants. In fact, in our repeatedly stressed desert shrubs, we see C_i/C_a values that are very similar to those of mesic plants (Gutschick *et al.*, unpubl.; also, the differences in $\Delta^{13}\text{C}$ seen by Hamerlynck *et al.* between plants differing in chronic stress are smaller than those between unstressed and acutely stressed plants. They amount to no more than 2‰, corresponding to a change in C_i/C_a of a bit less than 0.1).

Beyond phenotypic acclimation in a fixed genotype, plants can show genetic adaptation, including divergence among populations in $\Delta^{13}\text{C}$ and thus in WUE. Ehleringer (1993) found two such different populations of a sub-shrub, *Encelia farinosa*, in Death Valley. The high-WUE populations grew more slowly overall but had essentially no mortality in a once-in-20-year severe drought, while the higher-photosynthesis / lower-WUE population suffered a 46% mortality. In subsequent studies, Sandquist and Ehleringer (2003) demonstrated a genetic basis for the differences in discrimination and WUE.

Speaking of acclimation to long-term stresses, we should discuss how plant performance, and the isotopic signature thereof, varies with precipitation, atmospheric humidity, elevation (air pressure), and rising atmospheric CO_2 . Let's consider humidity first. Stomata respond to humidity in a positive fashion: the higher the relative humidity, the greater the stomatal conductance. This is adaptive because higher humidity means a smaller vapor-pressure deficit, $e_i - e_a$ (recall Eq. 34), and improved WUE (Eq. 35). All else equal, stomata should be open more during times of high relative humidity and more photosynthesis should be done then. A quantitative theory for this was propounded decades ago by Cowan and Farquhar (1977; yes, Graham has his finger in every pie...but he did start out as a nuclear physicist). The response is captured in a robust empirical model of stomatal conductance, the Ball-Berry model (Ball *et al.*, 1987 and hundreds of citations thereafter):

$$g_s = m \frac{A h_s}{C_s} + b \quad (39)$$

Here, A is the assimilation rate as we have used it before, h_s is the relative humidity at the leaf surface (beneath the leaf and canopy boundary layer; many times it is fairly close to relative humidity in bulk air), C_s is the CO_2 mole fraction in the air at the leaf surface (about 350×10^{-6} , of course), and m and b are the empirical slope and intercept. Interestingly, m is commonly very close to 10 for all unstressed C_3 plants, even desert shrubs, and b is small. Of course, for a given environment and a given photosynthetic capacity, A itself responds to g_s , so this is an implicit equation for g_s (or for A). There are mathematical methods to solve the simultaneous equations for g_s (Eq. 39), for A as a function of internal C_i , etc., and for the temperature of the leaf (responding to transpirational cooling, hence to g_s ; affecting enzyme activity, hence affecting A)(Collatz *et al.*, 1991; Gutschick, unpubl.).

Let's consider changes in h_s in the short term (daily, for example). Let's drop the b term in the Ball-Berry model. To express $A = k_C C_i$, let's use Eq. (38) to express how C_i responds to g_s . We get for A :

$$A = \frac{g_s' k_c}{g_s' + k_c} C_a \quad (40)$$

and we can plug this into the Ball-Berry equation (39) to get

$$g_s = m \frac{k_c g_s'}{k_c + g_s'} \frac{C_a h_s}{C_s} \quad (41)$$

Since C_s is commonly close to C_a , we can cancel these two out. Now, knowing that g_s is $1.6 g_s'$, and dividing out g_s' , we get

$$1.6 = \frac{m k_c}{k_c + g_s'} h_s \quad (42)$$

Some algebra (bringing the terms in g_s' together, etc.) gets us to

$$\frac{g_s'}{k_c} = m h_s - 1.6 \quad (43)$$

We can use this in Eq. (38), second line, to express C_i/C_a as

$$\frac{C_i}{C_a} = 1 - \frac{1.6}{m h_s} \quad (44)$$

This is a remarkably simple expression. It says that C_i/C_a increases as m increases (plants with a "drive" to have high γ and lots of photosynthesis) or as h_s increases (a response to good times for photosynthesis at high WUE). Let's carry this through to WUE:

$$\text{WUE} = \frac{0.62 C_a \left(1 - \frac{C_i}{C_a}\right)}{(e_i - e_a)} \quad (45)$$

If the leaf is near ambient air temperature and e_a is close to the vapor pressure at the exterior surface of the leaf (when boundary layer conductance is high: small leaves and/or good windspeed), we can write

$$e_i - e_a \approx e_{\text{sat}}(1 - h_s) \quad (46)$$

Now we can complete our approximation for WUE:

$$\begin{aligned} \text{WUE} &= \frac{0.62 C_a \frac{1.6}{m h_s}}{e_{\text{sat}}(1 - h_s)} \\ &= \frac{C_a}{m e_{\text{sat}} h_s (1 - h_s)} \end{aligned}$$

While it is approximate in many respects, Eq. (46) is a powerful summary of WUE control by the plant and by its environment. First, it says that WUE increases directly with the CO_2 content of the air, C_a . This means that increasing global CO_2 should be raising plant WUE around the world (at least by C_3 plants), and there is plenty of evidence from controlled experiments, including outdoor experiments, such as with free-air CO_2 enrichment (FACE; see, e.g.,

Gunderson *et al.*, 1993; Beerling, 1999; similar experimental methods used by Anderson *et al.*, 2001). It is also known that plant physiology, particularly photosynthetic physiology, acclimates to long-term exposure to elevated CO₂ (*e.g.*, Sage, 1994; ; overall, I found 578 references to acclimation at high CO₂ from 1993-2005, using SciSearch). This acclimation may alter the gains in WUE.

Furthermore, as one goes up in elevation, total air pressure decreases while the mole fraction or mixing fraction of CO₂ in air changes little. Plants at high elevations are expected to have lower WUE inherently...they should compensate by having lower C_i/C_a (by reducing the Ball-Berry slope *m*; this is also done by plants under water stress: Gutschick and Simonneau, 2002 and refs. therein). The patterns do exist along elevational gradients (Van de Water *et al.*, 2002; unusual contrary example found by Morecroft and Woodward, 1990), though they are complicated by the change in precipitation and water stress with elevation (*ibid.*). Basically, once precipitation is sufficient (at some moderate elevation), one does see decreasing C_i/C_a, but below this elevation, the increasing aridity gives the opposite trend. Note also another complication: at high elevations, not only is WUE affected by low C_a, so is photosynthesis. Nitrogen is used less efficiently for photosynthesis at low C_i (a result of low C_a). If N is limiting, this effect can drive a higher C_i/C_a. This trend is not common, for two reasons: first, N limitation is often less at higher elevations where precipitation is higher and growth (therefore, nutrient demand) is slower. Second, an increase in C_i/C_a disfavors WUE relatively much more than it favors A; recall the discussion following Eq. (38).

More inferences can be drawn from Eq. (46). Let's look at the dependence of WUE on humidity. It varies approximately as the inverse of the function $h_s (1 - h_s)$. This function varies relatively little over a wide range of h_s : between $h_s = 0.3$ and 0.7 , it only varies from 0.21 to 0.25, and at $h_s = 0.2$ or 0.8 , it equals 0.16, which is only about 1/3 smaller than at the peak of 0.25 when $h_s = 0.5$. Because the function occurs in the inverse, we see that WUE reaches high values when humidity is high...but also when it is low (because stomata close down so well). The stomatal response does acclimate to plant growth in various conditions, including elevated CO₂ (Bunce, 1998; Sage, 1994), apparently rebalancing the value of assimilation vs. WUE.

C. Tracking other aspects of the environment using plant (and air) isotopic signatures.

Respiration releases CO₂ from plant tissues, and this CO₂ has a lower $\delta^{13}\text{C}$ than bulk air in the environment. The respired CO₂ commonly mixes in well with bulk air, so that most plants see air of similar $\delta^{13}\text{C}$ in all parts of an ecosystem. This might tell us the rate of soil and root respiration, but this rate is not readily discerned from the small change in $\delta^{13}\text{C}$. In some conditions, such as dense forests, the respiratory CO₂ and its isotopic signature do accumulate, showing a significant gradient with height above the forest floor. This gradient can be used to estimate respiration itself (Sternberg *et al.*, 1989).

One important inquiry is the fate of the extra CO₂ we inject into the atmosphere by burning fossil fuel and burning material cleared during deforestation. We know the rate of input rather well, from fuel consumption and satellite monitoring of burned areas. We also know fairly well

how much of this extra CO₂ the ocean is taking up. Land plants might be expected to take up significant CO₂, based on the increase of photosynthesis with increasing CO₂, although from one year to the next, the increase should be quite small. The magnitude of the so-called terrestrial sink is sought empirically, by looking at gradients of CO₂ over large scales across the globe; the "sinks" are where CO₂ is drawn down in concentration. The isotopic gradients add power to this method (Ciais et al., 1995), which indicates that about 30% of injected CO₂ is taken up by the land biota, and that North America might be a major sink (Fan *et al.*, 1998; but see Field *et al.*, 1999). Note that the reason that North America appears to be a sink is that we have deforested large areas earlier. Houghton (2003) gives an interesting overview and synthesis.

Some parting words

I've focused on carbon isotope discrimination, but there is a wealth of literature on deuterium / hydrogen ratios, oxygen isotope ratios, and nitrogen isotope ratios to track processes from leaves to ecosystems. The reasons for discrimination and for variations in environmental isotopic composition are very different from those applying to carbon, and the insights are very different. I leave it to you to examine this very interesting literature.

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