CONVERGENT EVOLUTION

Shining fresh light on the evolution of photosynthesis

There are two types of photosynthesis, C3 and C4, and computational techniques have been used to explore how C4 plants evolved from their C3 ancestors.

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Related research article Williams BP, Johnston IG, Covshoff S, Hibberd JM. 2013. Phenotypic landscape inference reveals multiple evolutionary paths to C4 photosynthesis. eLife 2:e00961. doi: 10.7554/eLife.00961

Image Different evolutionary paths showing traits being added to an initial phenotype (bottom left) in different orders

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that allow these gases—and water vapour—to enter and leave the plant. This, in turn, keeps the loss of water to a minimum and helps the plant to resist drought: it is widely thought that the ability to survive droughts was important for the evolutionary emergence of C4 plants (Tipple and Pagnani, 2007; Kadereit et al., 2012). Much of the interest in C4 photosynthesis is motivated by

**Figure 1.** Photosynthesis in C3 and C4 plants. (A) There is competition between the Calvin-Benson cycle (photosynthesis) and photorespiration in C3 plants because the enzyme RuBisCO (ribulose-1,5-bisphosphate carboxylase oxygenase) is involved in both processes. When carbon dioxide is plentiful, the Calvin-Benson cycle converts carbon dioxide and water into carbohydrate (sugar) and oxygen: the energy needed to drive this process comes from the sun via ATP and NADPH molecules. However, when levels of carbon dioxide are low, photorespiration consumes energy and carbon dioxide without producing any carbohydrates. Acronyms are spelt out at the end of the caption: the number of carbon atoms in a molecule of the metabolite is given on the right, and the number of molecules involved in the reaction is on the left. (B) In C3 plants the Calvin-Benson cycle and photorespiration both occur in mesophyll cells, which are next to the pores (shown in yellow) that allow the plant to exchange gases with the atmosphere (and also allow water vapour to escape from the plant). Different organelles within the cells are illustrated in different colours: chloroplasts in green; peroxisomes in blue; mitochondria in pink. (C) In C4 plants mesophyll cells absorb atmospheric gases and harness energy from the sun using chlorophyll, while the fixation of carbon dioxide by RuBisCO generally takes place in bundle sheath cells, which have no direct interactions with the atmosphere. Two variations of the C4 cycle are shown: reactions unique to the enzyme NADP-ME are labelled 1, and those unique to enzyme NAD-ME are labelled 2. Acronyms: ALA, ASP, GLY and SER are all amino acids; PEPC (phosphoenolpyruvate carboxylase) is an enzyme; the other metabolites are BPGA (1,3-bisphosphoglycerate), G3P (glyceric acid 3-phosphate), GLYC (glycerate), GLYCLT (glycolate), GLX (glyoxylate), MAL (malate), OAA (oxaloacetate), PEP (phosphoenolpyruvate), PG (phosphoglycolate), PGA (3-phosphoglycerate), PYR (pyruvate), R5P (ribulose 5-phosphate) and RuBP (ribulose-1,5-bisphosphate).
the goal of transferring the higher rates of photosynthesis and growth found in C4 plants, along with their reduced need for water, to C3 plants such as rice.

To understand how C4 plants could have evolved from C3 plants, the Cambridge-Imperial team—which also includes Sarah Covshoff and Julian Hibberd—explored how C4 plants acquired a number of the traits that distinguish them from C3 plants. The 16 traits included in the study ranged from differences in the ways that cells accumulated various enzymes to differences in their size. The study exploited data from 72 plant species, including a number with phenotypes that are intermediate between C3 and C4.

The team used computational and statistical techniques to infer the probabilities of evolutionary trajectories that go from C3 to C4 phenotypes. In their framework, the probability of a trajectory can be calculated in terms of the probability of going from an arbitrary intermediate phenotype to a phenotype with one extra trait (see image on page 1). Since these ‘transition probabilities’ are unknown, Williams, Johnston et al. took a Bayesian approach and sampled these probabilities. In practice, this involved applying a technique known as Markov Chain Monte Carlo to adjust the set of transition probabilities to make them compatible with the experimental data—in this case the experimental data were the intermediate C3–C4 phenotypes.

Once formulated, this mathematical framework allowed questions about patterns in evolutionary trajectories to be answered objectively, with no human biases to skew the conclusions. Williams, Johnston et al. wanted to know if the evolutionary trajectories were strongly constrained (Lobkovsky et al., 2011; Heckmann et al., 2013). To answer this question they needed to know if the C3–C4 intermediate phenotypes had emerged from more than one evolutionary path. The answer to this question was yes. Next they asked in what order do C4 traits tend to be acquired. Interestingly, they found clear patterns: any given trait tends to be acquired at a characteristic time but, nonetheless, the order in which traits are acquired is not strict, which leads to a large amount of flexibility in the evolutionary trajectories. Williams, Johnston et al. also found that there were different classes of trajectories associated with the two major groups of flowering plants (monocots vs. eudicots).

What forces cause certain evolutionary trajectories to be more likely than others in the emergence of C4 plants? Selective pressures that favoured drought resistance and carbon capture in spite of high levels of oxygen were certainly present, but Williams, Johnston et al. give evidence that other forces may also have had a role. Furthermore, it is quite plausible that simple traits are acquired faster than more complex traits. Indeed, the acquisition of new traits typically involves modifying regulatory circuits and recruiting existing molecules to perform new functions (Barve and Wagner, 2013), which will require fewer changes for some traits than others.

It will take time to perform the sort of in-depth molecular investigation that is needed to clarify the details of how the different traits were acquired. In addition to testing some of the conclusions reported by Williams, Johnston et al., such experiments would also be of help to researchers trying to transfer C4 traits to C3 crops, be it via genetic engineering or breeding programmes using well chosen C3–C4 intermediates.

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