Understanding the evolution of complex innovations remains one of the most challenging problems in biology (Lynch, 2007; Wagner, 2014). Insights often stem from experimental lab studies that manipulate systems under ‘directed evolution’ (Weinreich et al., 2006; Blount et al., 2012; Finnigan et al., 2012). However, complex traits that have evolved many times over independent lineages present a different—yet equally powerful—opportunity to infer the evolutionary trajectories of novel traits.

In flowering plants, C₄ photosynthesis—a complex adaptation that increases photosynthetic efficiency—may have evolved first to correct an intercellular nitrogen imbalance, and only later evolved a central role in carbon fixation.

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Related research article Mallmann J, Heckmann D, Bräutigam A, Lercher MJ, Weber APM, Westhoff P, Gowik U. 2014. The role of photorespiration during the evolution of C₄ photosynthesis in the genus Flaveria. eLife 3:e02478. doi: 10.7554/eLife.02478

Image C₄ plants (Fb and Ft) have higher levels of certain enzymes than plants that use other forms of photosynthesis

Understanding the evolution of complex innovations remains one of the most challenging problems in biology (Lynch, 2007; Wagner, 2014). Insights often stem from experimental lab studies that manipulate systems under ‘directed evolution’ (Weinreich et al., 2006; Blount et al., 2012; Finnigan et al., 2012). However, complex traits that have evolved many times over independent lineages present a different—yet equally powerful—opportunity to infer the evolutionary trajectories of novel traits.

In flowering plants, C₄ photosynthesis is a well-studied, complex adaptation that has independently evolved over 60 times (Sage et al., 2011). Many key, shared stages along the C₄ evolutionary trajectory have been identified by studying multiple C₄-evolving plant groups (e.g., Kennedy et al., 1980; Ku et al., 1983; Vogan et al., 2007; Williams et al., 2013). Now, in eLife, Udo Gowik and colleagues at Heinrich-Heine-Universität—including Julia Mallmann and David Heckmann as joint first authors—present a compelling new hypothesis for how the final evolutionary steps were realized (Mallmann et al., 2014).

Although atmospheric carbon dioxide (CO₂) levels are currently rising, the last 30 million years witnessed great declines in CO₂, which has limited the efficiency of photosynthesis. Rubisco, the critical photosynthetic enzyme that catalyses the fixation of CO₂ into carbohydrate, also reacts with oxygen when CO₂ levels are low and temperatures are high. When this occurs, plants activate a process known as photorespiration, an energetically expensive set of reactions that—importantly for this story—release one molecule of CO₂.

C₄ photosynthesis is a clever solution to the problem of low atmospheric CO₂. It is an internal plant carbon-concentrating mechanism that largely eliminates photorespiration: a ‘fuel-injection’ system for the photosynthetic engine. C₄ plants differ from plants with the more typical ‘C₃’ photosynthesis because they restrict Rubisco activity to an inner compartment, typically the bundle sheath, with atmospheric CO₂ being fixed into a 4-carbon acid in the outer mesophyll. This molecule then travels to the bundle sheath, where it is broken down again, bathing Rubisco in CO₂ and limiting the costly process of photorespiration.
The evolution of the C₄ pathway requires many changes. These include the recruitment of multiple enzymes into new biochemical functions, massive shifts in the spatial distribution of proteins and organelles, and a set of anatomical modifications to cell size and structure. It is complex, and it is also highly effective: C₄ plants include many of our most important and productive crops (maize, sorghum, sugarcane, millet) and are responsible for around 25% of global terrestrial photosynthesis (Still et al., 2003).

A key intermediate step in the evolution of C₄ is the establishment of a rudimentary carbon-concentrating mechanism. Termed ‘C₂ photosynthesis’, this mechanism limits certain reactions of the photosynthetic cycle to the bundle sheath cells. A byproduct of these reactions is CO₂, creating a slightly elevated CO₂ concentration and increasing Rubisco efficiency in these cells. Though much rarer than C₄ plants, C₂ plants have been discovered in a variety of C₄-evolving lineages, and are thought to represent a common, if not requisite, intermediate step along the C₄ trajectory (Sage et al., 2012).

One implication of a restricted photorespiratory cycle is the development of a severe nitrogen imbalance between the mesophyll and the bundle sheath cells. This occurs because every molecule of CO₂ produced in the bundle sheath is accompanied by a molecule of ammonia. While this nitrogen imbalance has previously been recognised (Monson and Rawsthorne, 2000), it has never been closely studied, and certainly never considered as potentially important to the evolutionary assembly of the C₄ pathway.

To investigate this, Mallmann, Heckmann et al. combined a mechanistic model of C₂ physiological function with a metabolic model, which allowed them to predict the build-up of certain metabolites based on the rates of Rubisco and photorespiratory activity. They then modelled the various biochemical pathways that could potentially be
induced to balance metabolic fluxes between the mesophyll and bundle sheath cells. This creative combination of models allowed them to evaluate the various metabolic pathways for re-balancing nitrogen in terms of which pathways resulted in the highest biomass yield (a proxy for fitness).

Remarkably, when low levels of C₄ enzyme activity are permitted in the model, key elements of the C₄ cycle are favoured as the nitrogen-balancing pathway. What’s more, this model predicts that with a C₄ cycle established, increasing the activity of the enzymes results in a linear increase in biomass yield. Allowing for low levels of C₄ enzyme activity is biologically reasonable, as these enzymes are routinely present in C₃ leaves. Mallmann, Heckmann et al. support their model predictions with experimental gene expression data from a set of C₃, C₂, C₄, and other C₂-C₄ intermediate types in the plant lineage Flaveria, which show elevated C₄ cycle activity even in intermediates that are not using the enzymes to capture carbon.

In other words, once a C₂ cycle is established, the evolution of a fully realized C₄ process is fairly trivial. Once C₂ enzymes are recruited to shuttle nitrogen back to the mesophyll, it is all but inevitable. This can explain in part why C₄ has evolved such a startling number of times, and why many of these origins are highly clustered across the tree of life. Many C₄ evolutionary clusters likely share an ancestor that had already acquired an elevated likelihood of evolving the pathway (Figure 1).

This may also explain why C₂ species are so rare relative to C₃ species—C₂ is likely to be a step along the trajectory with a relatively short evolutionary lifespan. At the same time, it raises the question of why a handful of C₄ species are persistent—the C₂ Mollugo verticillata group may be up to 15 million years old (Christin et al., 2011). A testable hypothesis would be that these C₂ plants have solved their nitrogen problem a different way, thereby limiting their own evolutionary accessibility to C₄ photosynthesis. If so, this highlights the key role of contingency in adaptation, and our growing power to understand and predict macroevolutionary processes.

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References

Blount ZD, Barrick JE, Davidson CJ, Lenski RE. 2012. Genomic analysis of a key innovation in an experimental Escherichia coli population. Nature 489:513–518. doi: 10.1038/nature11514.

Christin PA, Osborne CP, Chattele DT, Columbus JT, Besnard G, Hodkinson TR, Garrison LM, Vorontsova MS, Edwards EJ. 2013. Anatomical enablers and the evolution of C₄ photosynthesis in grasses. Proceedings of the National Academy of Sciences of the USA 110:1381–1386. doi: 10.1073/pnas.1216777110.

Christin P, Sage T, Edwards E, Ogburn R, Khoshravesh R, Sage R. 2011. Complex evolutionary transitions and the significance of C₃-C₄ intermediate forms of photosynthesis in Molluginaceae. Evolution; International Journal of Organic Evolution 65:643–660. doi: 10.1111/j.1558-5646.2010.01168.x.

Finnigan GC, Hanson-Smith V, Stevens TH, Thornton JW. 2012. Evolution of increased complexity in a molecular machine. Nature 481:360–364. doi: 10.1038/nature10724.

Heckmann D, Schulze S, Denton A, Gowik U, Westhoff P, Weber AP, Lercher MJ. 2013. Predicting C₄ photosynthesis evolution: modular, individually adaptive steps on a Mount Fuji fitness landscape. Cell 153:1579–1588. doi: 10.1016/j.cell.2013.04.058.

Kennedy RA, Eastburn JL, Jensen KG. 1980. C₃-C₄ photosynthesis in the genus Mollugo: structure, physiology and evolution of intermediate characteristics. American Journal of Botany 67:1207–1217. doi: 10.2307/2442363.

Ku MSB, Monson RK, Robert O Littlejohn J, Nakamoto H, Fisher DB, Edwards GE. 1983. Photosynthetic characteristics of C₃-C₄ intermediate Flaveria species: I. leaf anatomy, photosynthetic responses to O₂ and CO₂ and activities of key enzymes in the C₃ and C₄ Pathways. Plant Physiology 71:944–948. doi: 10.1104/pp.71.4.944.

Lynch M. 2007. The frailty of adaptive hypotheses for the origins of organismal complexity. Proceedings of the National Academy of Sciences of the USA 104:8597–8604. doi: 10.1073/pnas.0702207104.

Mallmann J, Heckmann D, Bräutigam A, Lercher M, Weber A, Westhoff P, Gowik U. 2014. The role of photorespiration during the evolution of C₄ photosynthesis in the genus Flaveria. eLife 3:e02478. doi: 10.7554/eLife.02478.

Monson R, Rawsthorne S. 2000. CO₂ assimilation in C₃-C₄ intermediate plants. In: Leegood R, Sharkey T, Caemmerer S, editors. Advances in photosynthesis and respiration. Dordrecht: Kluwer Academic. p. 533–550.

Sage RF, Christin PA, Edwards EJ. 2011. The C₄ plant lineages of planet Earth. Journal of Experimental Botany 62:3155–3169. doi: 10.1093/jxb/err048.

Sage RF, Sage TL, Kocacinar F. 2012. Photorespiration and the evolution of C₄ photosynthesis. Annual Review of Plant Biology 63:19–47. doi: 10.1146/annurev-arplant-042811-105511.

Still CJ, Berry JA, Collatz GJ, Defries RS. 2003. Global distribution of C-3 and C-4 vegetation: carbon cycle implications. Global Biogeochem. Cycles 17:1006. doi: 10.1029/2001GB001807.

Vogin PJ, Frohlich MW, Sage RF. 2007. The functional significance of C₃-C₄ intermediate traits in Heliotropium L. (Boraginaceae): gas exchange perspectives. Plant, Cell and Environment 30:1337–1345. doi: 10.1111/j.1365-3040.2007.01706.x.

Wagner GP. 2014. Homology, genes, and evolutionary innovation. Princeton, NJ:Princeton University Press.
Weinreich DM, Delaney NF, DePristo MA, Hartl DL. 2006. Darwinian evolution can follow only very few mutational paths to fitter proteins. *Science* **312**:111–114. doi: 10.1126/science.1123539.

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.