

Once again, a great ape has demonstrated behaviour that is disconcertingly 'human'. Powerful males manipulate access to sex in order to buy support in their politics. Now where have we heard of that sort of thing before? Shakespeare would have loved it. But does this sort of manipulation by favours really require any special cognitive skills of the alpha male chimpanzee? Maybe not. A would-be Machiavelli has to distinguish his peers individually and remember who has been his supporter and how far that support went, and keep the score updated regularly. With each male characterized by his 'supporter rating', the alpha-male needs

simply to show tolerance in linear proportion to the measure of past support, for each male. Measure for measure, one might say.

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C₄ Photosynthesis: Convergence upon Convergence upon...

C₄ photosynthesis has evolved independently numerous times in plants. New work suggests that these multiple origins are the result of recurring selection on a few amino acid positions in the key enzyme phosphoenolpyruvate carboxylase.

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Our understanding of the mechanisms by which plants fix CO₂ has changed dramatically over the last 40 years [1,2]. In addition to the typical 'C₃' photosynthesis, in which CO₂ is fixed into three-carbon molecules by ribulose bis-phosphate carboxylase (Rubisco), modified systems known as 'C₄ metabolism' and 'crassulacean acid metabolism' (CAM) are not only present, but have been derived multiple times from the general C₃ system [3]. C₄ metabolism and CAM are both adaptations that enable plants to carry out the gas exchange required for photosynthesis while minimising water loss through stomata. C₄ photosynthesis involves the co-option of one member of the phosphoenolpyruvate carboxylase (PEPC) family, which is used for initial fixation of CO₂ into a four-carbon molecule [4].

Detailed characterization of the C₄ carbon-concentrating mechanism in a diversity of lineages has greatly increased our understanding of the physiological and biochemical diversity in higher plants, and has also led to interesting insights into evolutionary dynamics of complex biochemical pathways. Recent studies have suggested that there have been at least 24 separate origins of the C₄ pathway in monocots alone, with at least 45 angiosperm origins [5,6].

While the biochemical process of C₄ photosynthesis has been understood for quite some time, and the non-monophyly of the C₄ system has also been abundantly clear given its wide phylogenetic distribution and the large numbers of intervening C₃ lineages, we have only recently begun to understand the evolutionary dynamics that have led to these parallel origins of the C₄ pathway [7]. Conflicting evidence of species relationships and PEPC gene family

relationships have previously confused the origins of C₄. Evidence from gene phylogenies, morphological phylogenies and a host of additional systematic data suggest multiple origins of C₄ photosynthesis [3]. But previous phylogenetic analyses of the PEPC gene family have suggested that all, or most, of the C₄-type PEPC copies form a monophyletic group, at least within major clades, such as the grasses [8,9]. This has led researchers to hypothesize that an ancestral C₄ PEPC isoform might have predated the monocot-dicot divergence (discussed in [4]).

Parallelisms and convergences in genetic and phenotypic traits are well documented (reviewed in [10]). While the traditional expectation is for selection to drive convergence in form through different quantitative trait combinations (Fisher's infinitesimal model [11]), evidence from empirical studies provides evidence for parallel genotypic adaptations at multiple hierarchical levels [10]. It should be noted, though, that most of this evidence comes from artificial selection experiments, little of it has been evaluated statistically, and how artificial selection correlates to selection in more complex natural environments is unclear.

When PEPC functionality for C₄ photosynthesis is considered in

terms of selection, there are three relatively plausible explanations: first, the origins fit Fisher's traditional model, with the specialized physiological function of PEPC being achieved in different ways in different lineages; second, the specialised C₄ use of PEPC reflects a single (or small number of) ancestral origins of the C₄ isoform, as supported by the monophyly of C₄ isoforms in many phylogenetic analyses; and third, the C₄ PEPC isoforms are derived from more recent, parallel but identical, changes in the PEPC amino-acid sequence for C₄ functionality. This conundrum has been studied by Christin *et al.* [7], in their study reported recently in *Current Biology*, where they found that the strongly supported monophyly of most C₄-type PEPCs is the result of strong selective pressure on particular amino-acid positions, not shared history. Selection is clearly driving convergence at a small number of amino-acid positions in distant lineages, suggesting that selection for PEPC C₄ functionality does not fit Fisher's infinitesimal model.

These results are important for several reasons. They provide a clear, although non-parsimonious, explanation as to why C₄ isoforms from distantly related species form monophyletic groups when analyzed with the entire coding region. The compelling evidence provided by Christin *et al.* [7] is that, when the PEPC gene sequences are analysed, the 'true' phylogenetic relationships, with the C₄ isoforms being non-monophyletic, are reconstructed when the analyses are done using only the non-coding intron and 'silent' third position sites. Furthermore, the results provide a clear selection mechanism for achieving C₄ functionality in PEPC gene copies without inferring repeated loss of the C₄ isoform in C₃ lineages.

These results raise several important questions regarding inferences of phylogeny, molecular evolution and physiological pathway function. First, the use of PEPC sequences for phylogenetic reconstruction in lineages which include C₄ species [12] is

questionable, and relationships in these lineages need to be verified using genes that are not as likely to be under selective pressure. Where phylogenetic analyses based on PEPC gene sequences conflict with other estimates of relationships in the lineage, as in the Chenopodiaceae [13,14], these need to be revisited to determine if similar kinds of selective pressures are affecting phylogeny reconstruction as found by Christin *et al.* [7]. Preliminary results in *Eleocharis* (Cyperaceae) suggest a similar pattern of selection pressure skewing phylogenetic relationships inferred from PEPC genes when compared to other gene phylogenies (my unpublished data). These results from outside of the grasses suggest that this might be a widespread phenomenon in the PEPC gene family.

Specialized PEPC isoforms are not restricted to C₄ plants — CAM plants also use PEPC in the initial fixation of CO₂ [15]. Whether or not there has been similar selection pressure on CAM PEPC isoforms driving parallel amino-acid substitutions is not clear, but needs to be explored. Phylogenetic studies of the PEPC genes in lineages including CAM species [8,16] suggest CAM PEPC isoforms are monophyletic, but the results of Christin *et al.* [7] suggest that these phylogenies should be viewed with suspicion until they have been verified using other molecular phylogenies.

Christin *et al.* [7] have clearly demonstrated that selection played a role in parallel changes to the C₄ PEPC isoform in the grasses, and evidence for parallel changes in non-grasses needs to be further explored. More detailed studies of PEPC gene family diversity and tests of selection pressure are necessary to better understand this issue in other lineages, with a focus on much denser sampling of C₄, CAM, and C₃ gene copies from C₄, CAM, and C₃ species across lineages. While use of PEPC for reconstructing phylogenetic relationships has become popular in recent years [17,18], and while its use in C₃ clades may not be unduly influenced by positive selection, rigorous tests for selection and

comparisons with other gene phylogenies should be conducted to verify that selection is not skewing inferences of evolutionary relationships in these lineages.

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