Current Biology 22, 445–449, March 6, 2012 ©2012 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2012.01.054

Report

Adaptive Evolution of C_4 Photosynthesis through Recurrent Lateral Gene Transfer

Pascal-Antoine Christin,[1,*] Erika J. Edwards,[1] Guillaume Besnard,[2] Susanna F. Boxall,[3] Richard Gregory,[4] Elizabeth A. Kellogg,[5] James Hartwell,[3] and Colin P. Osborne[6]

[1]Department of Ecology and Evolutionary Biology, Brown University, 80 Waterman Street, Providence, RI 02912, USA
[2]Laboratoire Evolution & Diversité Biologique, CNRS-UPS-ENFA, UMR 5174, 1062 Toulouse, France
[3]Department of Plant Sciences
[4]Centre for Genomic Research
Institute of Integrative Biology, University of Liverpool, Liverpool L69 7ZB, UK
[5]Department of Biology, University of Missouri, St. Louis, One University Boulevard, St. Louis, MO 63121, USA
[6]Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, UK

Summary

C_4 photosynthesis is a complex trait that confers higher productivity under warm and arid conditions [1–3]. It has evolved more than 60 times via the co-option of genes present in C_3 ancestors followed by alteration of the patterns and levels of expression and adaptive changes in the coding sequences [4–12], but the evolutionary path to C_4 photosynthesis is still poorly understood. The grass lineage *Alloteropsis* offers unparalleled opportunities for studying C_4 evolution, because it includes a C_3 taxon and five C_4 species that vary significantly in C_4 anatomy and biochemistry [13, 14]. Using phylogenetic analyses of nuclear genes and leaf transcriptomes, we show that fundamental elements of the C_4 pathway in the grass lineage *Alloteropsis* were acquired via a minimum of four independent lateral gene transfers from C_4 taxa that diverged from this group more than 20 million years ago. The transfer of genes that were already fully adapted for C_4 function has occurred periodically over at least the last 10 million years and has been a recurrent source for the optimization of the C_4 pathway. This report shows that plant-plant lateral nuclear gene transfers can be a potent source of genetic novelty and adaptation in flowering plants.

Results and Discussion

Multiple Lateral Acquisitions of Key C_4 Genes

We sampled geographically separated populations of four *Alloteropsis* taxa (Figure 1). Three taxa are reported as C_4 (*A. angusta*, *A. cimicina*, and *A. semialata* subspecies *semialata*), whereas the fourth is C_3 (*A. semialata* subspecies *eckloniana*). The history of photosynthetic transitions in this group is unclear [13], because the spatial compartmentalization of carbon assimilation and reduction that is instrumental to C_4 photosynthesis is completely absent from the C_3 *A. s. eckloniana* and involves nonhomologous cell types in the different C_4 *Alloteropsis* lineages [14]. Several multigene families were investigated by PCR amplification from genomic DNA, whereas gene transcripts from leaves were analyzed in representatives of the different taxa (see Supplemental Information available online).

We isolated genes from all *A. angusta* and *A. semialata* accessions for the C_4 carbon-fixing enzyme phosphoenolpyruvate carboxylase (*ppc*; Figure 2) that are positioned in phylogenies near those of affiliated C_3 taxa (Figure 3A). The vertically acquired *ppc* of *A. angusta* has many amino acid residues that are characteristic of C_4-specific forms (Figure 4A) [8], suggesting that the C_4 *ppc* used by this species evolved via adaptive changes to the gene inherited from its C_3 ancestor. However, the vertically acquired *ppc* of all *A. s. semialata* accessions has no C_4-characteristics; it was present only at a very low transcript level and was not found at all in *A. cimicina*, ruling out a C_4 function in these taxa.

C_4-specific *ppc* genes from different *Alloteropsis* accessions were identified as the copy most abundantly transcribed in leaves (Supplemental Information). The *ppc* used for C_4 photosynthesis in *A. cimicina* was not related to those of affiliated taxa but clustered with those of the distantly related Melinidinae (Figure 3A). This gene was also found in South African *A. s. semialata*, which suggests an ancient lateral *ppc* acquisition before the diversification of *Alloteropsis* (Figure 1). However, this gene appears to be nonfunctional in South African *A. s. semialata* (Supplemental Information), indicating that the C_4 function of this *ppc* was lost after the divergence of the *A. cimicina* and *A. semialata* lineages. The C_4 *ppc* of South African *A. s. semialata* was phylogenetically nested in the distantly related C_4 Cenchrinae, among the members of the *Setaria palmifolia* species complex (Figure 3A). It was therefore likely acquired laterally in tropical Africa, where *A. s. semialata* and members of the *Setaria palmifolia* species complex co-occur [15]. The C_4 *ppc* of the Australian population of *A. s. semialata* was nested in those of Andropogoneae (Figure 3A), a group of highly diversified C_4 grasses that diverged from *Alloteropsis* ~25 million years ago (Ma) (Figure 1). On an overlapping fragment of 719 bp, Australian *A. s. semialata* and the Andropogoneae taxon *Themeda quadrivalvis* differed at only four nucleotides, a level of polymorphism usually observed only among closely related species. The C_4 *ppc* of Australian *A. s. semialata* was thus likely acquired horizontally from *T. quadrivalvis* or a very close relative in regions of Australia where these taxa co-occur. Because each of these laterally acquired *ppc* had spent several million years in the genomes of other C_4 plants prior to their acquisition by *Alloteropsis* species (Figure 1), they had already accumulated numerous C_4-characteristic residues and were fine-tuned for function in the C_4 pathway (Figure 4A).

We also isolated a gene encoding phosphoenolpyruvate carboxykinase (*pck*; Figure 2), the decarboxylating enzyme of one of the C_4 biochemical subtypes, from all populations of *Alloteropsis*, that was most closely related to the *pck* from affiliated C_3 taxa (Figure 3B). However, this vertically transmitted gene had none of the C_4-specific amino acid residues and was present only at very low transcript levels in the leaf

*Correspondence: pascal-antoine_christin@brown.edu

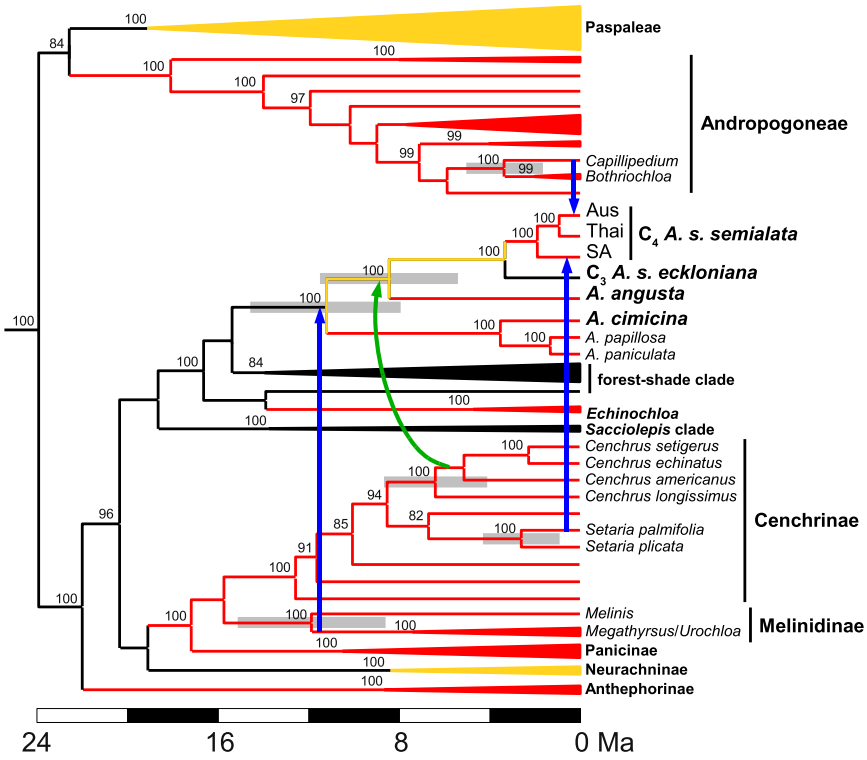

Figure 1. Time-Calibrated Phylogeny of Panicoideae and Inferred Lateral Gene Transfers

The main clades are compressed, with the size of wedges proportional to the number of species sampled, and colored in red when containing C_4 species only, black for C_3 species only, and yellow for both C_3 and C_4 species. Yellow branches indicate an uncertain ancestral state. Bayesian support values are shown as percentages near branches, when greater than 80%. These species relationships, deduced from multiple plastid markers, were also confirmed with several nuclear markers (Supplemental Information). The inferred lateral gene transfers are represented by arrows, in green for *pck* and blue for *ppc*. For *A. s. semialata*, geographically separated populations are indicated as follows: Aus, Australia; Thai, Thailand; SA, South Africa. Grey boxes indicate confidence intervals of the age estimates for the last node before the inferred lateral transfer and the first node after the transfer.

transcriptome of C_4 *A. s. semialata* (Supplemental Information), making a significant role in the C_4 pathway highly unlikely. Instead, we isolated a second *pck* gene from all accessions of *A. angusta* and *A. s. semialata*, which had most of the C_4-characteristic amino acid residues (Figure 4B) [9], and was the most abundant *pck* transcript in leaves of *A. s. semialata*. This C_4-specific gene was not closely related to the non-C_4 genes from *Alloteropsis* but instead was nested within C_4 *pck* from Cenchrinae (Figure 3B). This shows that a C_4 *pck* was transmitted from a member of Cenchrinae to the common ancestor of *A. angusta* and *A. semialata* and now fulfills a key function in the C_4 pathway of these taxa.

The different phylogenetic groupings all receive strong statistical support even when only introns or third positions of codons are considered (Supplemental Information), ruling out the possibility that selective pressures misled phylogenetic reconstructions. A first transfer of C_4 *ppc* from Melinidinae occurred prior to the divergence of all extant *Alloteropsis* species. It was followed by a lateral acquisition of a C_4 *pck* from Cenchrinae after the first speciation inside *Alloteropsis* (Figure 1). Two additional and recent transfers involved C_4 *ppc* from different origins and happened in geographically isolated populations of *A. s. semialata*.

Most of the Genome and Transcriptome Was Vertically Acquired

The lateral acquisitions of *ppc* and *pck* could have occurred during allopolyploidization events involving distantly related parents, a scenario compatible with the multiple ploidy levels observed in *A. semialata* [16]. After allopolyploidization, numerous genes from each parent are expected to persist in the descendants and contribute to their transcriptome even after several million years [17, 18]. We investigated the presence of additional foreign genes in *Alloteropsis* genomes by sequencing seven nuclear markers unrelated to C_4 photosynthesis, one of which is physically located adjacent to *ppc* in the genomes of *Setaria*, *Sorghum*, and *Brachypodium* (Supplemental Information). The isolated sequences were all positioned in the phylogeny near close relatives of *Alloteropsis*, and no laterally acquired gene was detected. In addition, we screened the South African *A. s. semialata* transcriptome for phylogenetically relevant markers and successfully positioned 3,279 transcripts in phylogenies including orthologous genes extracted from the *A. s. eckloniana* transcriptome and whole-genome sequences for *Setaria italica* (C_4 Cenchrinae), *Sorghum* (C_4 Andropogoneae), rice, and *Brachypodium* (Supplemental Information). If other genes were transmitted from a species of the Cenchrinae alongside *pck* and *ppc*, as would occur during allopolyploidization, multiple transcripts of *A. s. semialata* should group with *Setaria italica* sequences. However, 3,275 of the 3,279 investigated transcripts strongly grouped *A. s. semialata* with *A. s. eckloniana*, and only four transcripts encoding *pck* and *ppc* grouped *A. s. semialata* with *Setaria* (Supplemental Information). These analyses show that most of the genes used by the South African *A. s. semialata*, including all known genes of the C_4 pathway other than *ppc* and *pck* (Figure 2), were acquired vertically. Together with the analysis of low-copy genes, the vertical inheritance of the transcriptome therefore argues strongly against allopolyploid formation as a mechanism for the lateral transmission of *ppc* and *pck* into *Alloteropsis*.

Possible Mechanisms and Importance of Selection

Our data indicate that the horizontal transmission of *pck* and *ppc* genes to *Alloteropsis* included at most a very small amount of other genetic material. However, the high similarity between intron and untranslated region (UTR) sequences of the horizontally acquired genes and those of the putative donors (Supplemental Information) indicates that the whole genes were transmitted as genomic DNA. Reports of nuclear horizontal gene transfer between plants are rare, but several mechanisms have been suggested, including vector-mediated, plant-plant contact, transformation, and illegitimate

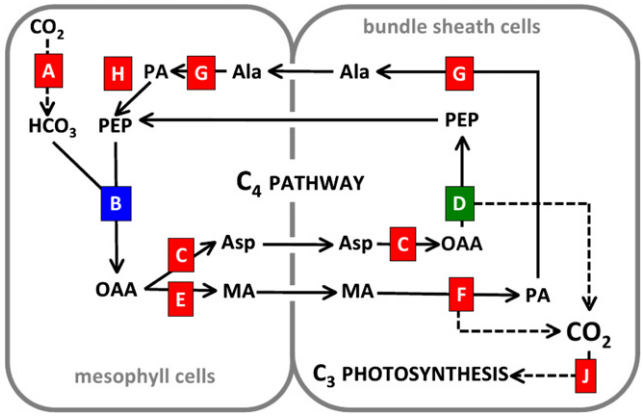

Figure 2. Key Biochemical Steps in the Putative C_4 Photosynthetic Pathway of *Alloteropsis semialata*

The CO_2-concentrating mechanism hinges on the enzyme PEP carboxylase (PEPC) (B), which fixes inorganic carbon to produce four-carbon organic acids in the mesophyll cells of the leaf (aspartate [Asp], malate [MA]). These acids diffuse into the bundle sheath cells, where CO_2 is liberated at high concentrations by decarboxylase enzymes including PEP carboxykinase (PCK) (D) and NADP-malic enzyme (NADP-ME) (F). The CO_2 is refixed by Rubisco (J), whereas the organic acceptor molecule returns to the mesophyll cells. The following metabolite abbreviations are used: Ala, alanine; Asp, aspartate; MA, malate; OAA, oxaloacetate; PA, pyruvate; PEP, phosphoenolpyruvate. The following enzymes (colored boxes) abbreviations are used: carbonic anhydrase (A); PEPC, PEP carboxylase (B); Asp animotransferase (C); PCK, PEP carboxykinase (D); NADP-MA dehydrogenase (E); NADP-ME, NADP-malic enzyme (F); Ala aminotransferase, (G); PA phosphate dikinase (H); Rubisco, ribulose-1,5-*bis*phosphate carboxylase/oxygenase (J).

pollination [19, 20]. The most commonly described is direct contact via a host-parasite relationship [21, 22], which is not a possibility in the present case, because neither the putative donors nor *Alloteropsis* species have been reported as parasitic. Multiple cases of lateral gene transfer have involved infecting agents, viral or bacterial [19, 20, 23], and an unknown agent has been proposed for the lateral transmission of nuclear genes among distantly related grasses [24, 25]. Vector-mediated transfers are similarly possible in the case of *Alloteropsis*, although the necessity of a first transfer from a plant to the agent followed by a second transfer from the agent to the germ line of *Alloteropsis* diminishes the likelihood of this scenario. The exact mechanism for the plant-plant gene transfers shown in the present study remains speculative and could differ among the multiple cases detected. We suggest that the most reasonable scenario for the transfer of *pck* and *ppc* genes may have been during direct contact after the deposition of foreign pollen on the *Alloteropsis* stigma.

All grasses are pollinated largely by wind, and putative donors of the laterally acquired genes are found in close contact with *Alloteropsis* populations. Introgression of these genes via ordinary hybridization and successive backcrossing is unlikely. The horizontally acquired genes do not seem to recombine with native homologs, suggesting they occupy different loci (Supplemental Information). Moreover, such an introgression would require recombination between paired chromosomes from the two parental genomes that last shared a common ancestor more than 20 Ma, a period sufficient for complete turnover of noncoding intergenic regions [26], which hampers chromosomal pairing. However, the temporary mingling of foreign and native genomes, even if limited to the

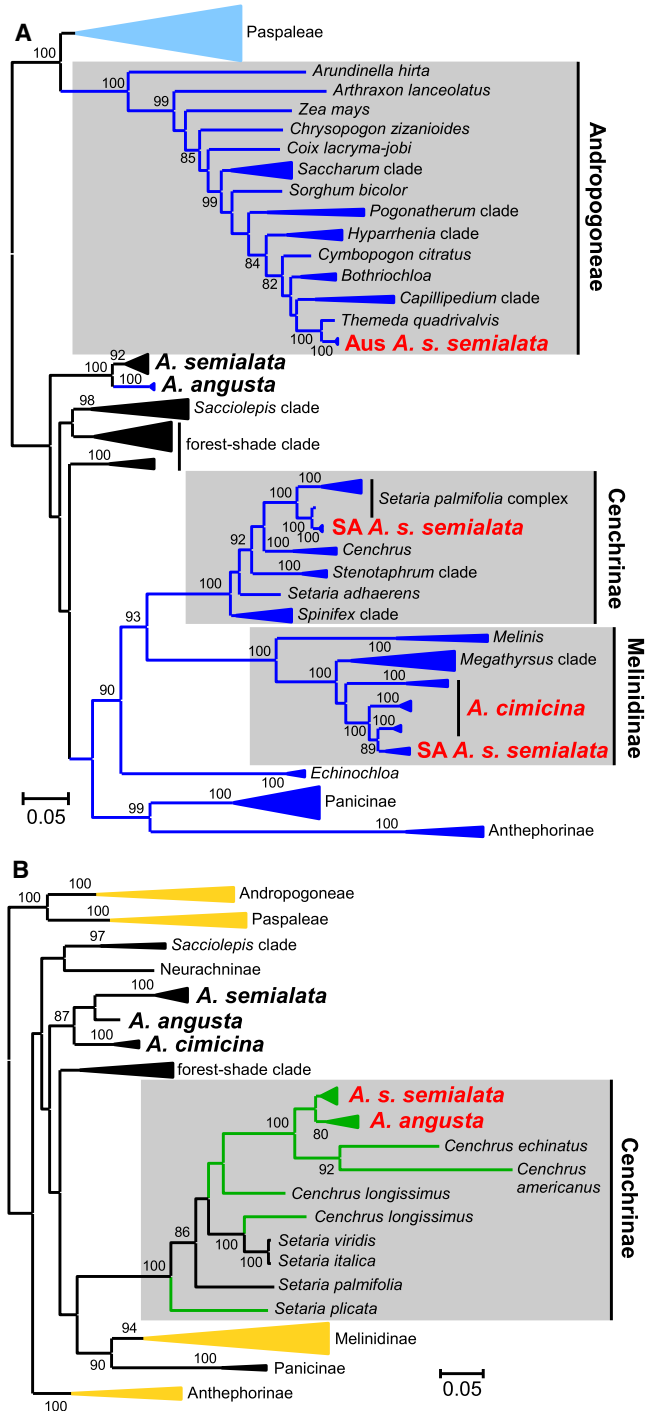

Figure 3. Phylogenetic Position of *Alloteropsis* Laterally Transmitted Genes

(A) Phylogeny of *ppc* in Panicoideae.

(B) Phylogeny of *pck* in Panicoideae.

Alloteropsis genes are highlighted with a larger font, with vertically acquired genes in black and laterally acquired genes in red. Both phylogenies were inferred from the third positions of codons. Branches leading to C_4-specific genes are in blue and green. Clades in light blue and yellow contain both C_4 and non-C_4 genes (see Supplemental Information for details). Several clades encompassing only C_4 taxa contain both C_4 and non-C_4 genes due to gene duplications (Supplemental Information). Bayesian support values are shown as percentages near branches when greater than 80%. Aus, Australia; SA, South Africa.

A

	466	517	531	560	577	579	625	637	761	780	794	807
A. semialata native gene	L	A	A	R	A	A	V	M	S	A	F	R
A. angusta native gene	L	A	P	R	S	E	V	M	S	S	V	R
Melinidinae gene	I	T	P	P	T	E	S	L	A	S	V	K
Cenchrinae gene	I	C	P	P	S	E	A	L	A	S	F	K
Andropogoneae gene	I	A	P	P	S	E	A	L	A	S	V	K

B

	149	150	184	206	242	264	421	505	519
Alloteropsis native gene	Y	E	A	T	S	E	H	Q	L
Cenchrinae gene	S	N	A	E	T	Q	R	T	Y

Figure 4. Presence of Putative C_4-Adaptive Amino Acids on Laterally Transmitted Genes

For native and foreign genes of *Alloteropsis*, amino acid residues are shown for positions that are known to be under positive selection for C_4 function [8, 9]. Putative C_4-adaptive residues are in black boxes. The amino acids at these positions are identical in the putative donors, with the exceptions of *ppc* 625, which is variable in Melinidinae, and *pck* 149, which is variable in Cenchrinae.

(A) Genes for PEPC (*ppc*). The 12 sites with the highest probability of C_4-specific selection [8] are shown. Numbering is based on the *Zea mays* sequence (CAA33317).

(B) Genes for PCK (*pck*). Numbering is based on the *Zoysia japonica* sequence (AB199899).

early stages of reproduction (e.g., pollen tube growth or early embryonic development), could provide sufficient opportunity for the transfer of genes via retrotransposons or other genomic rearrangements [27, 28]. In controlled conditions, foreign pollen from grasses that diverged more than 45 Ma can germinate and initiate embryo development, with the occasional transmission of paternal genetic material to mostly maternal descendants [29, 30]. Even if the fitness of the resulting offspring is reduced, subsequent crosses with other *Alloteropsis* individuals would allow the integration of foreign genes into the population, especially if the transmitted genes are strongly favored by natural selection.

The laterally acquired genes both encode key enzymes of the C_4 pathway (Figure 2) whose evolution from non-C_4 genes requires a series of important genetic changes [7–9]. Before their transmission to *Alloteropsis*, the foreign copies of *ppc* and *pck* had spent more than 15 Ma in the genomes of other C_4 plants and were highly optimized for C_4 function. We hypothesize that they were incorporated into *Alloteropsis* plants that operated a suboptimal C_4 cycle, with genes that were not fully adapted for function in the C_4 pathway, as is apparently still the case in the Thai *A. s. semialata* (Figure 4; Supplemental Information). The replacement of these vertically acquired genes by laterally acquired genes that were better optimized for the C_4 pathway improved the efficiency of *Alloteropsis* C_4 pathway, and natural selection led to a rapid fixation of the transferred genes in populations.

Concluding Remarks

The *Alloteropsis* case demonstrates a remarkable permeability of species boundaries in some grasses and shows that natural selection on the rare and random exchanges of genes between distantly related species can lead to the horizontal spread of adaptive biochemical novelties. The high number of lateral transfers reported here could indicate that *Alloteropsis* taxa are especially prone to lateral acquisitions, because of genomic plasticity or other lifestyle characteristics that increase the likelihood of lateral transfer in some species [31], such as the short time to first seed production in *Alloteropsis* taxa (typically less than 1 year). Additionally, the photosynthetic variation observed in *Alloteropsis* might indicate that the transition to full C_4 function occurred multiple times over a long evolutionary period from poorly optimized C_4 or C_3–C_4 intermediates (Figure 1), providing continued opportunity to receive foreign C_4 genes that further optimized the C_4 pathway. The extent to which this evolutionary process applies to other groups is difficult to evaluate. No conclusive evidence has been found for lateral acquisition in the C_4 taxa previously surveyed [7–9, 32], with the exception of the sedge *Eleocharis vivipara*, which acquired a C_4 *ppc* gene from a congeneric [32], but lateral gene transfer can be detected only with a large species sampling for which a solid understanding of the phylogenetic relationships is known.

Our discovery of lateral transfer of C_4 genes in *Alloteropsis* was possible for four important reasons. First, genomic data is available for certain grass crops, some of which happen to be close relatives of our donor species. Second, compared with other complex traits, we have a relatively good understanding of the enzymes involved in this metabolic pathway, and previous work has generated sequences of C_4 genes for a large number of grasses [8, 9]. Third, genes for C_4 enzymes are highly expressed and are thus easily captured by transcriptome analyses. Finally, a set of adaptive amino acid changes to enzymes recruited to the C_4 pathway have been previously identified, revealing the evolutionary significance of the genes acquired laterally [7–10]. Without this breadth and depth of background knowledge, the present case would likely have remained undetected. There is no reason to think that this mechanism would be unique to the evolution of the C_4 pathway. As more genomic data accumulate for other taxa, additional cases of adaptive plant-plant nuclear gene transfers may be discovered, in a great variety of adaptive traits.

Accession Numbers

The sequences generated in this paper were deposited in GenBank database, under the accession numbers FR845806–FR845993 and FR872768–FR872793. The alignments used for phylogenetic inference, the inferred phylogenetic trees, and the 454 contigs used for phylogenomic analyses have been deposited in the Dryad Repository under the doi 10.5061/dryad.m1h5c85d.

Supplemental Information

Supplemental Information includes three figures, two tables, and Supplemental Experimental Procedures and can be found with this article online at doi:10.1016/j.cub.2012.01.054.

Acknowledgments

This work was supported by the Marie Curie International Outgoing Fellowship 252568 and the Swiss National Science Foundation grant PBLAP3-129423 to P.-A.C., and the Natural Environment Research Council Molecular Genetics Facility small projects scheme grant MGF234 to C.P.O. and J.H. The US Department of Energy Joint Genome Institute provided sequencing and analyses under the Community Sequencing Program supported by the Office of Science of the US Department of Energy under contract no. DE-AC02-05CH11231. We thank Jon Slate, Terry Burke, Julie Gray, David Beerling, Richard Leegood, and Christian Parisod for useful discussions and critical comments on this manuscript.

Received: December 5, 2011
Revised: January 12, 2012
Accepted: January 25, 2012
Published online: February 16, 2012

References

1. Ehleringer, J.R., Sage, R.F., Flanagan, L.B., and Pearcy, R.W. (1991). Climate change and the evolution of C_4 photosynthesis. Trends Ecol. Evol. (Amst.) *6*, 95–99.
2. Sage, R.F. (2004). The evolution of C_4 photosynthesis. New Phytol. *161*, 341–370.
3. Edwards, E.J., Osborne, C.P., Strömberg, C.A.E., Smith, S.A., Bond, W.J., Christin, P.A., Cousins, A.B., Duvall, M.R., Fox, D.L., Freckleton, R.P., et al.; C4 Grasses Consortium. (2010). The origins of C_4 grasslands: integrating evolutionary and ecosystem science. Science *328*, 587–591.
4. Finnegan, P.M., Suzuki, S., Ludwig, M., and Burnell, J.N. (1999). Phosphoenolpyruvate carboxykinase in the C_4 monocot *Urochloa panicoides* is encoded by four differentially expressed genes. Plant Physiol. *120*, 1033–1042.
5. Hibberd, J.M., and Quick, W.P. (2002). Characteristics of C4 photosynthesis in stems and petioles of C3 flowering plants. Nature *415*, 451–454.
6. Monson, R.K. (2003). Gene duplication, neofunctionalization, and the evolution of C4 photosynthesis. Int. J. Plant Sci. *164*, S43–S54.
7. Svensson, P., Bläsing, O.E., and Westhoff, P. (2003). Evolution of C_4 phosphoenolpyruvate carboxylase. Arch. Biochem. Biophys. *414*, 180–188.
8. Christin, P.A., Salamin, N., Savolainen, V., Duvall, M.R., and Besnard, G. (2007). C_4 Photosynthesis evolved in grasses via parallel adaptive genetic changes. Curr. Biol. *17*, 1241–1247.
9. Christin, P.A., Petitpierre, B., Salamin, N., Büchi, L., and Besnard, G. (2009). Evolution of C_4 phosphoenolpyruvate carboxykinase in grasses, from genotype to phenotype. Mol. Biol. Evol. *26*, 357–365.
10. Wang, X., Gowik, U., Tang, H., Bowers, J.E., Westhoff, P., and Paterson, A.H. (2009). Comparative genomic analysis of C4 photosynthetic pathway evolution in grasses. Genome Biol. *10*, R68.
11. Hibberd, J.M., and Covshoff, S. (2010). The regulation of gene expression required for C_4 photosynthesis. Annu. Rev. Plant Biol. *61*, 181–207.
12. Brown, N.J., Newell, C.A., Stanley, S., Chen, J.E., Perrin, A.J., Kajala, K., and Hibberd, J.M. (2011). Independent and parallel recruitment of preexisting mechanisms underlying C_4 photosynthesis. Science *331*, 1436–1439.
13. Ibrahim, D.G., Burke, T., Ripley, B.S., and Osborne, C.P. (2009). A molecular phylogeny of the genus *Alloteropsis* (Panicoideae, Poaceae) suggests an evolutionary reversion from C_4 to C_3 photosynthesis. Ann. Bot. (Lond.) *103*, 127–136.
14. Christin, P.A., Freckleton, R.P., and Osborne, C.P. (2010). Can phylogenetics identify C_4 origins and reversals? Trends Ecol. Evol. (Amst.) *25*, 403–409.
15. Clayton, W.D. (1979). Notes on *Setaria* (*Gramineae*). Kew Bull. *33*, 501–509.
16. Liebenberg, E.J.L., and Fossey, A. (2001). Comparative cytogenetic investigation of the two subspecies of the grass *Alloteropsis semialata* (Poaceae). Bot. J. Linn. Soc. *137*, 243–248.
17. Adams, K.L., Cronn, R., Percifield, R., and Wendel, J.F. (2003). Genes duplicated by polyploidy show unequal contributions to the transcriptome and organ-specific reciprocal silencing. Proc. Natl. Acad. Sci. USA *100*, 4649–4654.
18. Akhunova, A.R., Matniyazov, R.T., Liang, H., and Akhunov, E.D. (2010). Homoeolog-specific transcriptional bias in allopolyploid wheat. BMC Genomics *11*, 505.
19. Keeling, P.J., and Palmer, J.D. (2008). Horizontal gene transfer in eukaryotic evolution. Nat. Rev. Genet. *9*, 605–618.
20. Bock, R. (2010). The give-and-take of DNA: horizontal gene transfer in plants. Trends Plant Sci. *15*, 11–22.
21. Davis, C.C., and Wurdack, K.J. (2004). Host-to-parasite gene transfer in flowering plants: phylogenetic evidence from Malpighiales. Science *305*, 676–678.
22. Yoshida, S., Maruyama, S., Nozaki, H., and Shirasu, K. (2010). Horizontal gene transfer by the parasitic plant *Striga hermonthica*. Science *328*, 1128.
23. Dunning Hotopp, J.C., Clark, M.E., Oliveira, D.C.S.G., Foster, J.M., Fischer, P., Muñoz Torres, M.C., Giebel, J.D., Kumar, N., Ishmael, N., Wang, S., et al. (2007). Widespread lateral gene transfer from intracellular bacteria to multicellular eukaryotes. Science *317*, 1753–1756.
24. Diao, X., Freeling, M., and Lisch, D. (2006). Horizontal transfer of a plant transposon. PLoS Biol. *4*, e5.
25. Vallenback, P., Ghatnekar, L., and Bengtsson, B.O. (2010). Structure of the natural transgene *PgiC2* in the common grass *Festuca ovina*. PLoS ONE *5*, e13529.
26. Ammiraju, J.S.S., Lu, F., Sanyal, A., Yu, Y., Song, X., Jiang, N., Pontaroli, A.C., Rambo, T., Currie, J., Collura, K., et al. (2008). Dynamic evolution of *oryza* genomes is revealed by comparative genomic analysis of a genus-wide vertical data set. Plant Cell *20*, 3191–3209.
27. Bennetzen, J.L. (2000). Transposable element contributions to plant gene and genome evolution. Plant Mol. Biol. *42*, 251–269.
28. Stegemann, S., and Bock, R. (2009). Exchange of genetic material between cells in plant tissue grafts. Science *324*, 649–651.
29. Laurie, D.A., and Bennett, M.D. (1986). Wheat x maize hybridization. Can. J. Genet. Cytol. *28*, 313–316.
30. Riera-Lizarazu, O., Rines, H.W., and Phillips, R.L. (1996). Cytological and molecular characterization of oat x maize partial hybrids. Theor. Appl. Genet. *93*, 123–135.
31. Talianova, M., and Janousek, B. (2011). What can we learn from tobacco and other Solanaceae about horizontal DNA transfer? Am. J. Bot. *98*, 1231–1242.
32. Besnard, G., Muasya, A.M., Russier, F., Roalson, E.H., Salamin, N., and Christin, P.A. (2009). Phylogenomics of C_4 photosynthesis in sedges (Cyperaceae): multiple appearances and genetic convergence. Mol. Biol. Evol. *26*, 1909–1919.