Necessity is the mother of *re*-invention

The parallel evolution of C_4 photosynthesis

Steve Kelly
www.stevekellylab.com

Research summary

Evolution of gene expression regulation

Evolution of genome organisation and composition

Methods for transcriptomics, genomics and phylogenetics

Genetic regulation of photosynthesis and engineering photosynthesis in rice

Outline

- Introduction to convergent and parallel evolution in the tree of life

Biological properties of organisms poorly correlated with their evolutionary relationship.

- Introduction to C_4 photosynthesis

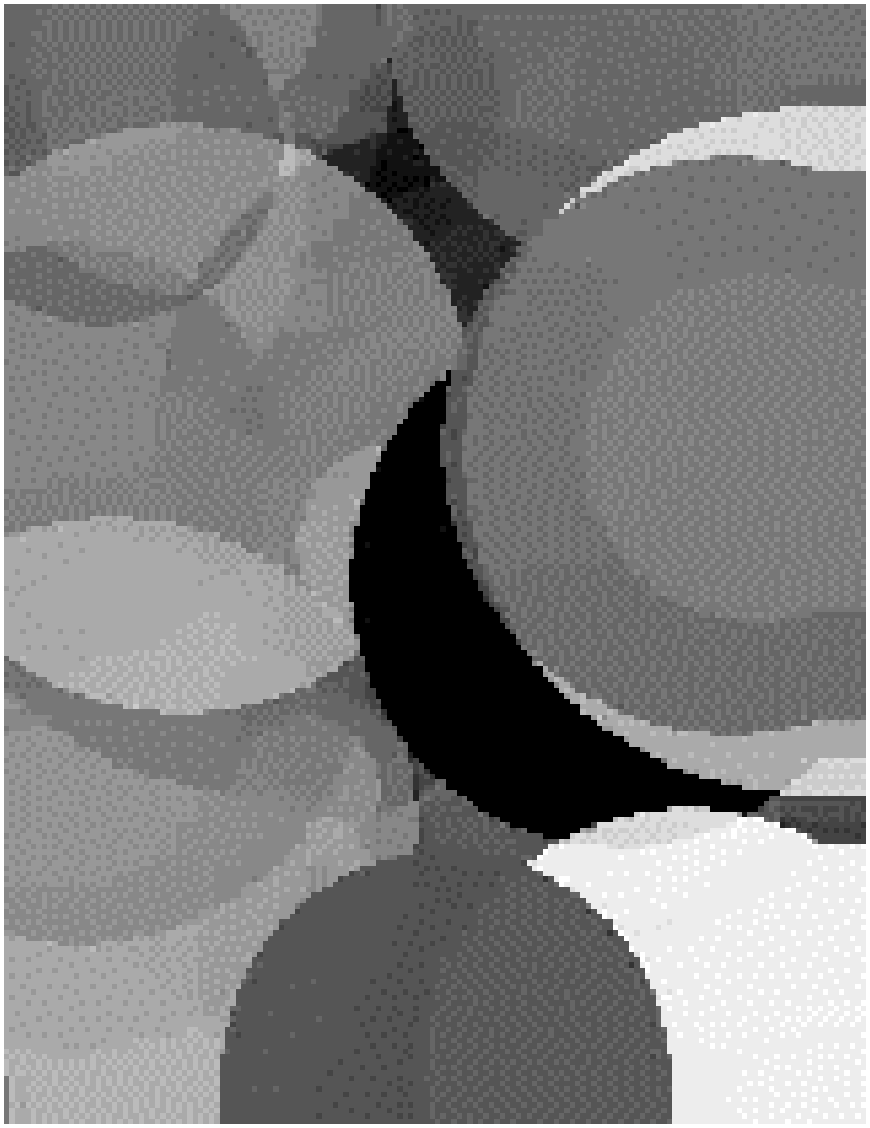
What, why, when, & who?

- Parallel changes in gene expression

Changes in the expression of the same genes in different origins of C_4 photosynthesis.

- Parallel evolution of new genes by duplication

Parallel duplication of the same genes in multiple independent origins of C_4 photosynthesis.



Same selective pressure leads to the same or similar solutions

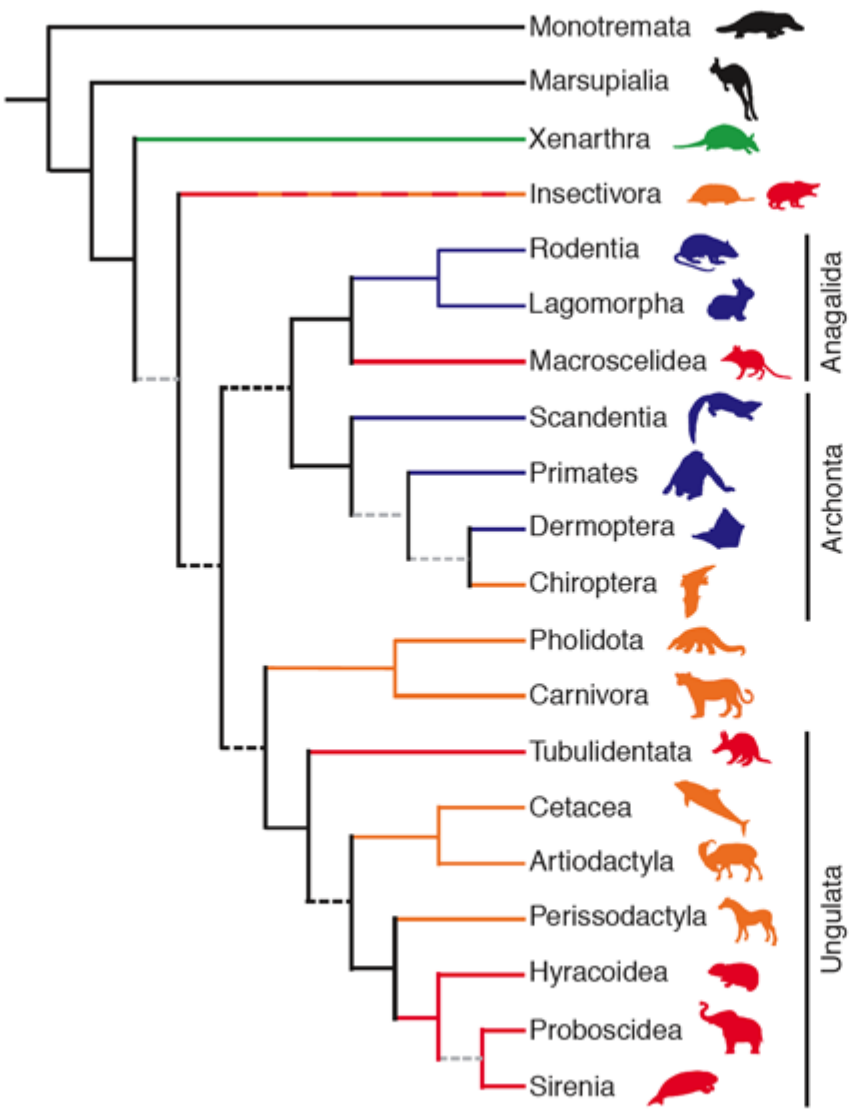
Original

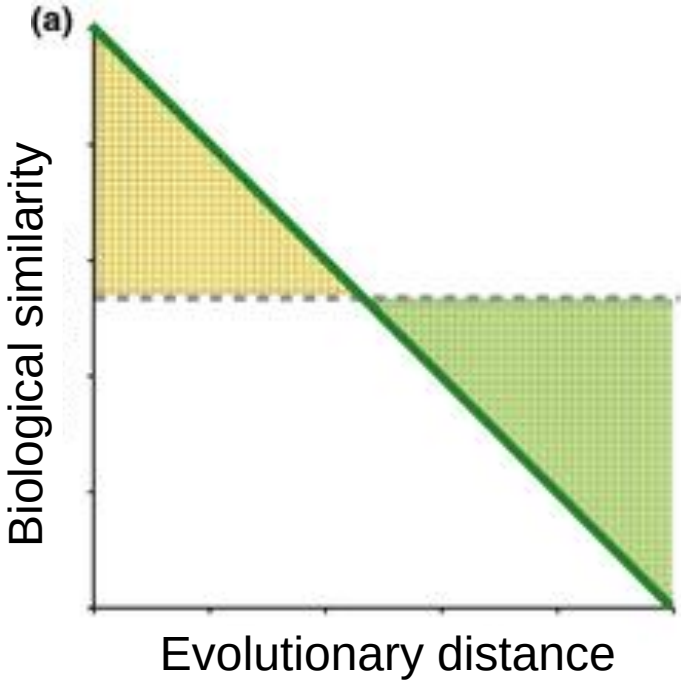
128 circles

256 circles

There are a limited number of environments on earth

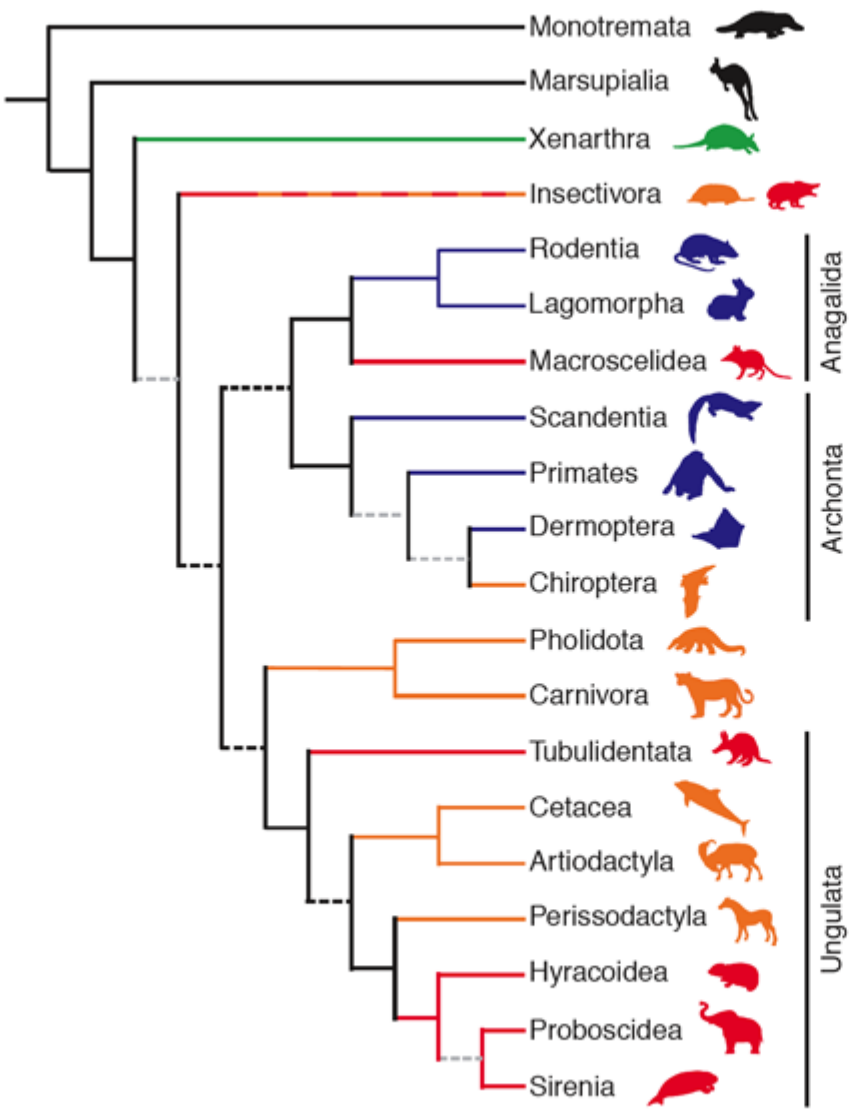
Our expectation is that close relatives have more similar biology

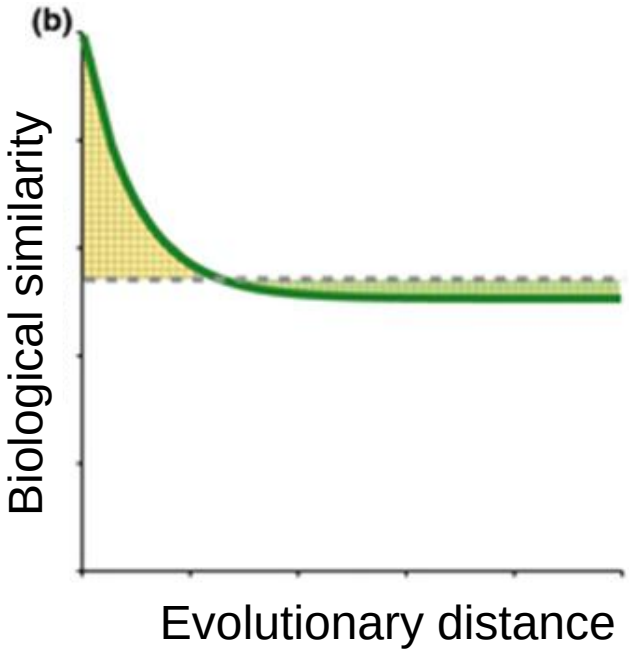




Kelly, S. *et al. Diversity and Distributions* 2014

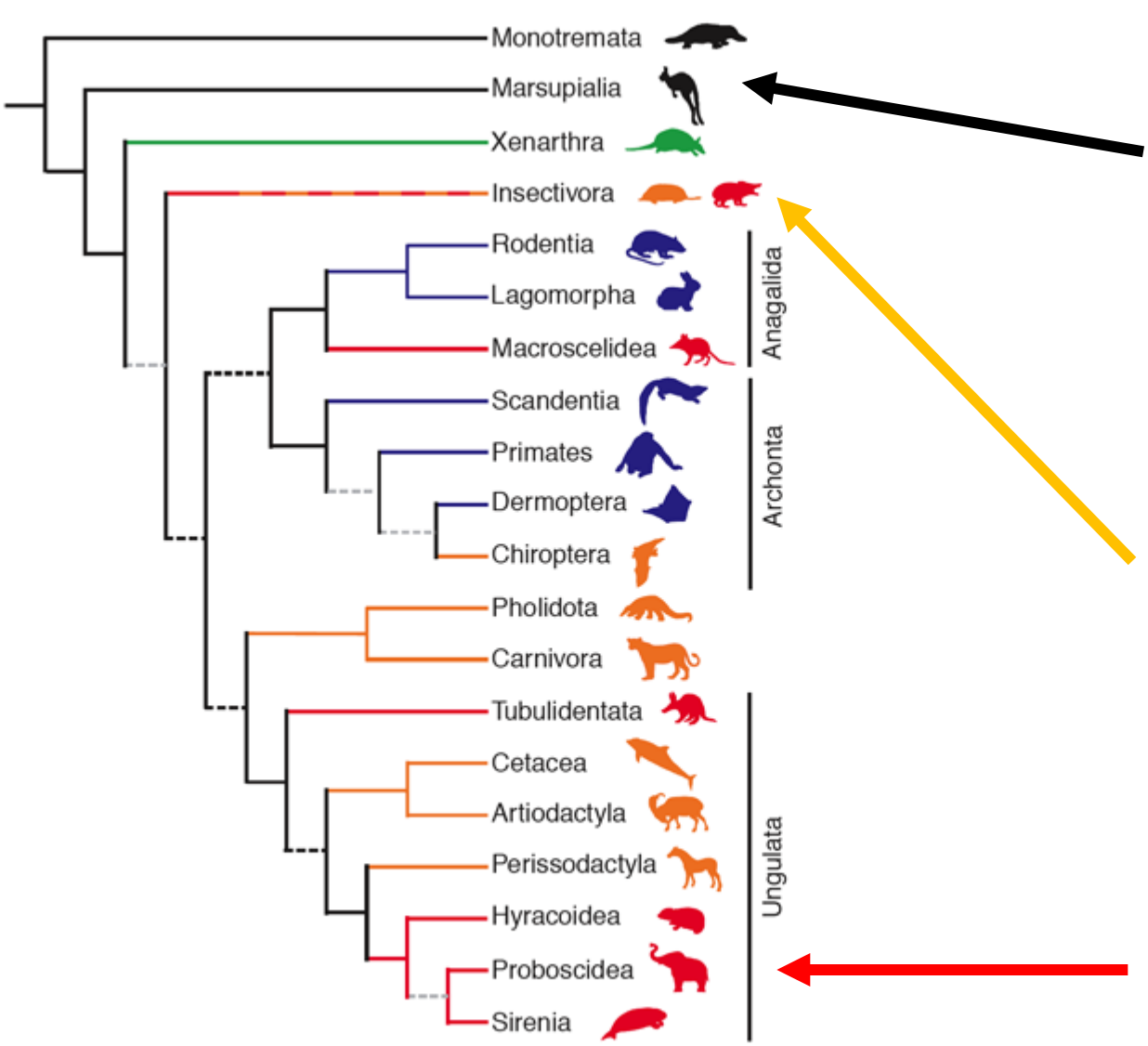
Adapting to same environment => distant relatives evolve same biology





Kelly, S *et al. Diversity and Distributions* 2014

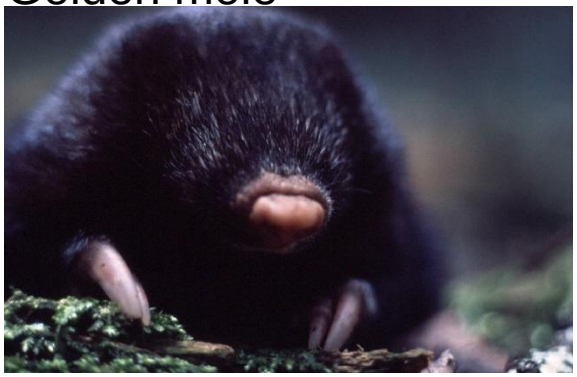
Adapting to same environment => distant relatives evolve same biology



Marsupial mole

European mole

Golden mole



Multiple examples of parallel/convergent evolution

Species	Kingdom	Taxonomic level	Phenotype	Types of evolution	Genes	Type of gene	Refs
ΦX174	Virus	Intraspecific (experimental evolution)	Adaptation to high temperature and a novel host	Parallel evolution	Multiple genes	NA	21,22
HIV	Virus	Intraspecific	Antiretroviral resistance	Parallel evolution	Reverse transcriptase gene	Effector	88,89
Escherichia coli	Monera	Intraspecific (experimental evolution)	Adaptation to glucose-limited medium	Parallel evolution	Multiple genes	NA	20
		Intraspecific (experimental evolution)	Adaptation to glycerol-based medium	Parallel evolution	Glycerol kinase (*glpK*) and RNA polymerase genes	Effector	86
Pseudomonas aeruginosa	Monera	Intraspecific (experimental evolution)	Adaptation to novel environments	Parallel evolution	Multiple genes	NA	24
		Intraspecific (experimental evolution)	Hyperswarming	Parallel evolution	Flagella synthesis regulator (*fleN*)	Regulatory	23
Saccharomyces cerevisiae	Fungi	Intraspecific (experimental evolution)	Adaptation to fluctuating glucose and galactose levels	Parallel evolution	*GAL80*	Regulatory	93
Diverse species of yeast	Fungi	Interspecific	Loss of galactose utilization	Parallel evolution	*GAL* genes	Regulatory	94
Ipomoea horsfalliae and *Ipomoea quamoclit*	Plantae	Intergeneric	Evolution of red flowers from blue flowers	Parallel evolution	Flavonoid 3′-hydroxylase	Effector	95
Arabidopsis thaliana and *Arabidopsis lyrata*	Plantae	Intraspecific and interspecific	Vernalization	Parallel evolution	*FRIGIDA*	Regulatory	96,97
Plants (multiple species)	Plantae	Interspecific	C_4 photosynthesis	Parallel evolution	Phosphoenolpyruvate carboxylases (PEPC) genes	Effector	43,98, 99
Alloteropsis spp. grasses	Plantae	Interspecific	C_4 photosynthesis	Collateral evolution by hybridization	PEPC and phophoenolpyruvate carboxykinase genes	Effector	52
Human (*Homo sapiens*)	Animalia	Intraspecific	Resistance to malaria	Parallel evolution	Glucose-6-phosphate dehydrogenase (*G6PD*)	Effector	100
		Intraspecific	Lactase persistence	Parallel evolution	Lactase (*LCT*)	Effector	101

Stern, D.L., Nature reviews genetics 2013, 14, 751

Exploit parallel evolution to understand fundamental biology

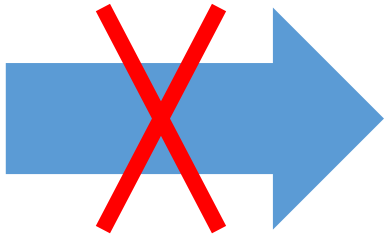
What are the genetic factors that underpin these re-invented traits?

Can we use this knowledge to engineer these traits into other organisms?

Exploit parallel evolution to understand fundamental biology

What are the genetic factors that underpin these re-invented traits?

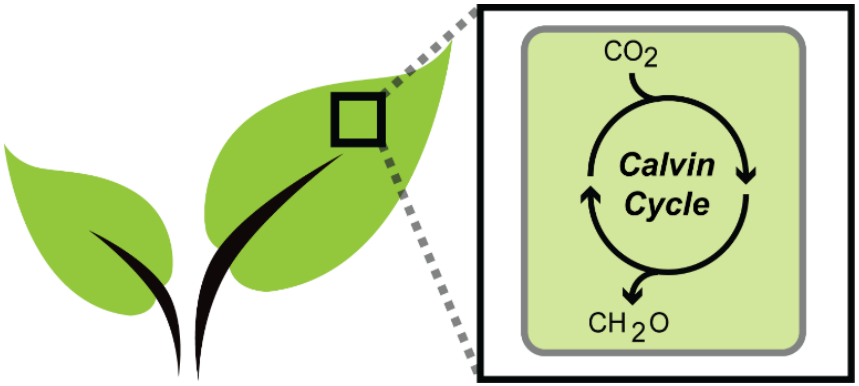
Can we use this knowledge to engineer these traits into other organisms?



C_4 photosynthesis

C_3 plants express all photosynthetic genes in all photosynthetic cells

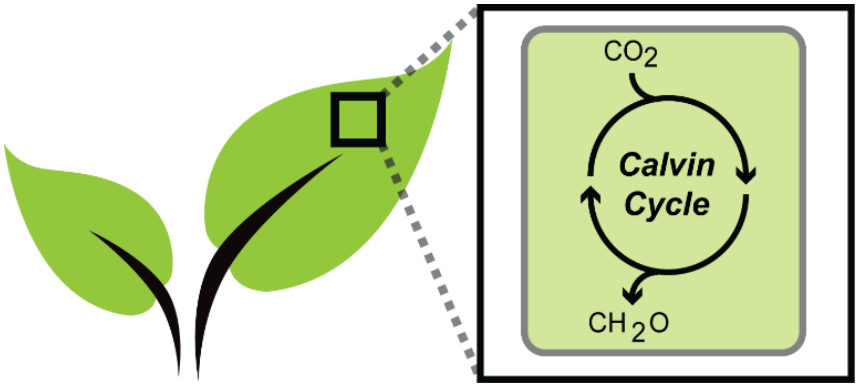
C_3



Photosynthesis in **one cell**

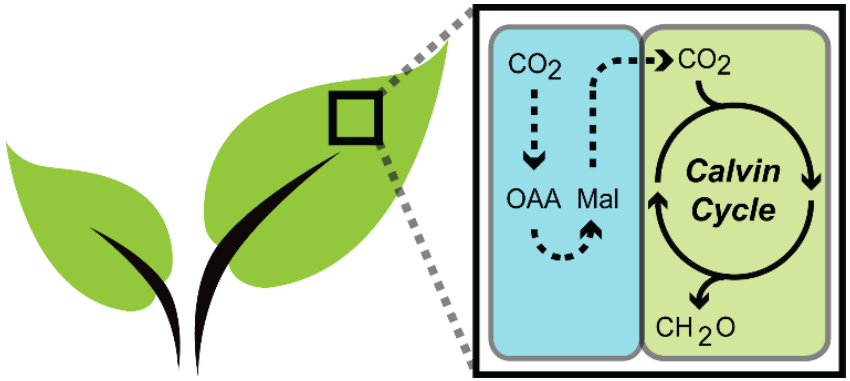
C_4 plants partition photosynthetic genes between specialised cells

C_3



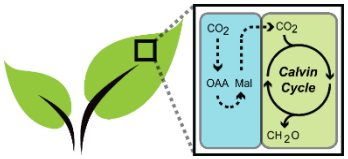
Photosynthesis in **one cell**

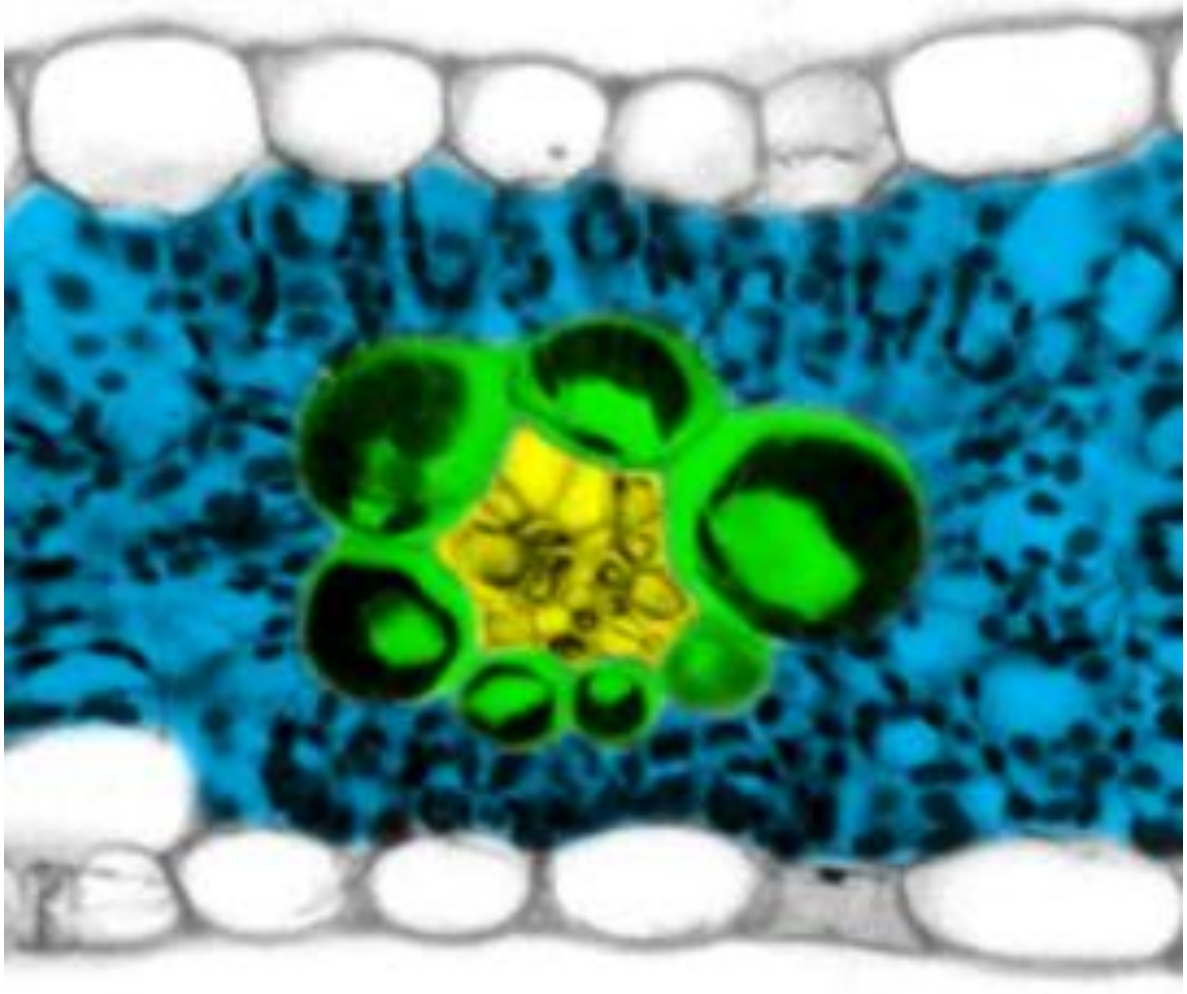
C_4



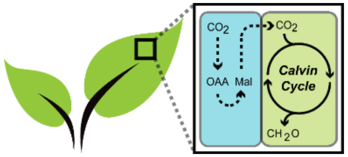
Photosynthesis distributed across **two cells**

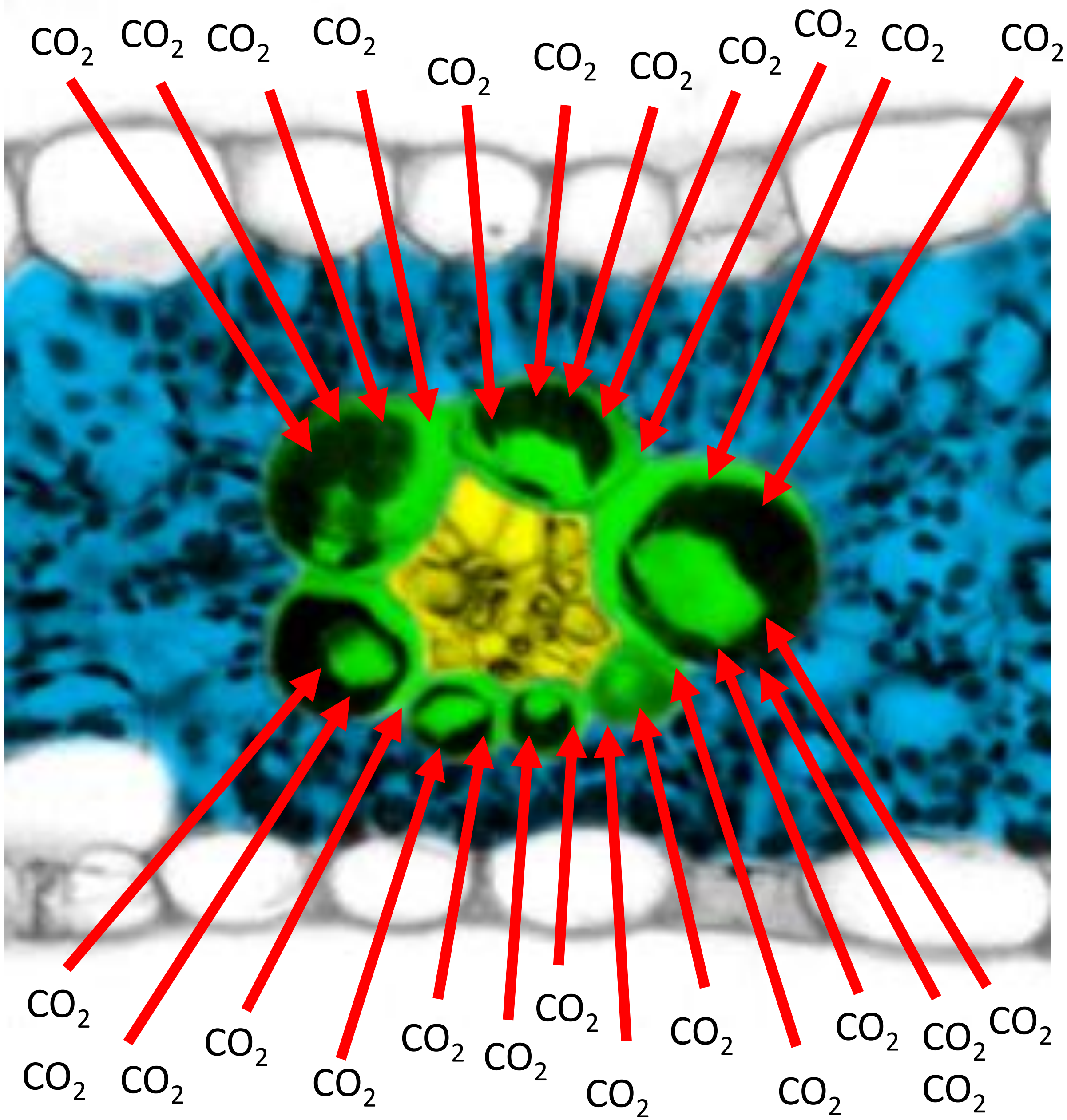
C_4 plants partition photosynthetic genes between specialised cells



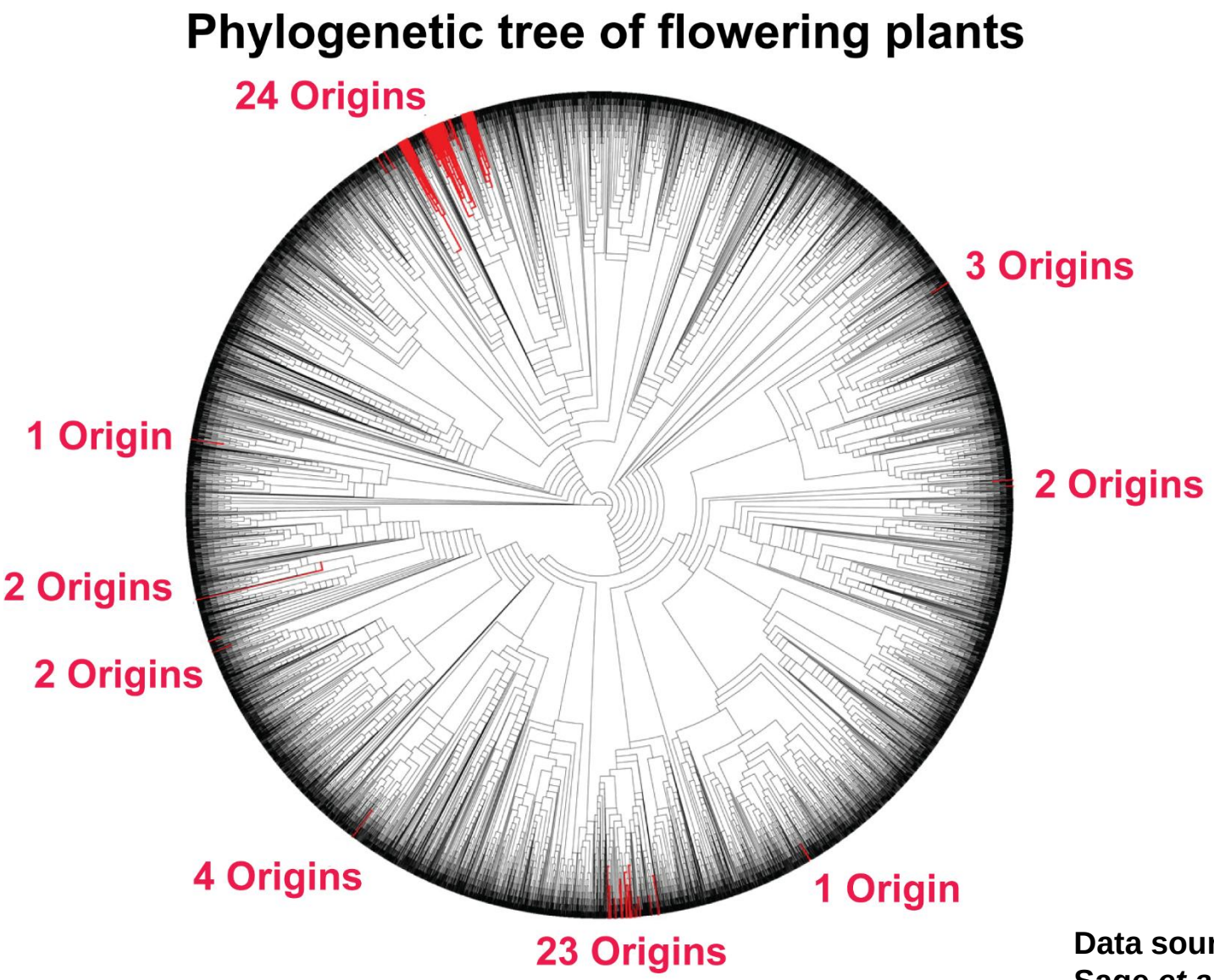


C_4 plants partition photosynthetic genes between specialised cells



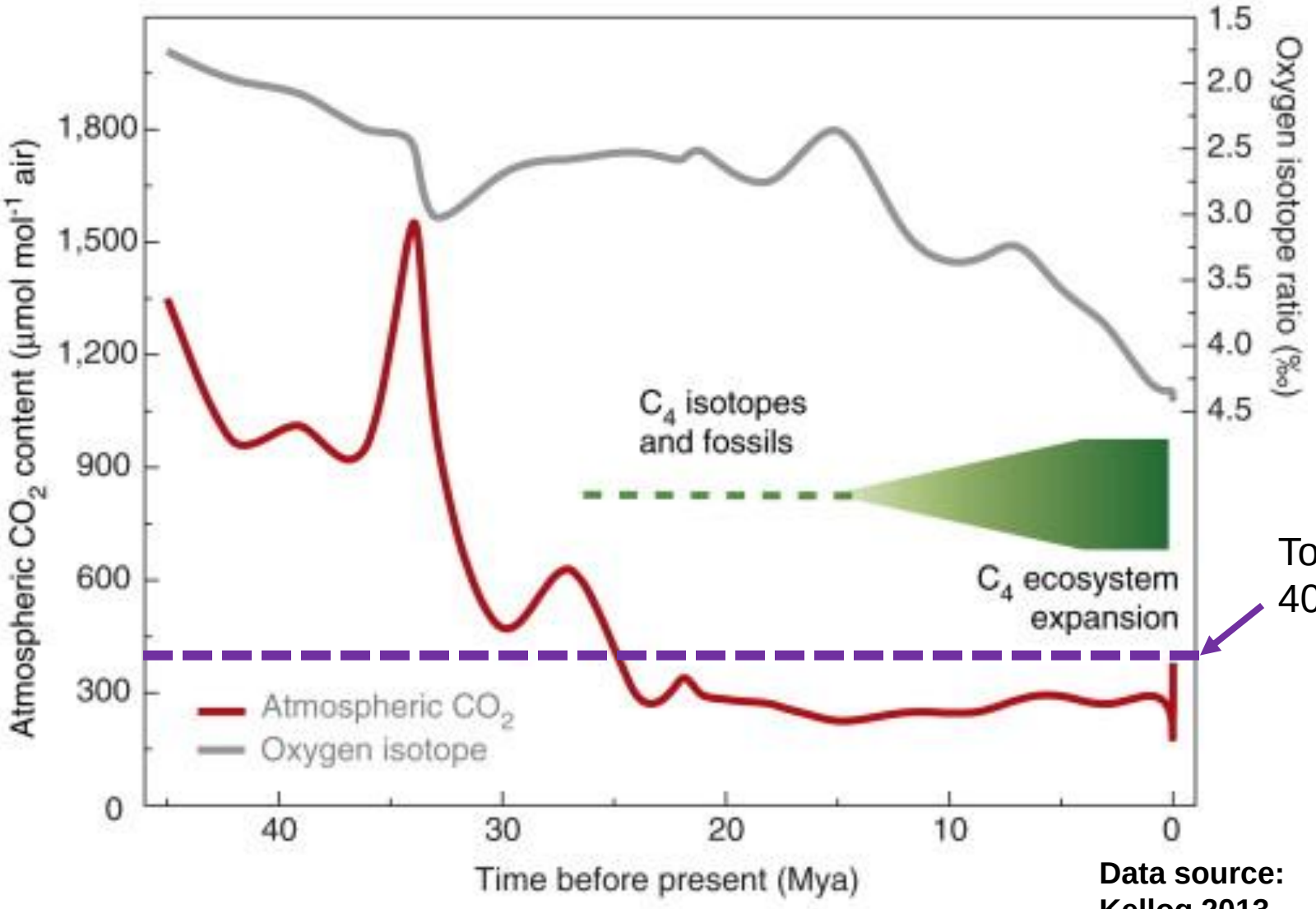


C_4 photosynthetic partitioning evolved independently >60 times



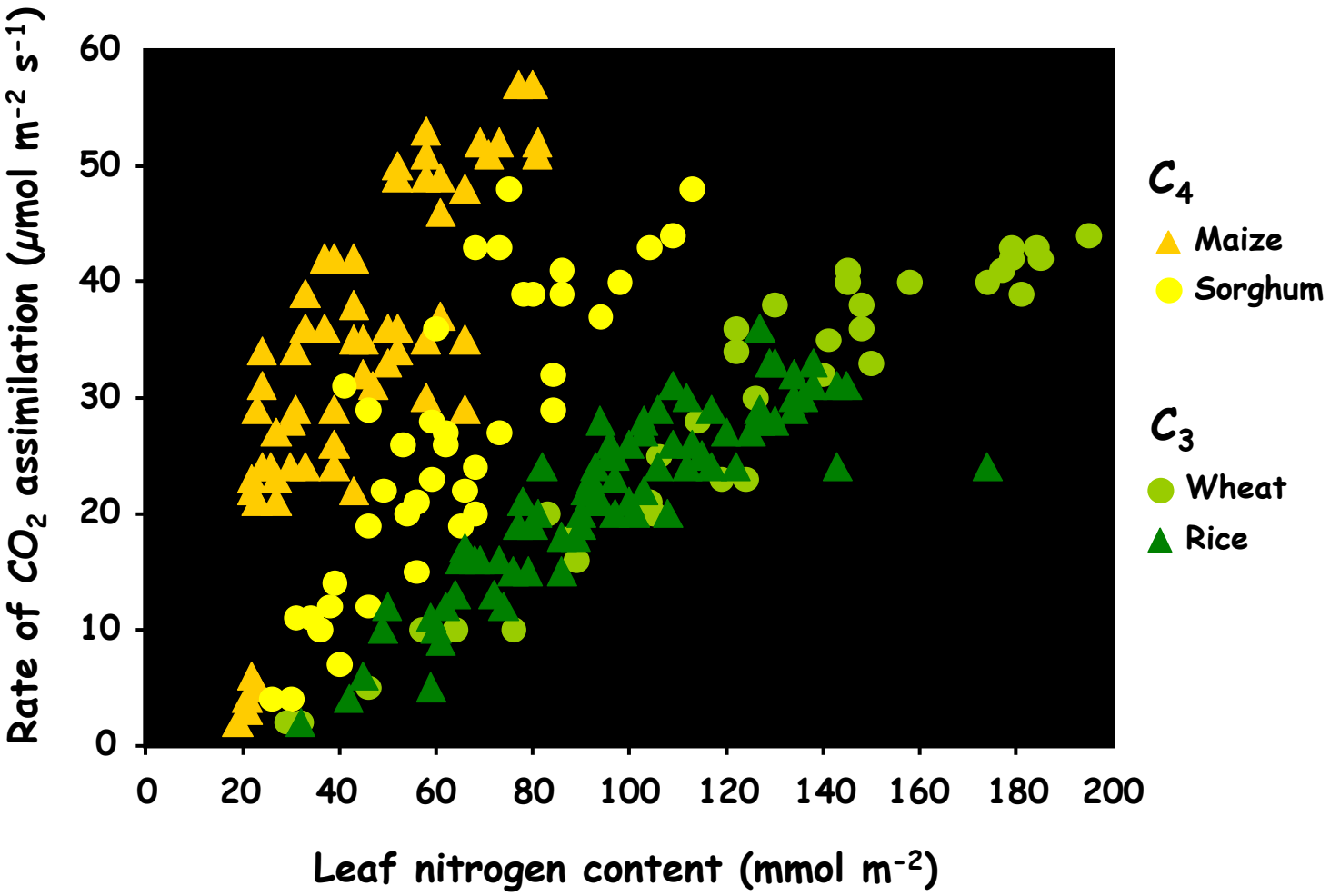
Data source:
Sage *et al.* 2011

C_4 photosynthesis evolved after a substantial drop in atmospheric CO_2



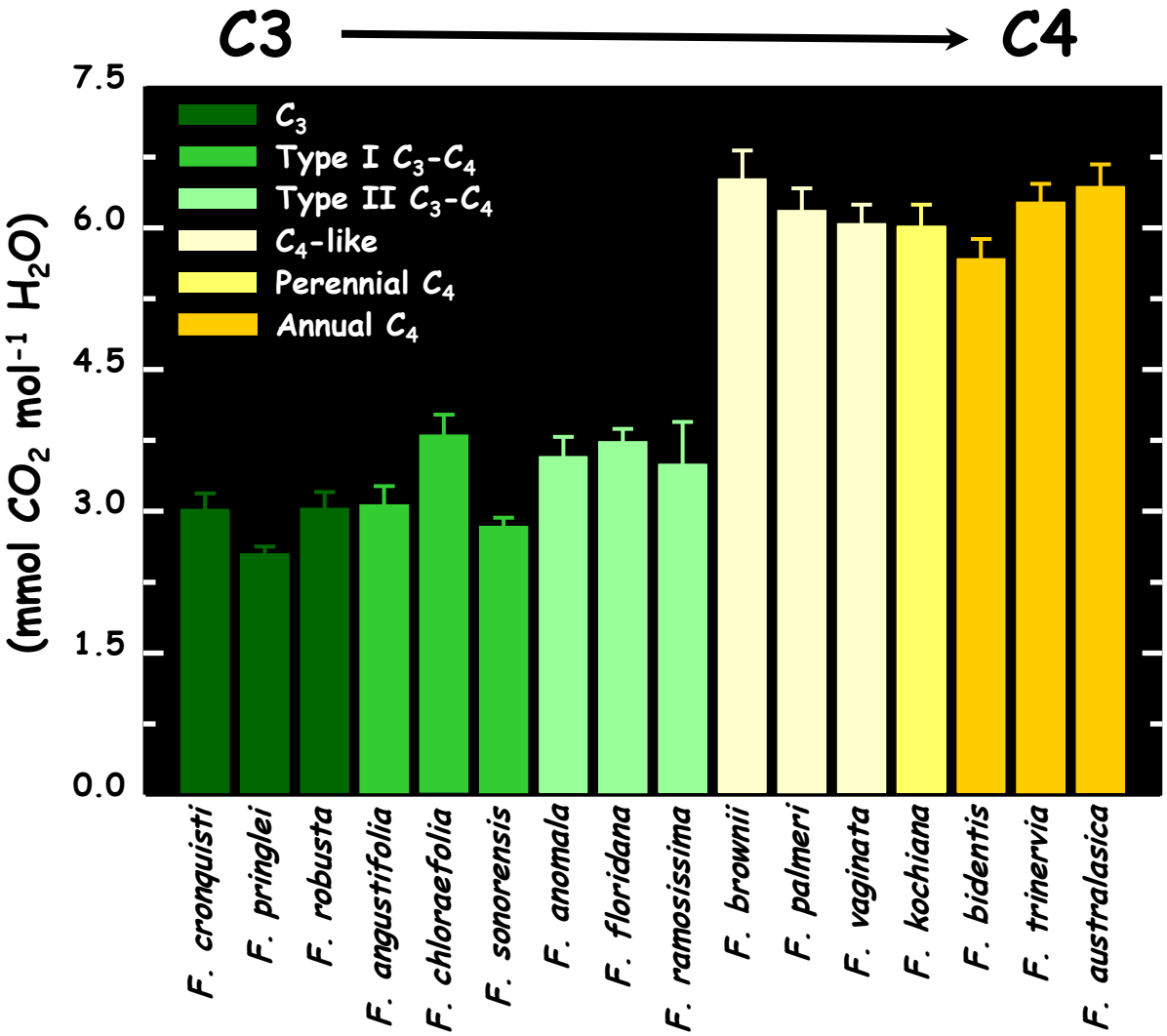
Data source:
Kellog 2013
Current Biology 23 (14)

Understanding the evolution of C_4 might be "useful"



Data Source:
Evans & von Caemmerer 2000

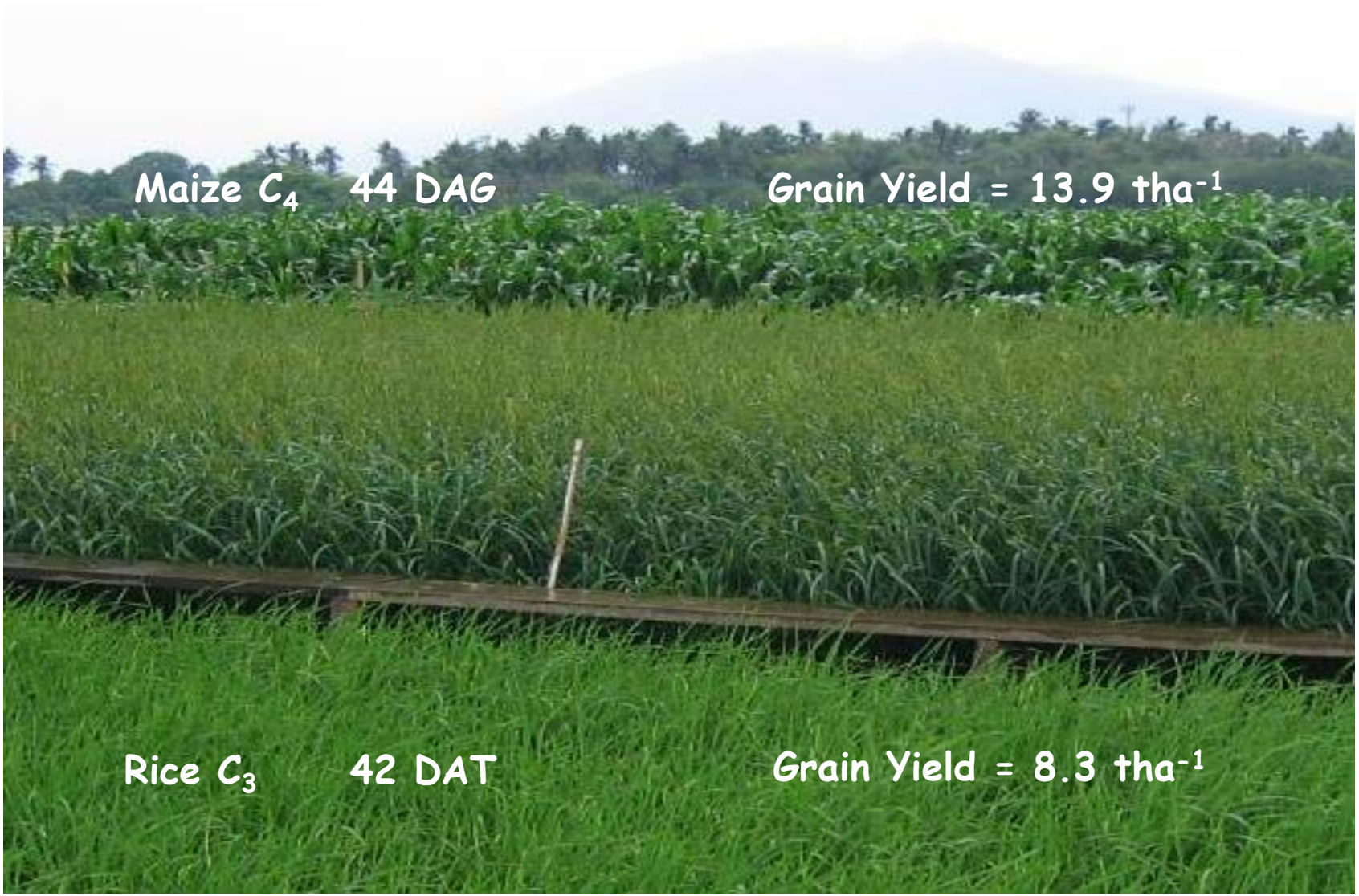
Understanding the evolution of C_4 might be "useful"



Species from genus *Flaveria*

Data Source:
Kocacinar et al 2008

Understanding the evolution of C_4 might be "useful"



What are the genetic causes of these re-invented traits?

Parallel changes in gene expression

Parallel duplication of genes

CRB-BLAST

Aubrey and Kelly *et al.* 2014
PLoS Genetics

Smith-Unna et al.
pre-sub available on
bioRxiv.org

OrthoFinder

Emms and Kelly 2015
GenomeBiology

iSplice

Novel splice intermediates
Kelly *et al.* 2015 **NAR**

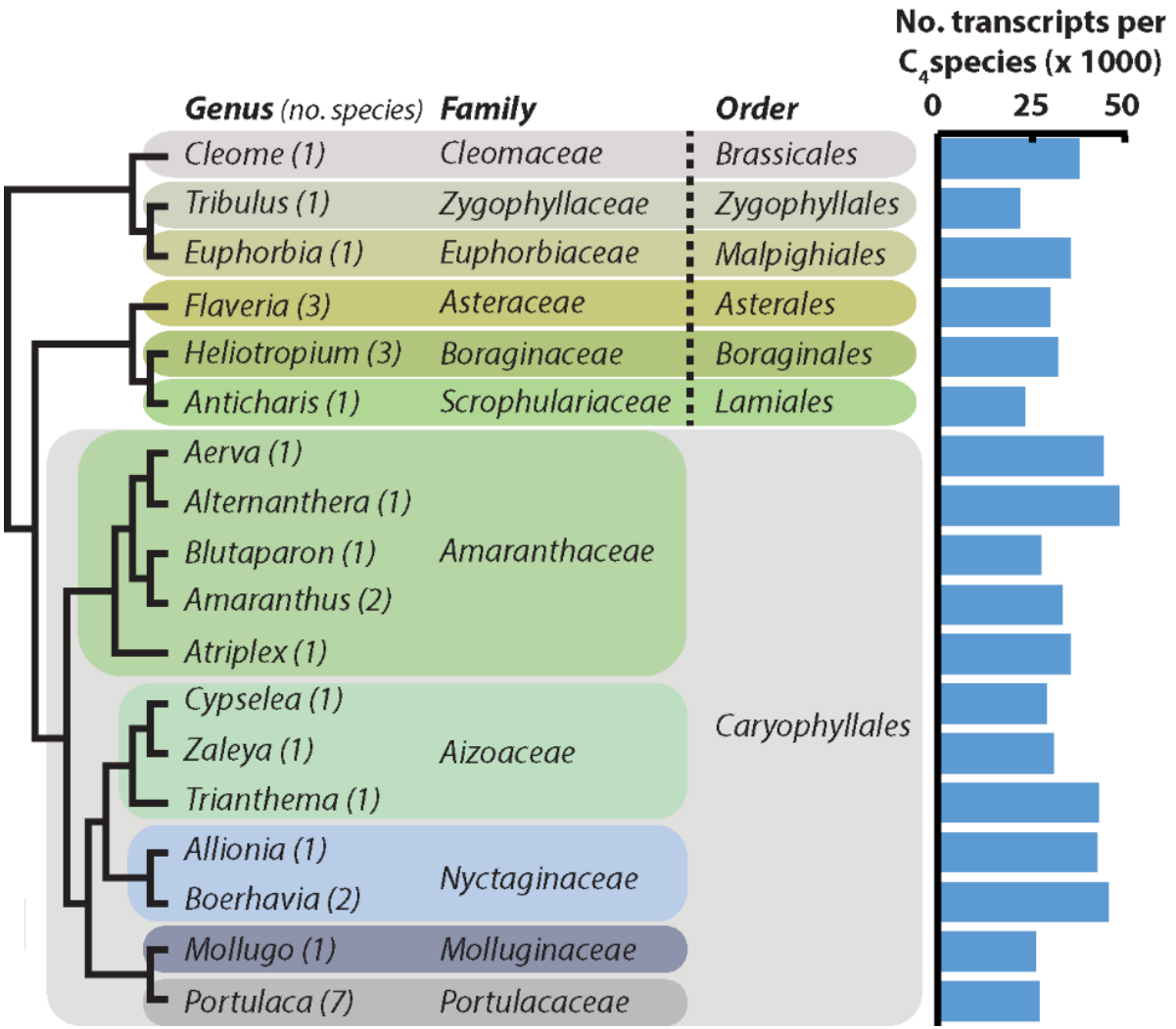
Alternative splicing
Kelly *et al.* 2015 **JMB**

http://www.stevekellylab.com/software/

Parallel changes in gene expression

Comparative transcriptomics of 63 different species (34 C_4 and 29 C_3)

2.2 million different genes (30,000 gene families)



7 orders

↓

11 families

↓

18 genera

↓

63 species

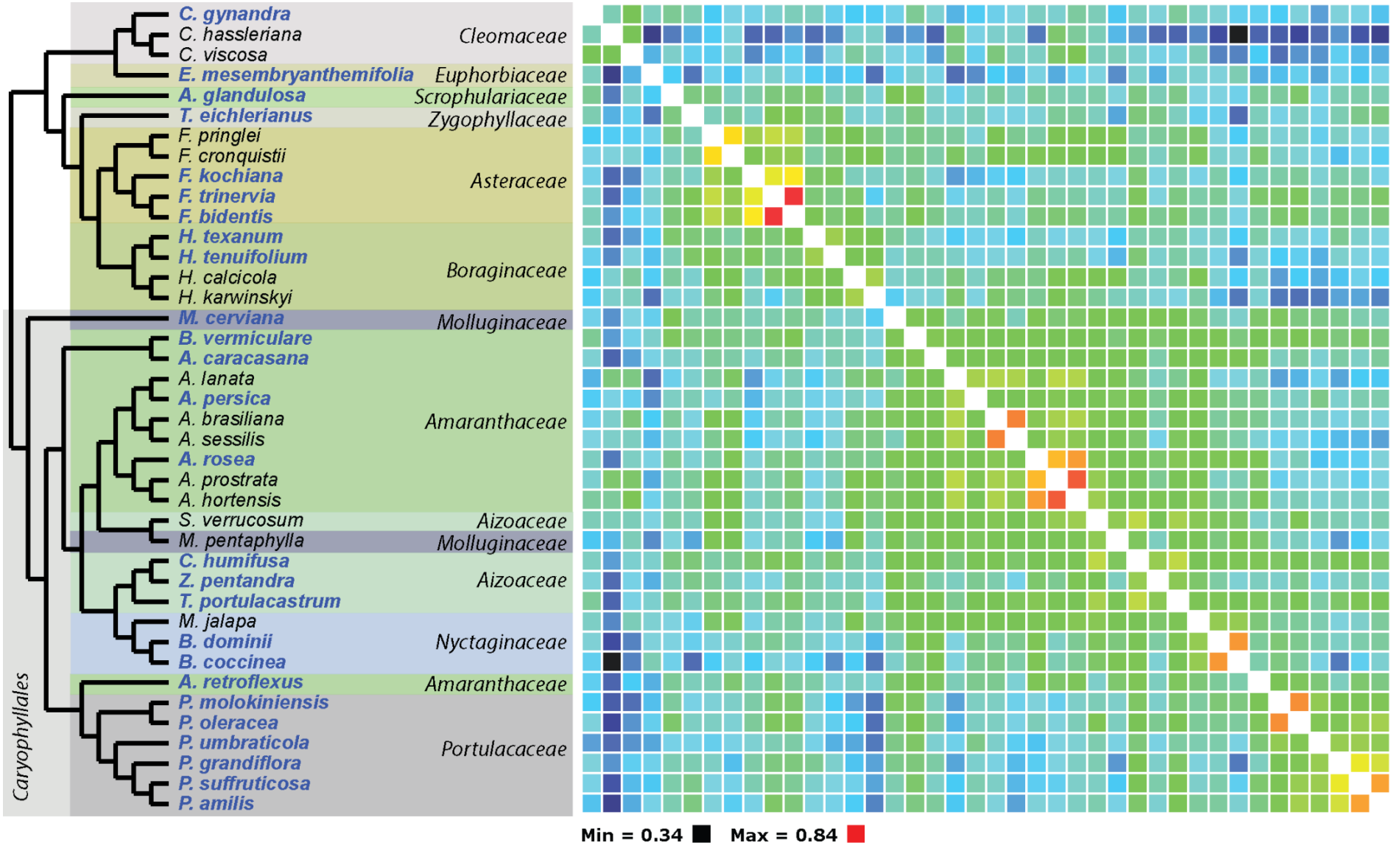
Sarah Covshoff
Vivek Thakur
Samart Wanchana

Julian Hibberd

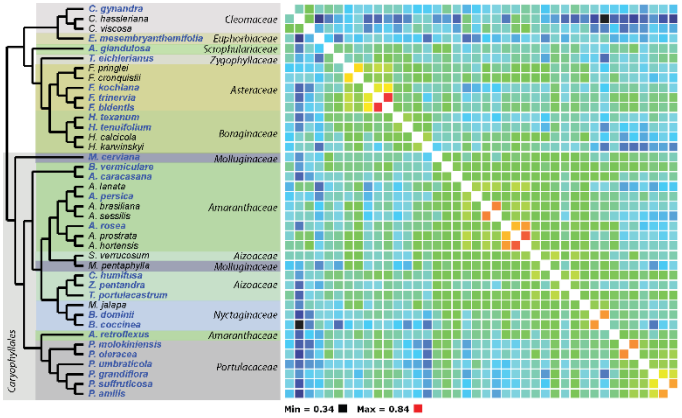
Paul Quick
Rowan Sage
Martha Ludwig
Gane Ka-Shu Wong

Parallel changes in gene expression

Global correlation in gene expression



149 parallel changes in gene expression are common to all C_4 dicots



113 genes up-regulated in all C_4 species

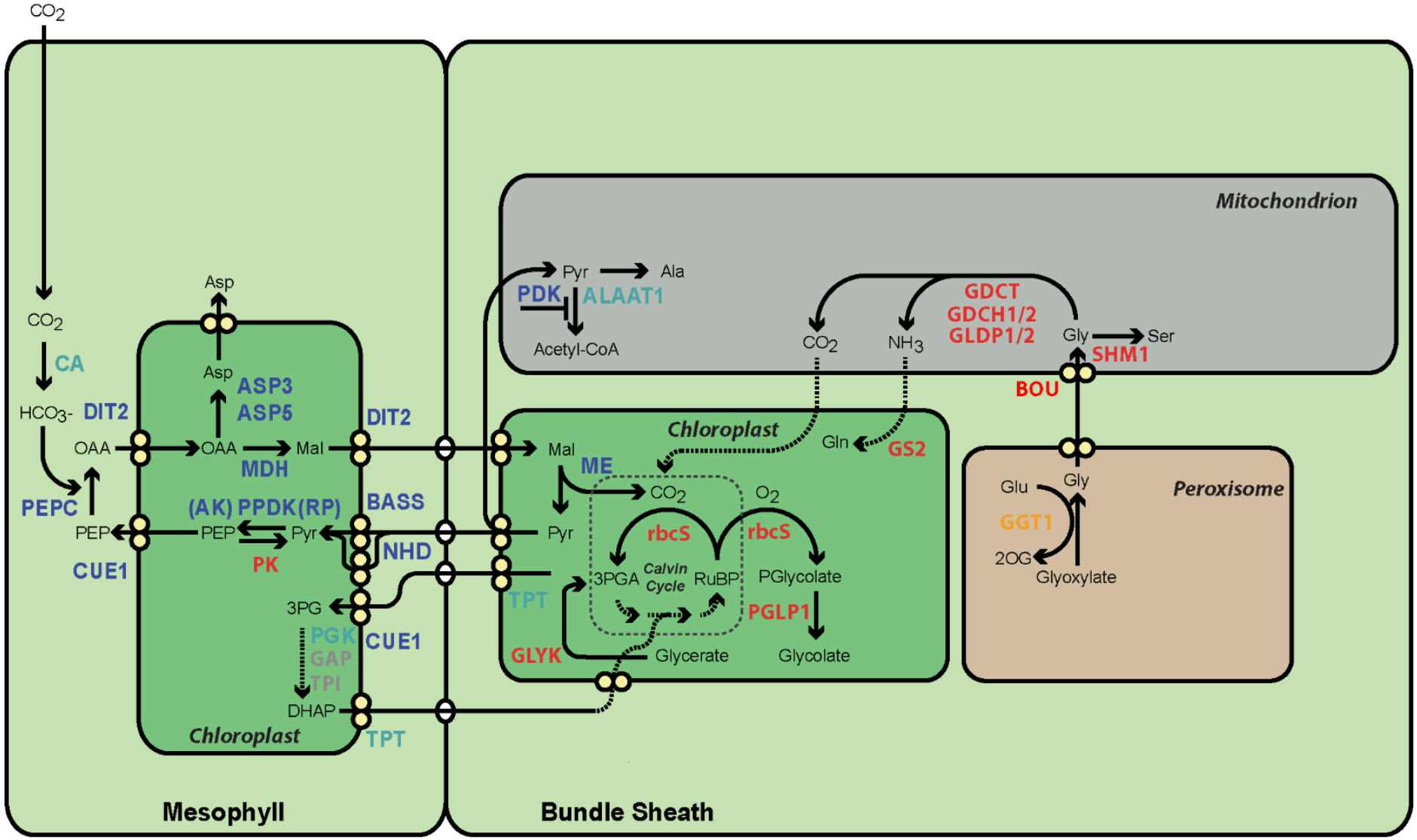
36 genes down-regulated in all C_4 species

Functional categorisation of differentially expressed genes

Category	Cell	Chloroplast	Mitochondrion
Metabolic components	15 (8)	29 (10)	6 (4)
Signalling components	18 (2)	7 (1)	0 (0)
Transporters	4 (1)	9 (1)	2 (0)
Transcription regulators	4 (1)	0 (2)	0 (0)
Post transcription regulators	3 (1)	0 (0)	0 (0)
Other	4 (0)	1 (2)	0 (0)
Unknown	9 (3)	1 (0)	0 (0)

n= 113, n = 36 No. up-regulated (No. down-regulated)

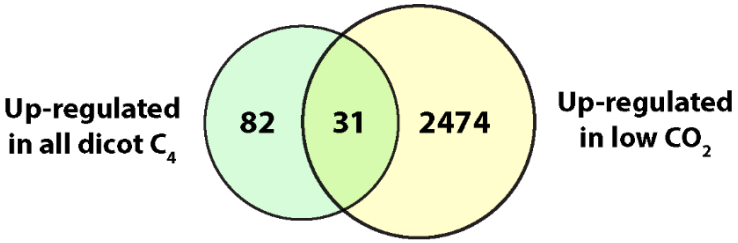
Changes include previously characterised C_4 cycle components



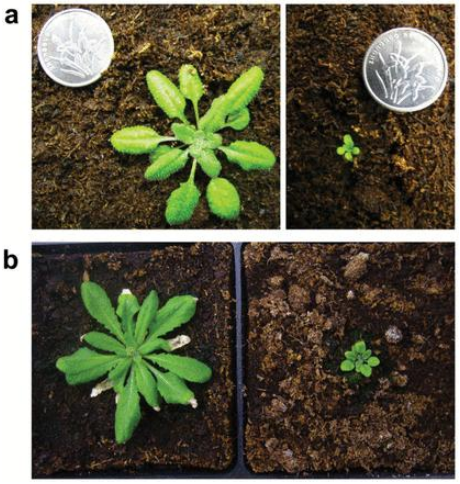
Blue = Up regulated in C_4 species Red = Down regulated in C_4 species

Changes provide clues into evolutionary drivers of C_4

Many C_4 up-regulated genes also up-regulated in arabidopsis in low CO_2

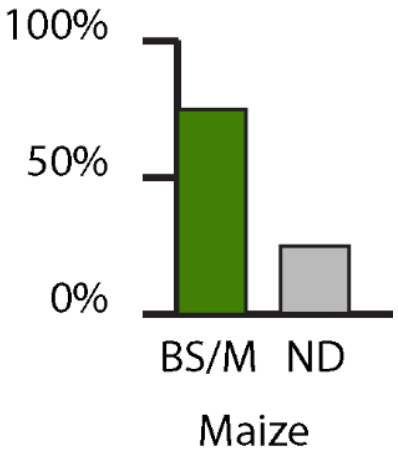


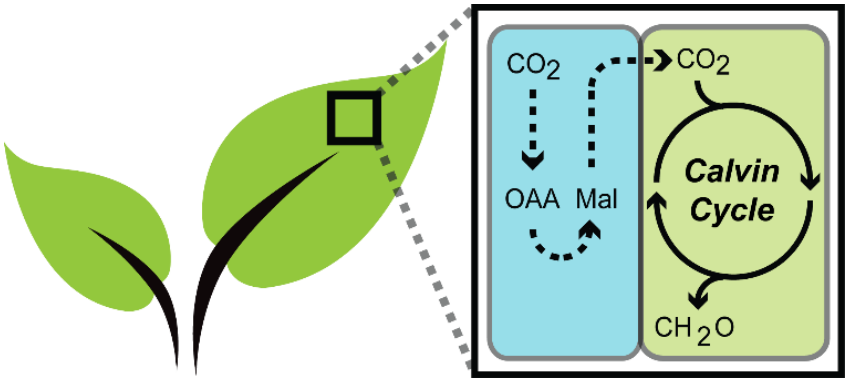
$p < 1 \times 10^{-5}$



Liu *et al.* (2014) *J. Exp. Bot.*

Many C_4 up-regulated genes preferentially expressed in BS or M cells





Maize: Chang *et al.* (2012) *Plant Physiol.*

Setaria: John *et al.* (2014) *Plant Physiol.*

Kelly *et al. in prep*

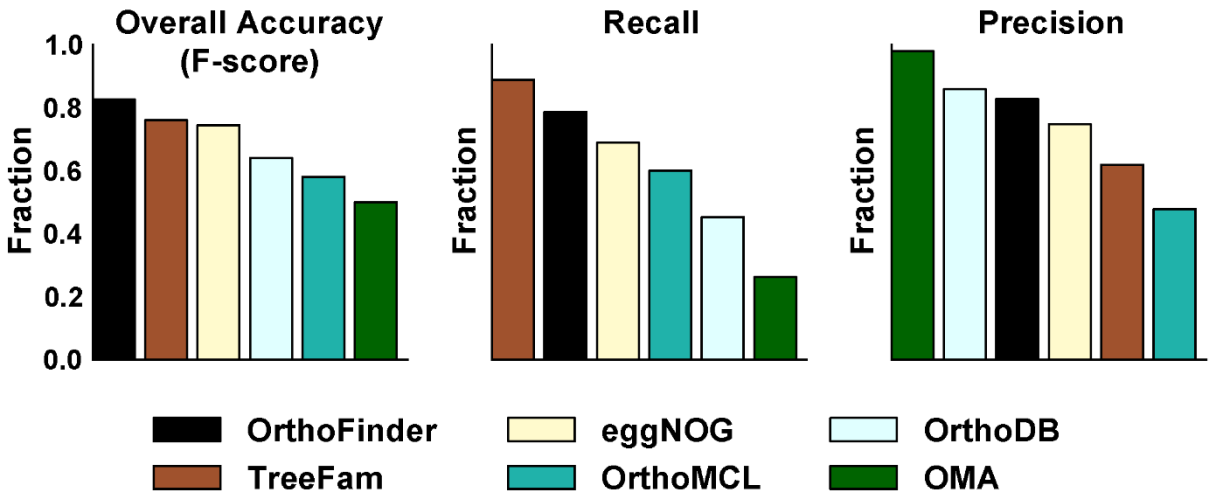
What are the genetic causes of these re-invented traits?

Parallel changes in gene expression

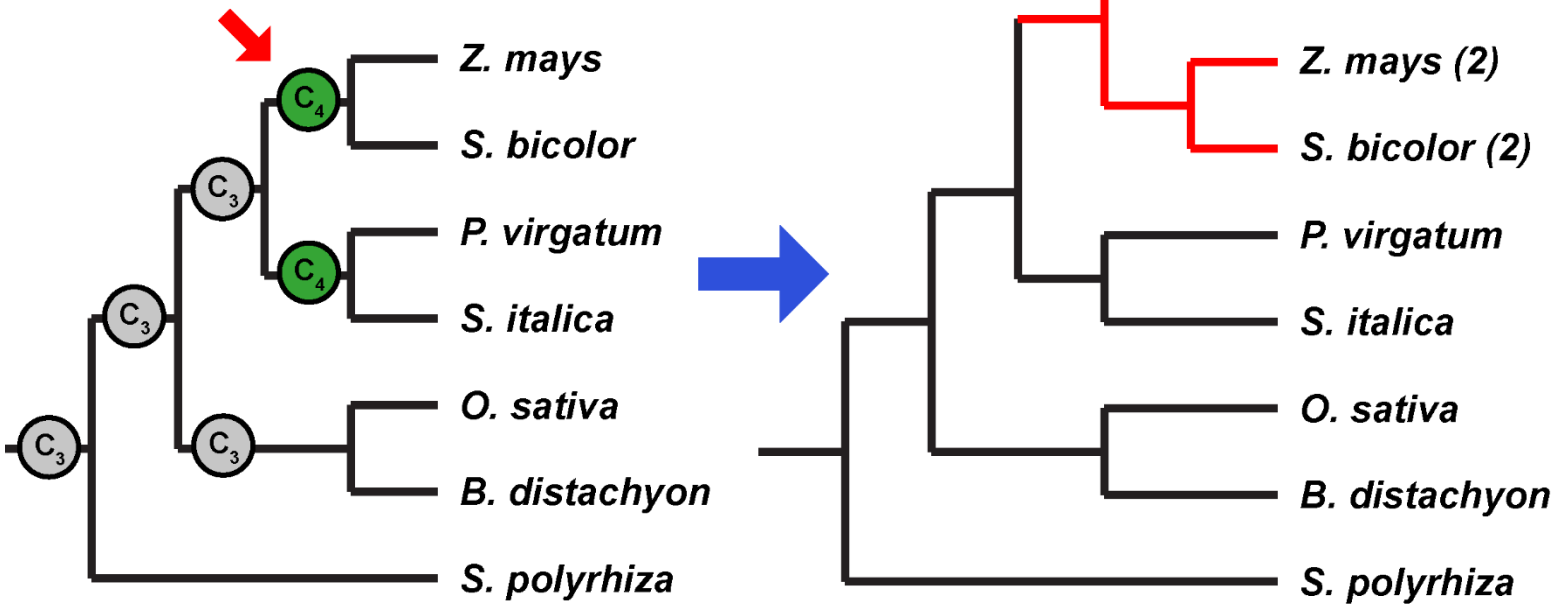
Parallel duplication of genes

OrthoFinder

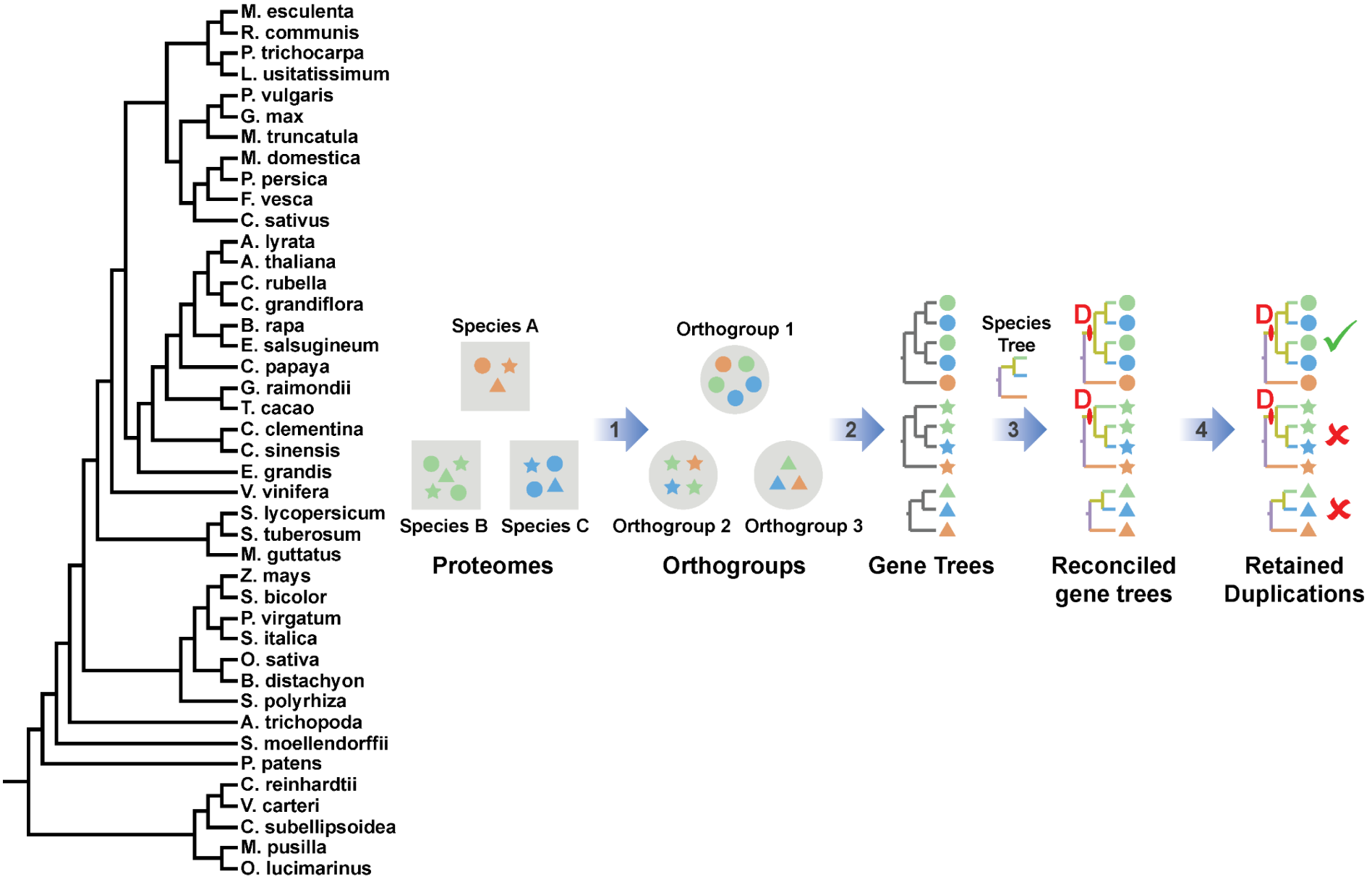
Emms and Kelly 2015
GenomeBiology



Which genes duplicated coincident with the evolution of C_4?

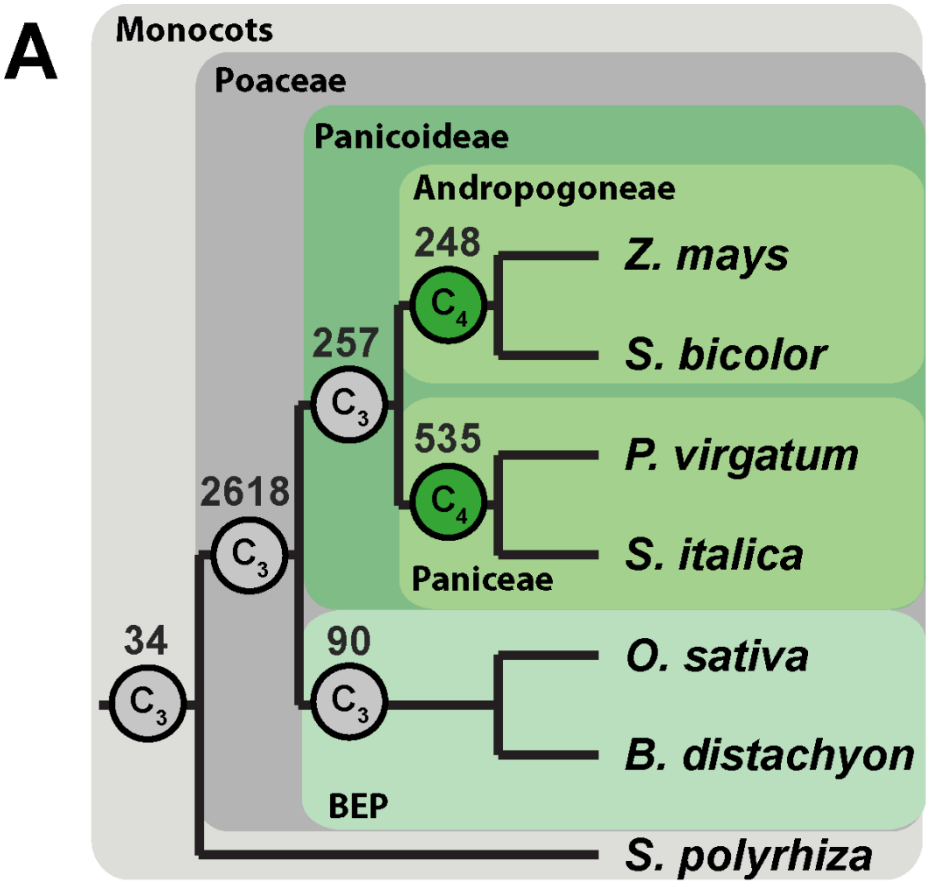


Which genes duplicated coincident with the evolution of C_4?

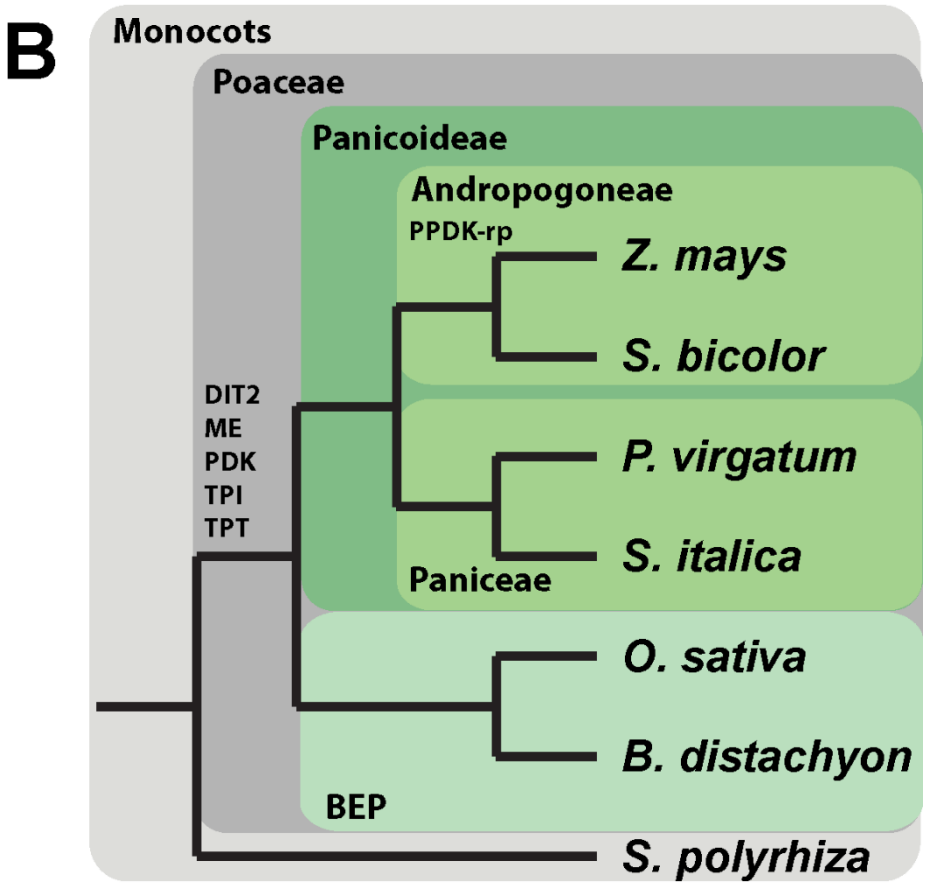


What genes duplicated coincident with the evolution of C_4?

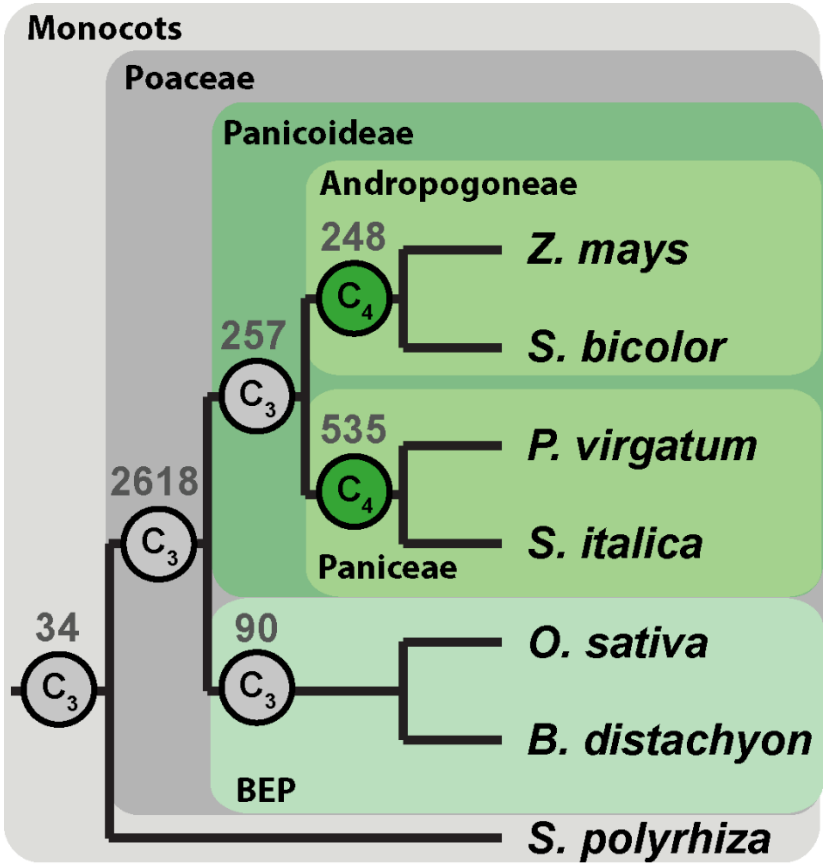
All retained duplication events

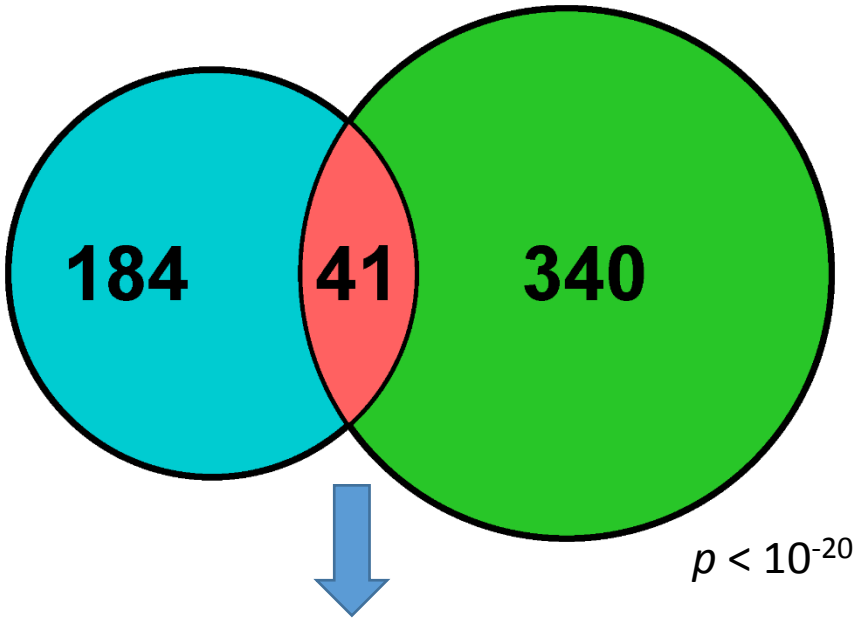


Genes associated with C_4



What genes duplicated coincident with the evolution of C_4?





$p < 10^{-20}$

21 same orthologue

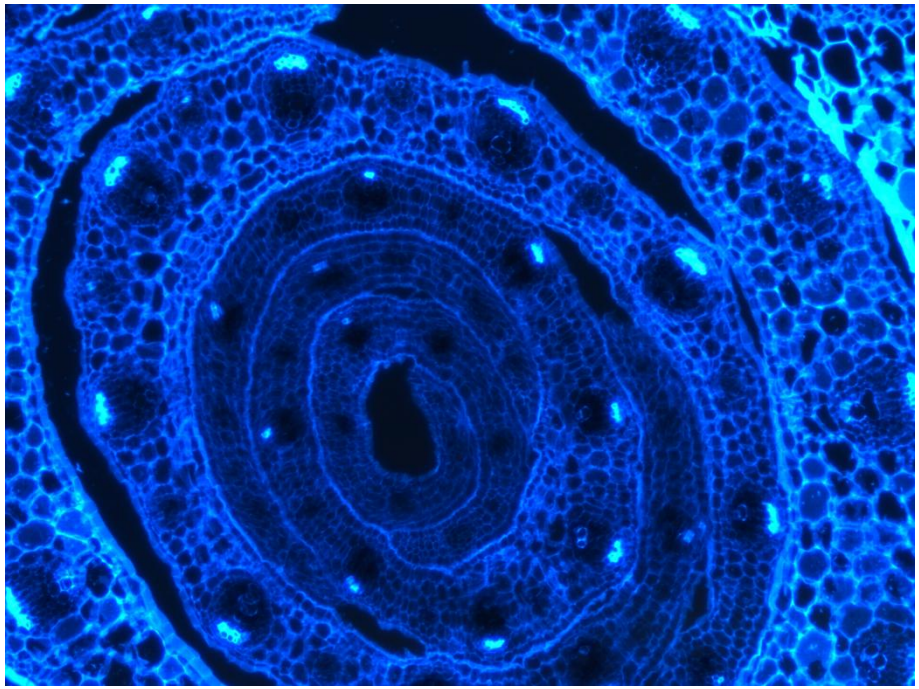
3 X Transcription factors
2 X Transporters
2 X Hormone metabolism
2 X Redox homeostasis

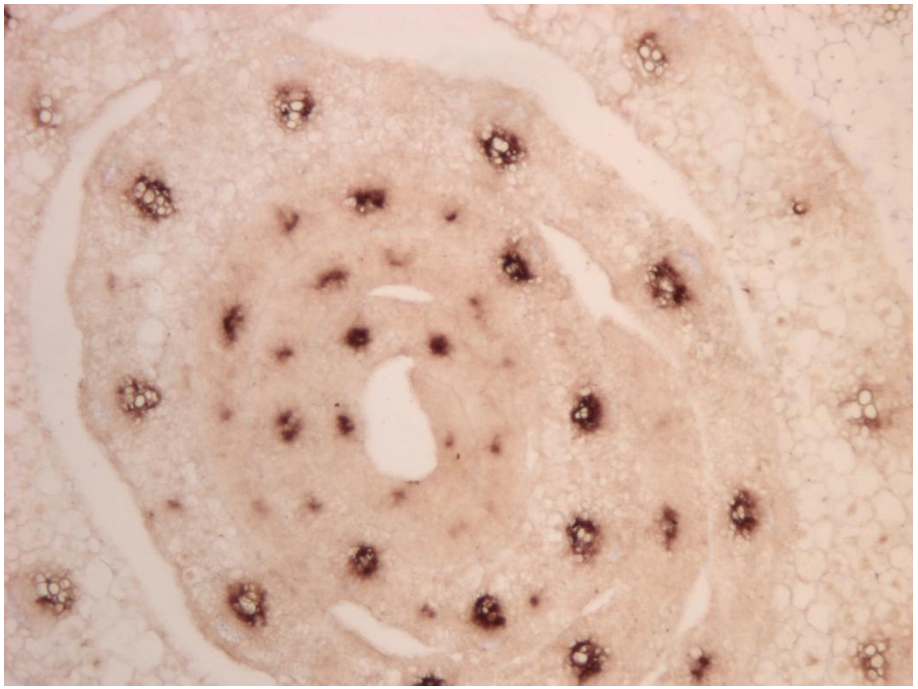
1 X Plastid import, signalling, lipid biosynthesis, plantacyanin, flavonoid biosynthesis

6 X Genes of unknown function

Emms and Kelly ***in prep.***

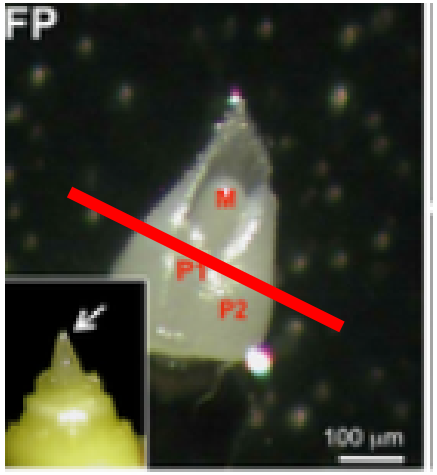
Regulator of primary vein vascular patterning

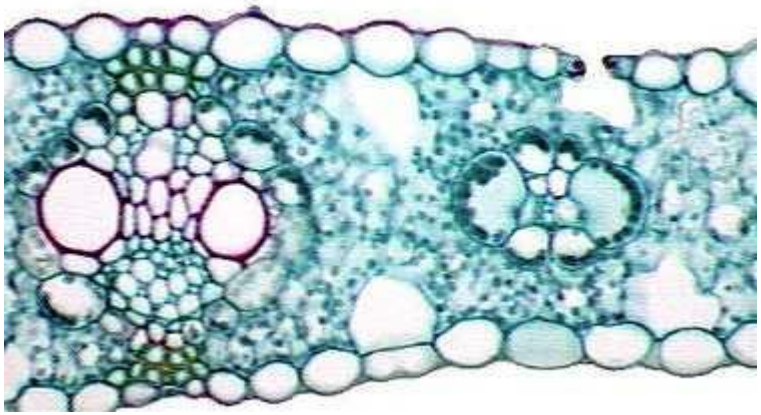




Olga Sedelnikova

Jane Langdale





Summary

The evolution of C_4 involved hundreds of parallel changes

- Parallel changes in gene expression

149 genes change expression common to all dicot C_4 origins.

- Parallel evolution of new genes by duplication

21 genes duplicated in parallel in two independent C_4 origins.