Evolution of C4 Photosynthesis and its Fitness Landscape

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Why I Think You Should Know About This Work

Theory

Cell

Predicting C_4 Photosynthesis Evolution: Modular, Individually Adaptive Steps on a Mount Fuji Fitness Landscape

David Heckmann,[1] Stefanie Schulze,[2] Alisandra Denton,[3] Udo Gowik,[2] Peter Westhoff,[2,4] Andreas P.M. Weber,[3,4] and Martin J. Lercher[1,4,*]

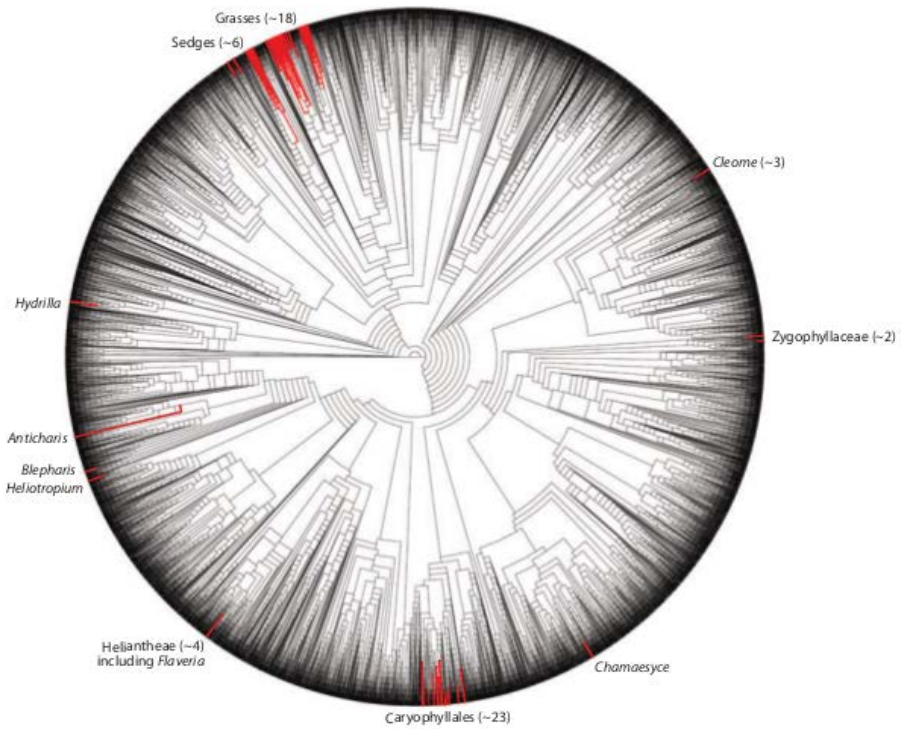
- Application of a simple model to study the evolutionary trajectory from C3 to C4 Photosynthesis
- An interesting story of convergent evolution
- Multi-scale model: chemical reactions, compartmentalization, Monte Carlo simulation to search for evolutionary trajectories
- Result is universal and can cope with different plants, having different enzymes and reactions

Content

- Background of C3-C4 evolution
  - Overview of the “C4 syndrome”
  - C3 photosynthesis and the photorespiration
  - Environmental changes / driving forces of C4 evolution
  - Empirical observations of evolutionary trajectory
- Modeling the C3-C4 transition
  - A simplified metabolic network
  - Verification of the model
  - Monte Carlo simulation and analysis on evolutionary trajectory

Background

- C4 plants are successful, constitute 3% of vascular plant species but account for ~25% of photosynthesis
- Highly repeatable, in ~7500 species, >60 independent lineages -> convergent evolution



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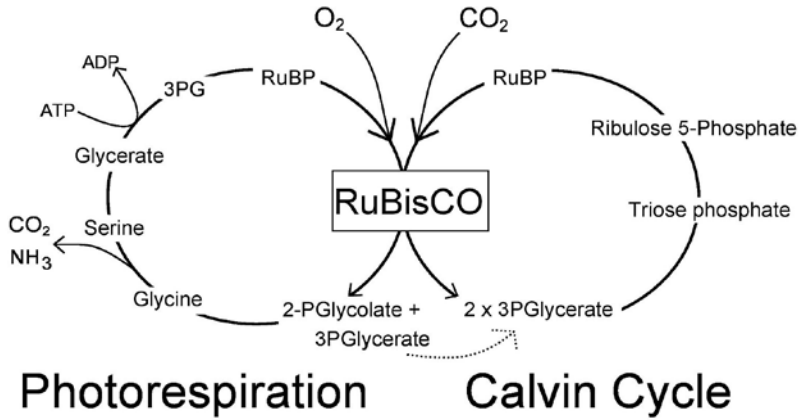
Biochemistry of C3 Cycle

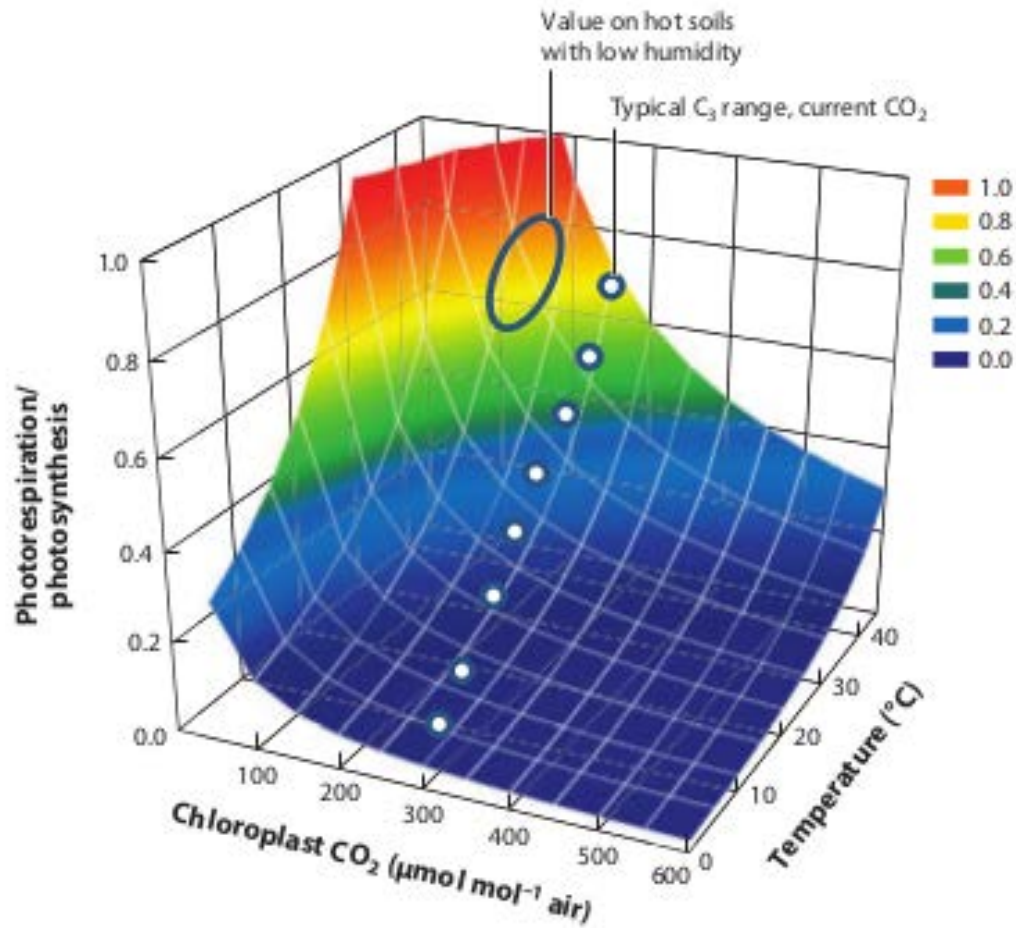
- Rubisco is essential for photosynthesis, it is most abundant protein on Earth; 30% of nitrogen and 50% of total protein investment in plants
- Carbon fixation begins with CO2 reacting with the enzyme rubisco and start the C3 cycle, and produces water and other useful organic compounds
- Rubisco is not specific, it also reacts with O2 and carries out photorespiration
- Photorespiration produces CO2 (reduces yield) and ammonia (toxic)



http://en.wikipedia.org/wiki/File:Simplified_photorespiration_diagram.jpg

Photorespiration Reduces Efficiency of C3 Photosynthesis

The ratio of C3 cycle / photorespiration depends on environmental conditions: CO2 partial pressure, heat, light …



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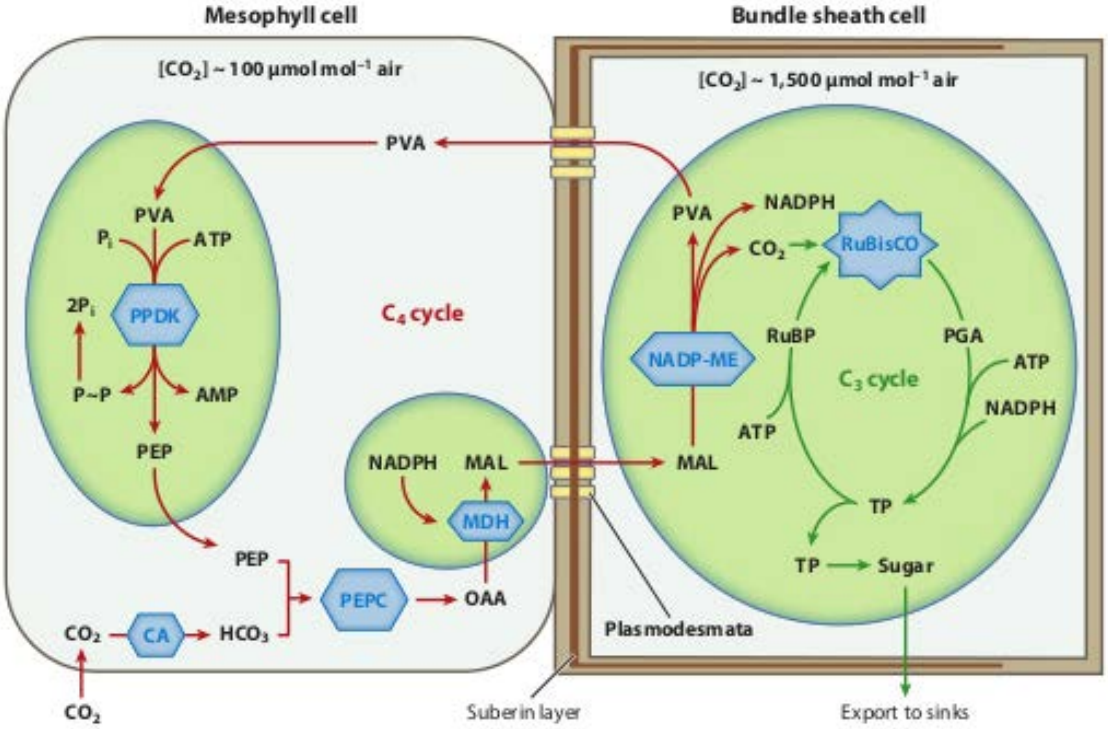
Advantages of C4 Photosynthesis

- C4 plants have lower amount of rubisco per leaf area -> lower nitrogen requirements
- Reduction of rubisco is compensated by higher CO2 assimilation rate
- C4 plants do not need to open stomata so often to maintain CO2 partial pressure -> reduces water loss -> helps the plants to adapt to dry / arid climate

Characteristics of C4 Photosynthesis

- Rubisco is shifted to bundle sheath (BS) cells
- CO2 enters from mesophyll (M) cell, and shuttled to BS cell through C4 cycle
- C4 cycle: hydration of CO2, PEP carboxylation in M, decarboxylation in BS (by different enzymes in different lineages)



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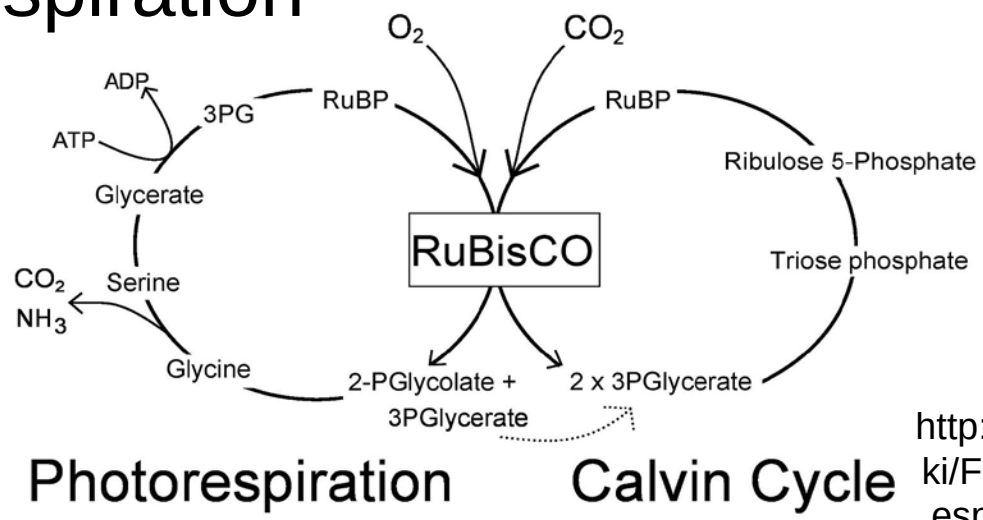
C3-C4 Intermediate Species

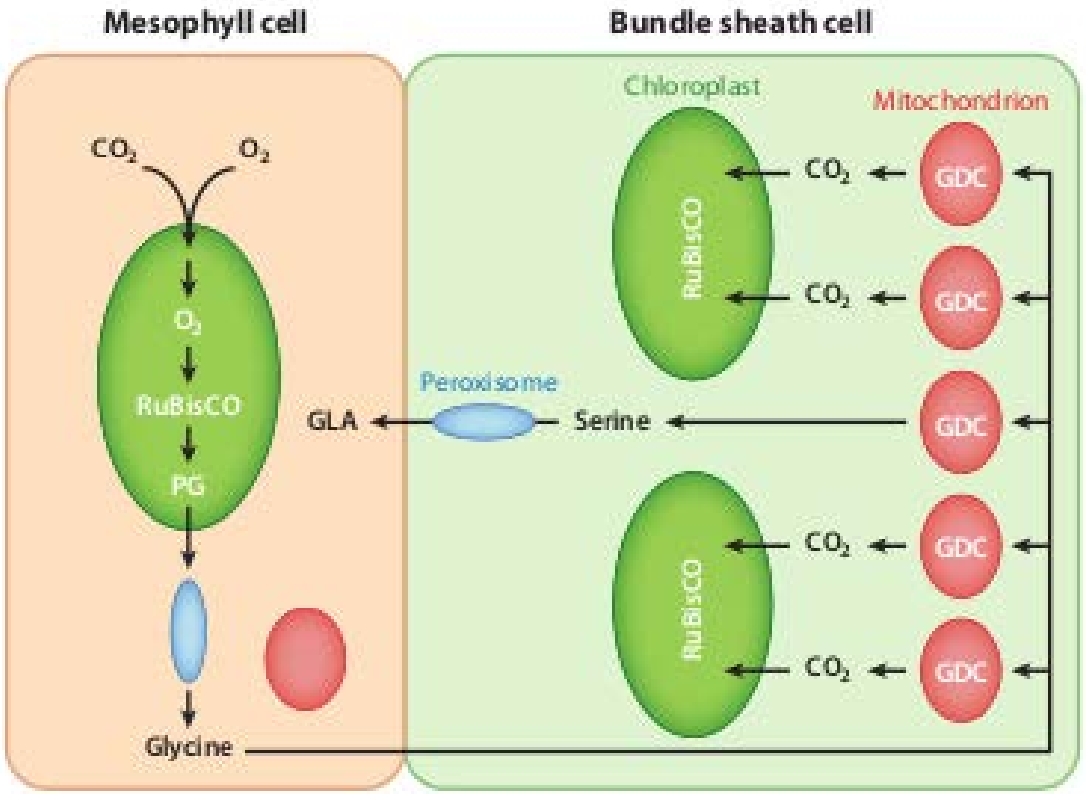
- C3-C4 intermediate species can be found in the clades having C4 species
- Photorespiration also produces CO2, and the C3-C4 intermediate species can capture these CO2 to compensate the loss due to photorespiration



http://en.wikipedia.org/wiki/File:Simplified_photorespiration_diagram.jpg

C3-C4 Intermediate Species

- GDC in mitochondria can convert glycine into CO2
- GDC activity is shifted to BS cell in the intermediate species



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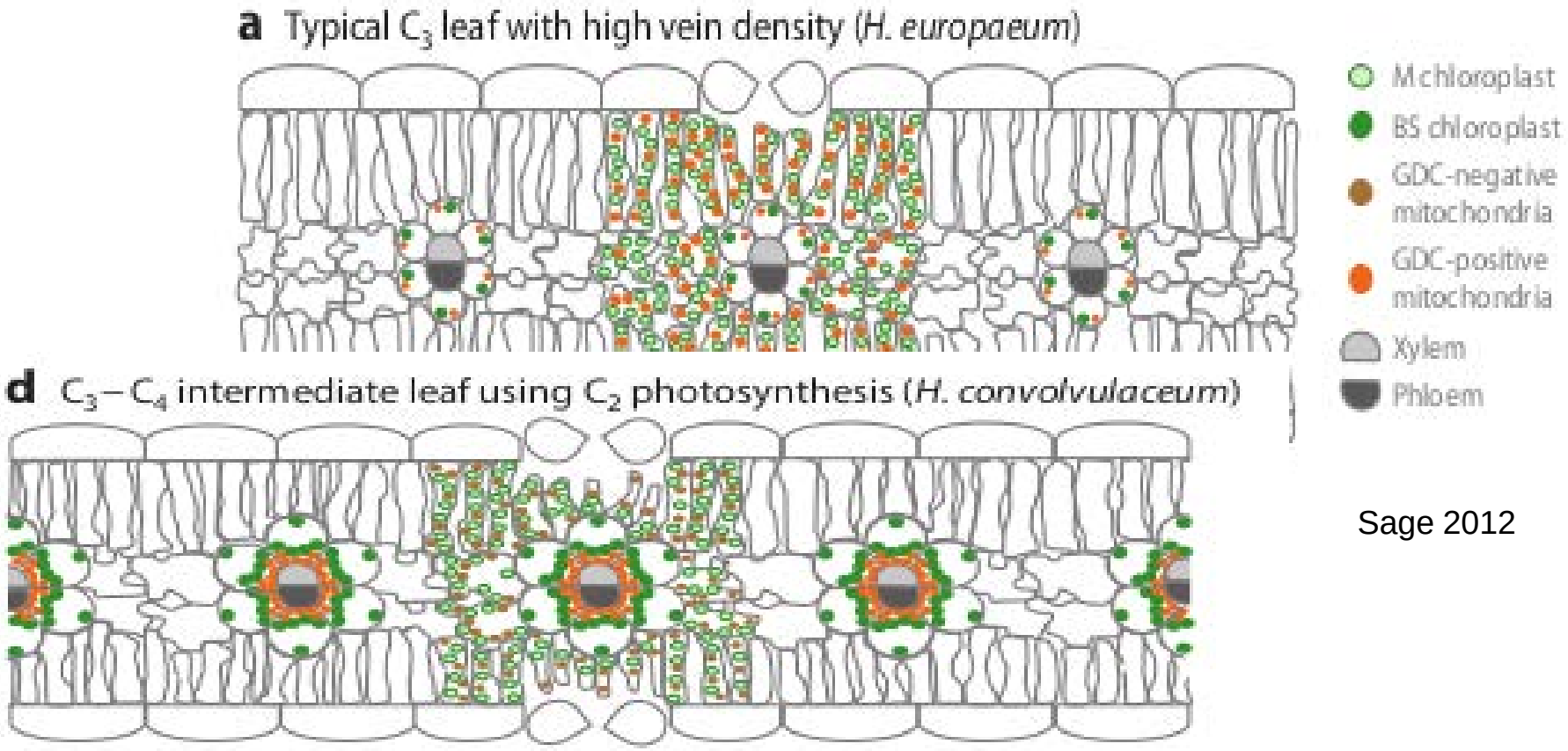
C3-C4 Intermediate Species

Anatomic change optimizes trapping of CO2 for photosynthesis



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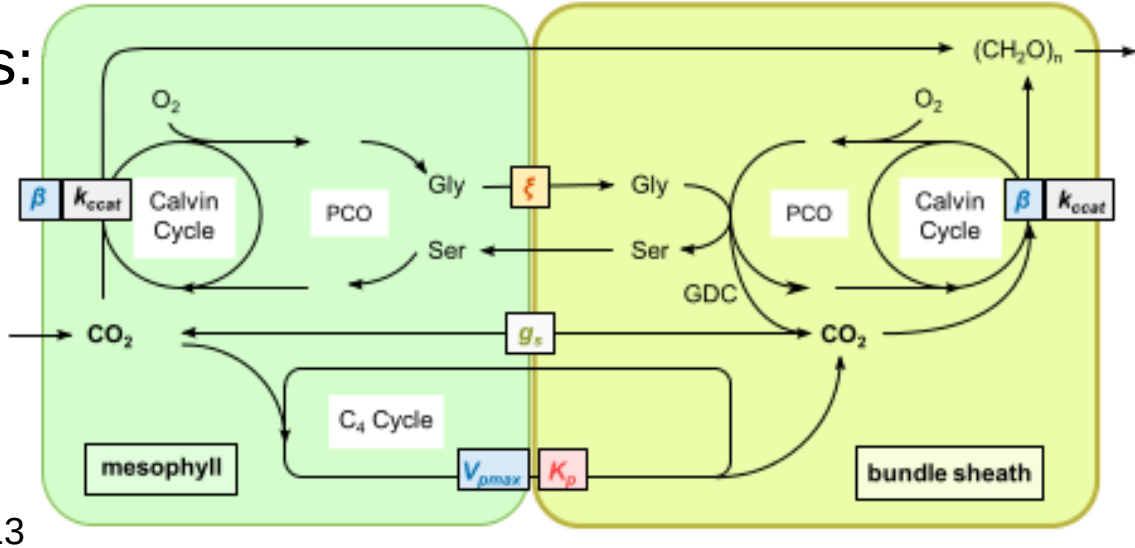
Modeling the C3-C4 Evolution

- A small metabolic network that describes both C3 and C4 states
- Fitness = amount of CO2 fixed using a given amount of rubisco per leaf area
- Rich environment with excess light and nitrogen
- A network of chemical reactions involving C3 / C4 cycle, CO2 / glycine diffusion, … in M and BS cell
- Assumption of Proto-Kranz anatomy: high vein density, enlarged BS cells with more organelles

Modeling the C3-C4 Evolution

- 6 free model parameters:
  - β: fraction of rubisco active sites in M, ~95% in C3 and 0% in C4;
  - kccat: turn over rate of rubisco, low in C3 due to trade off with photorespiration;
  - ξ: fraction of glycine produced in M due to photorespiration that is decarboxylated by GDC in BS, 0 in C3 and 1 in C3-C4 intermediate
  - Vpmax / Kp: Max rate / Michaelis constant for PEPC (C4 pump)
  - g_s: BS gas conductance, quantifies the effect of cell geometry and cell wall properties
- Non-free parameters:
  - rubisco Michaelis constant
  - O2, CO2 specificity



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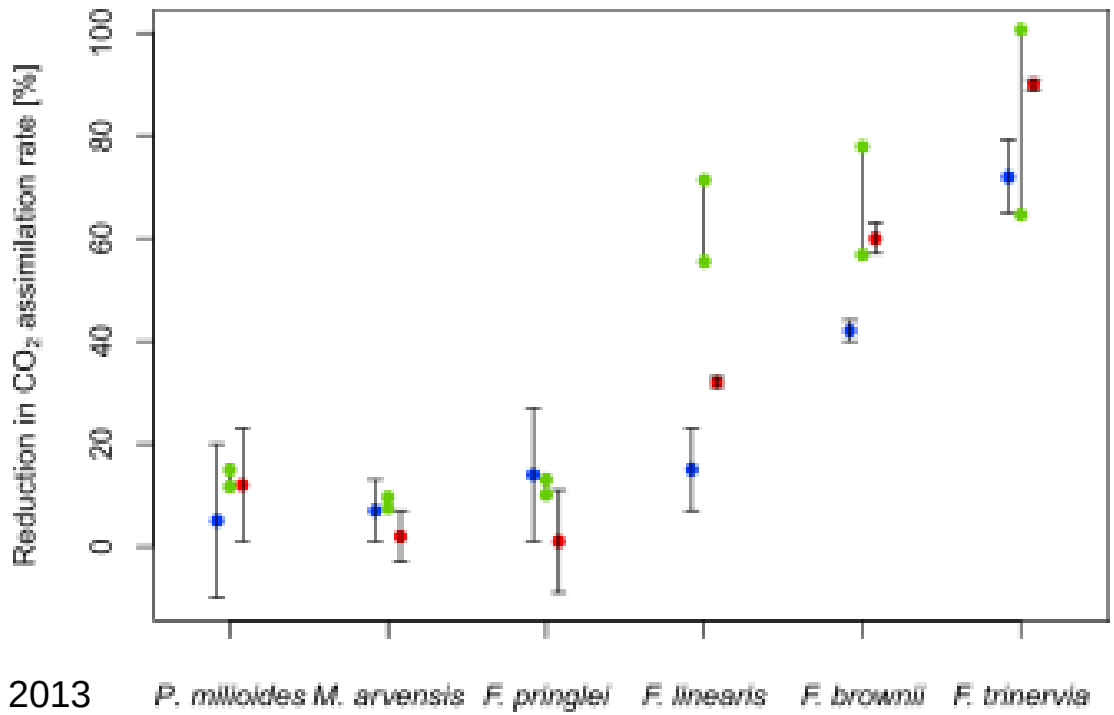
Model Verification

- Existence of different C4 subtypes -> couple the model with metabolic networks of different subtypes, perform flux balance calculation, and verify that the CO2 fixation rate is independent with different subtypes
- Suppress the C4 cycle, and compare model predictions with experiments



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Monte Carlo Simulation to Study Evolution Trajectory

- Each of the 6 dimensions is divided into 5 steps
- Monte Carlo simulation: random walk in the 6D space, starting from the C3 state
- Fitness gain: $s = (A_{c2}-A_{c1})/A_{c2}$, where A_c is the CO2 production rate
- Acceptance probability:
- N = 100,000

$$\pi = \begin{cases} \dfrac{1}{2N}, & s = 0 \\ \dfrac{1-e^{-s}}{1-e^{-2Ns}}, & s \neq 0 \end{cases}$$

C4 State is the Only Maximal Fitness State

- 6D space, max 6 direct to get closer to C4
- Distribution of number of directions from a point in the 6D space that will give a fitness increase
- The only point with no way out is C4 state
- There is no other local maximum to stop the evolution

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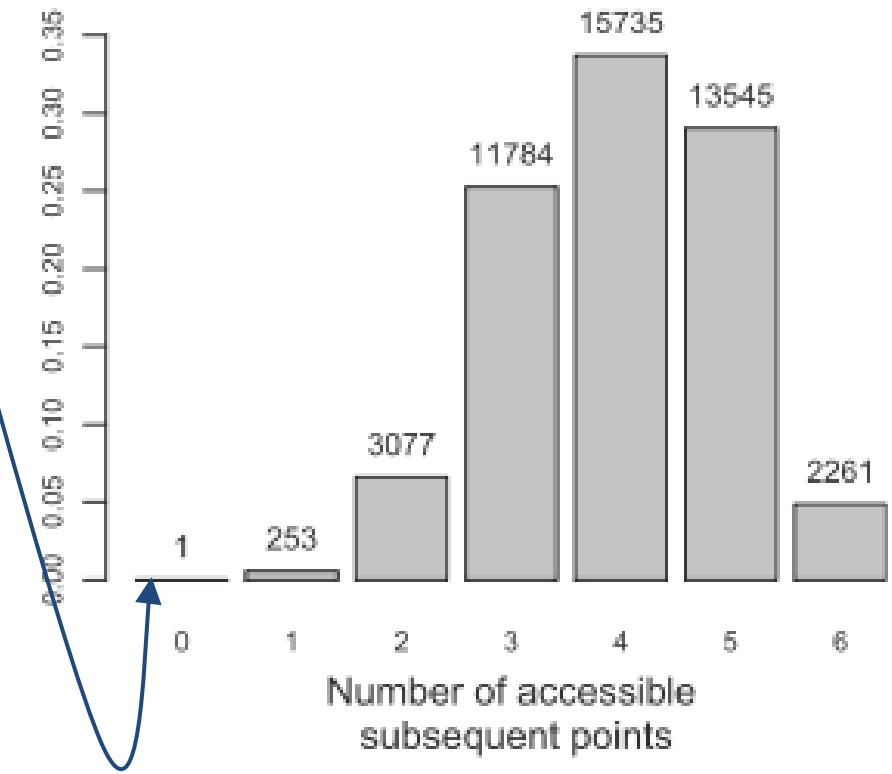
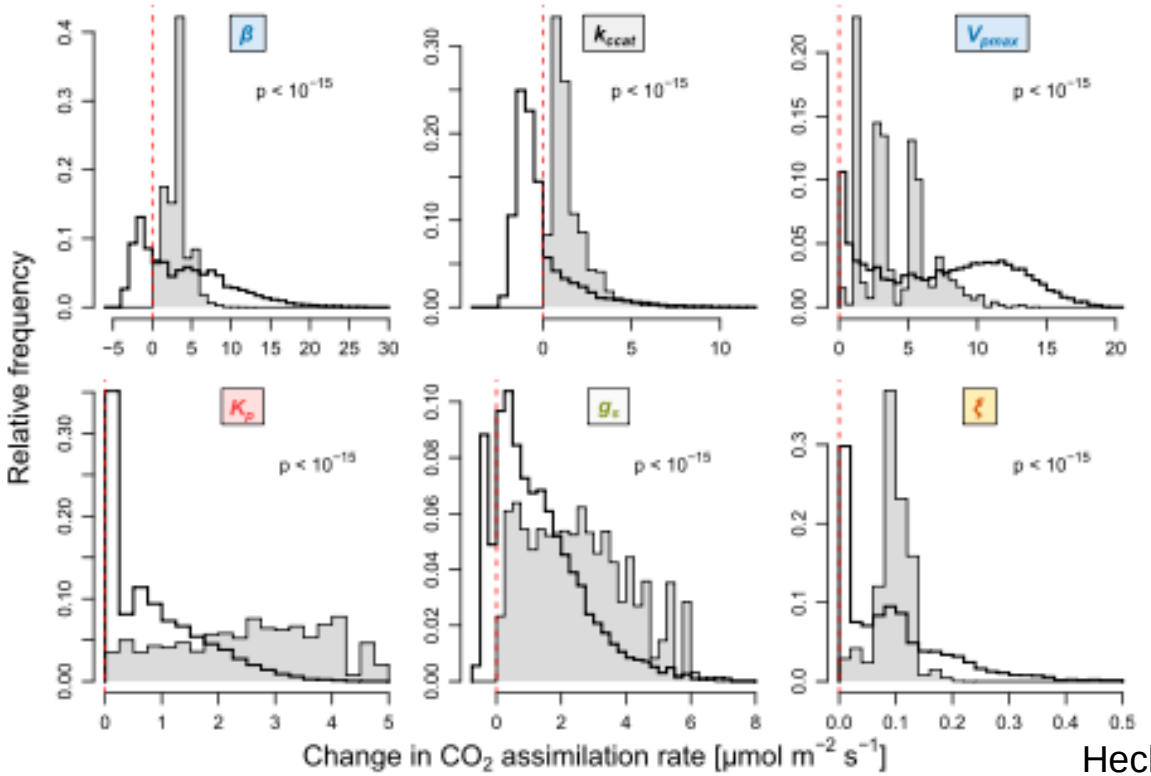


Extensive Epistasis

- Distribution of Fitness change along 6 different directions towards C4 state
- Extensive presence of uncrossable paths



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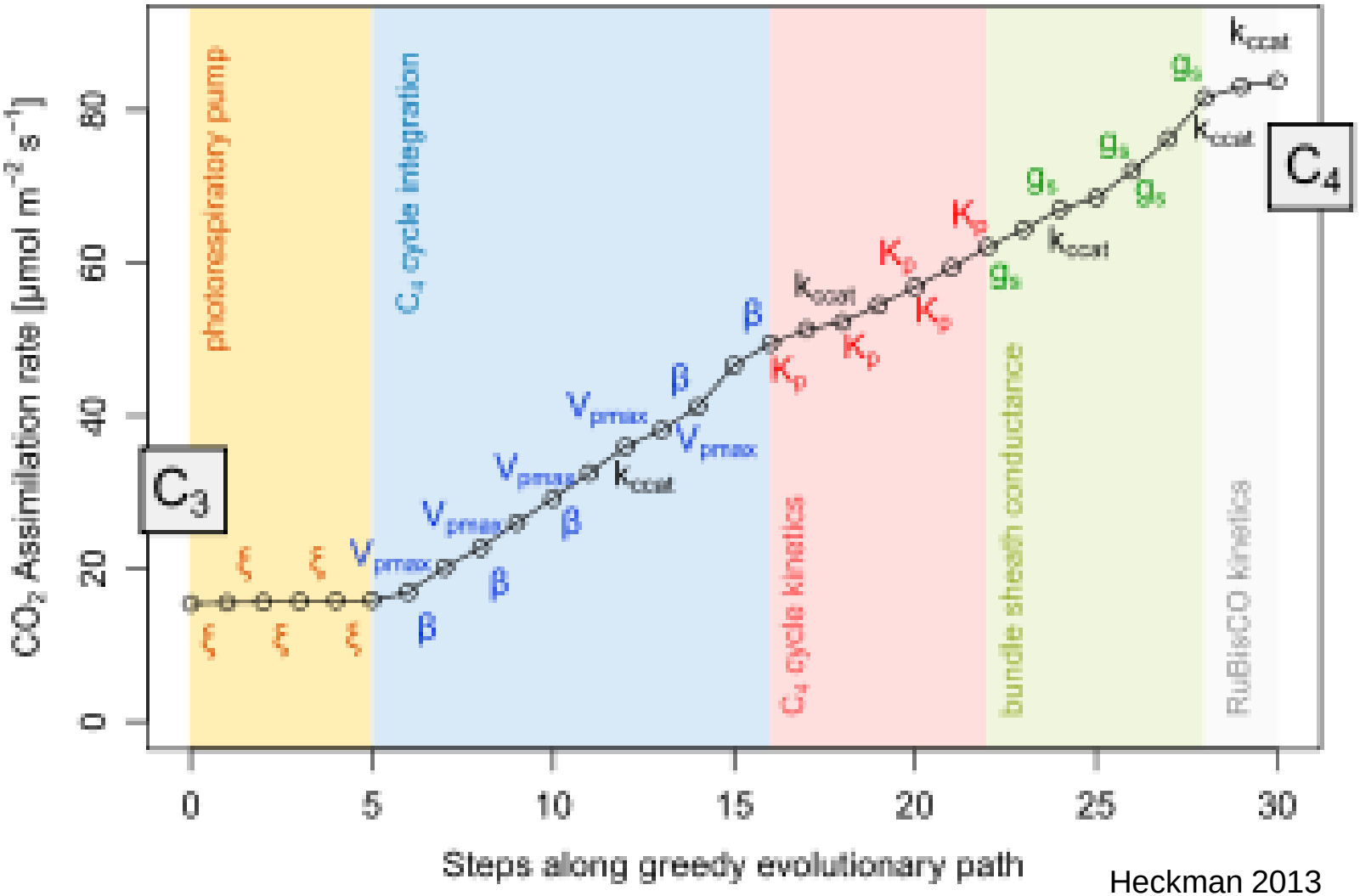
Modular Evolution in the Greedy Trajectory



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Modular Nature of Evolutionary Trajectories

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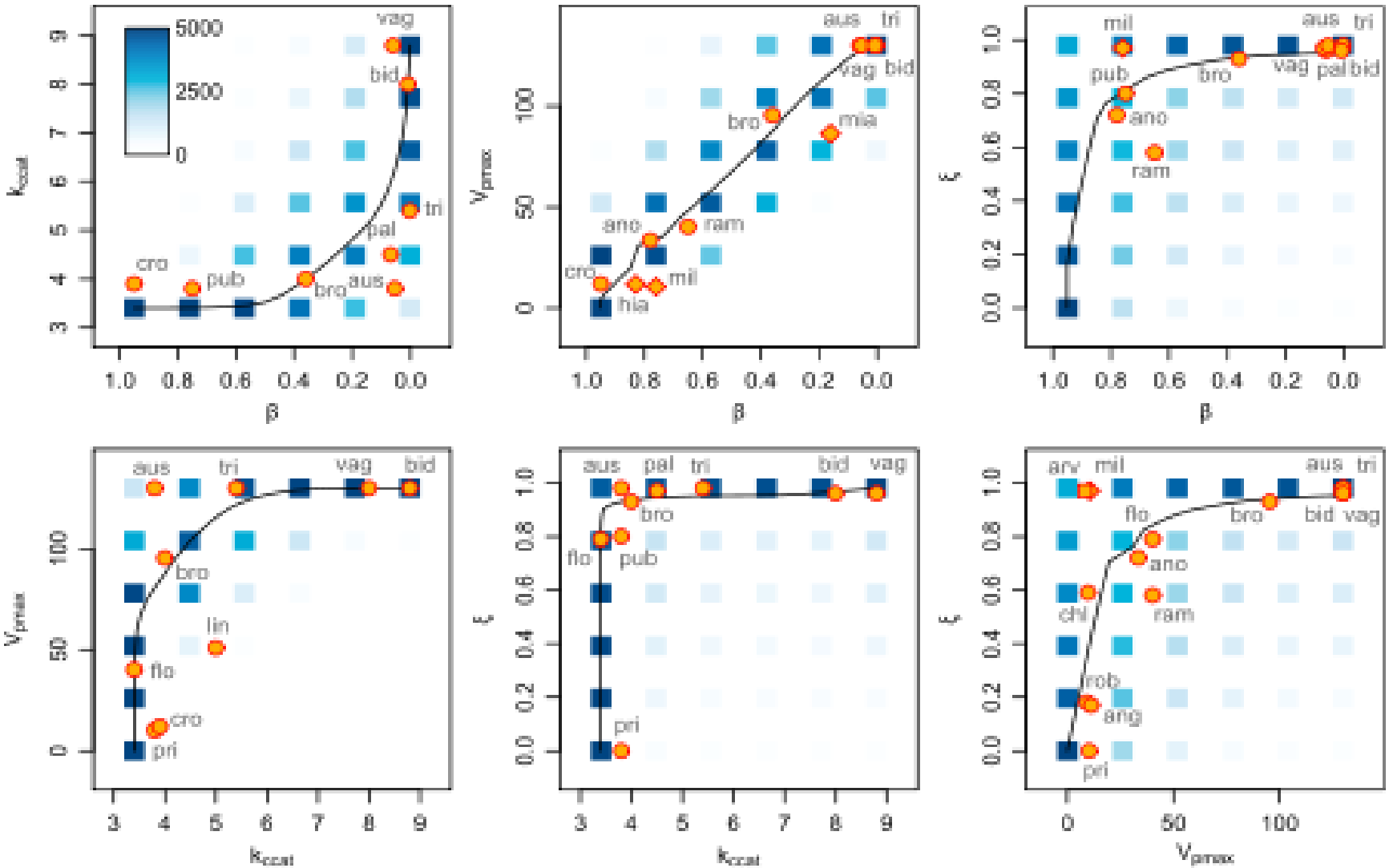
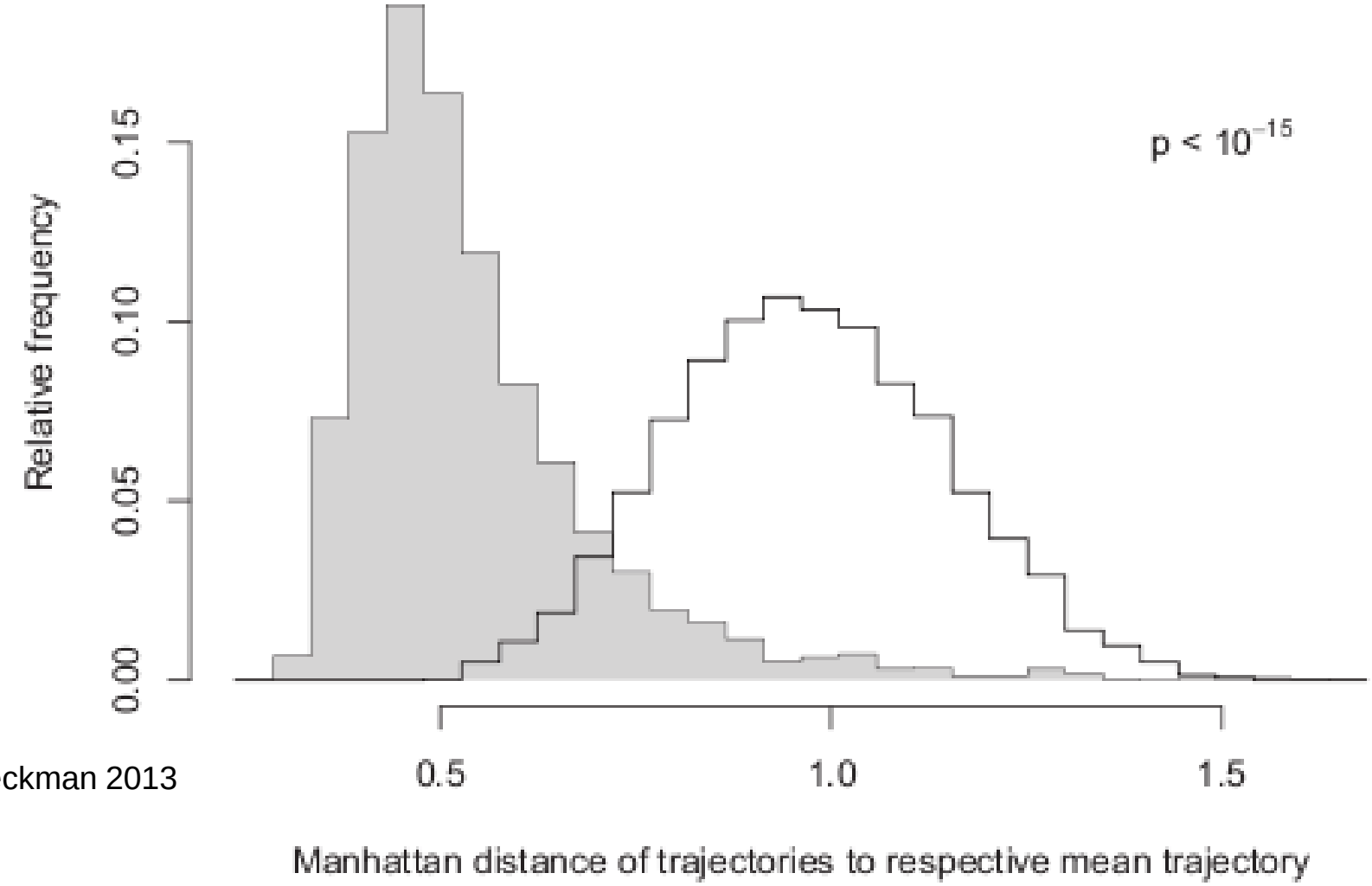


Existence of Preferential Evolutionary Trajectory



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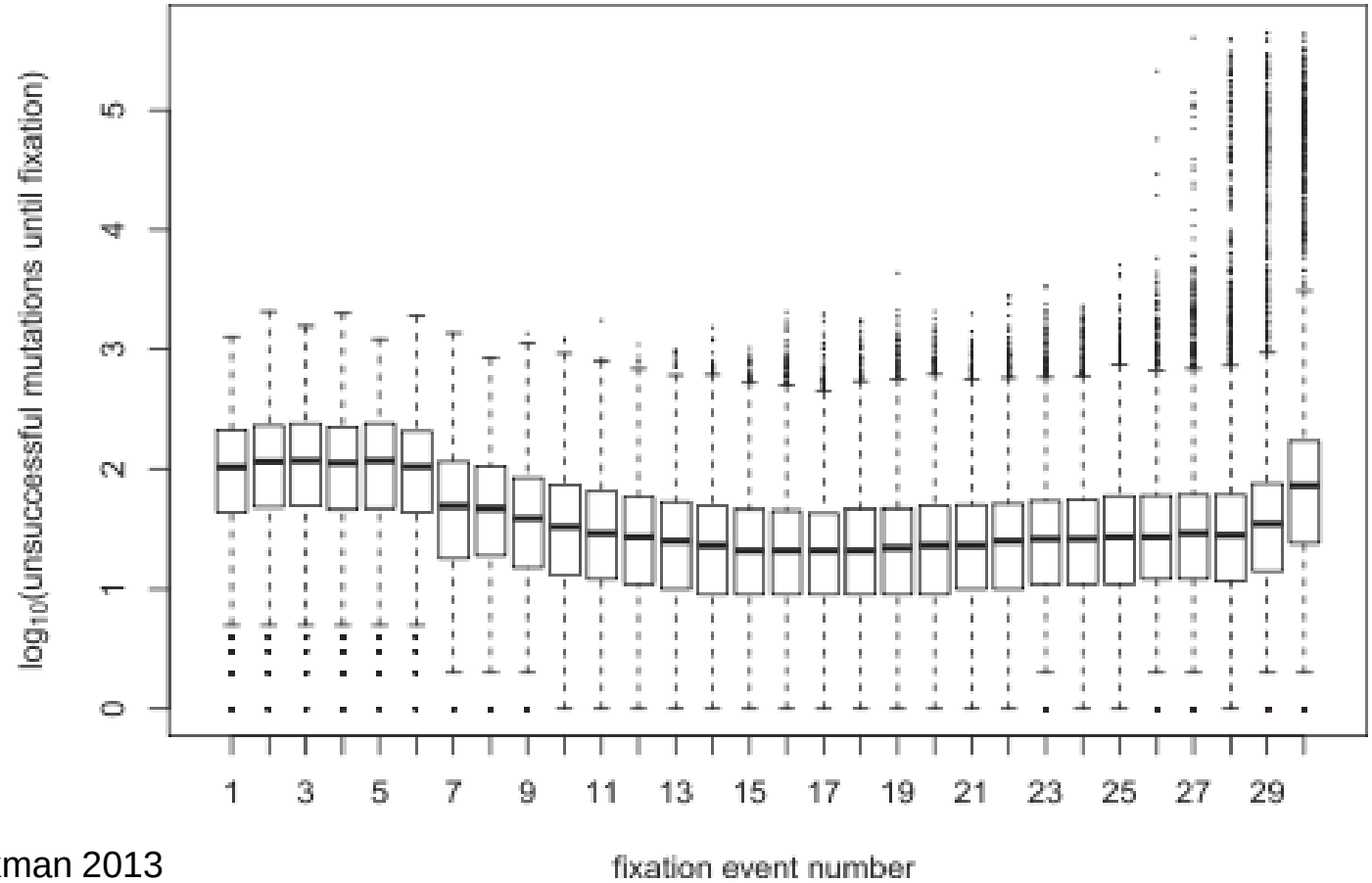
No Systematic Slow Down of Evolution

- Distribution of failure rate at each steps, out of 5000 simulations



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Conclusion

- The single peak in fitness landscape, C4 state is accessible from any point despite epistasis
- Extensive epistasis
- Modular evolution
- Preferential evolutionary trajectory exists

Take Home Message

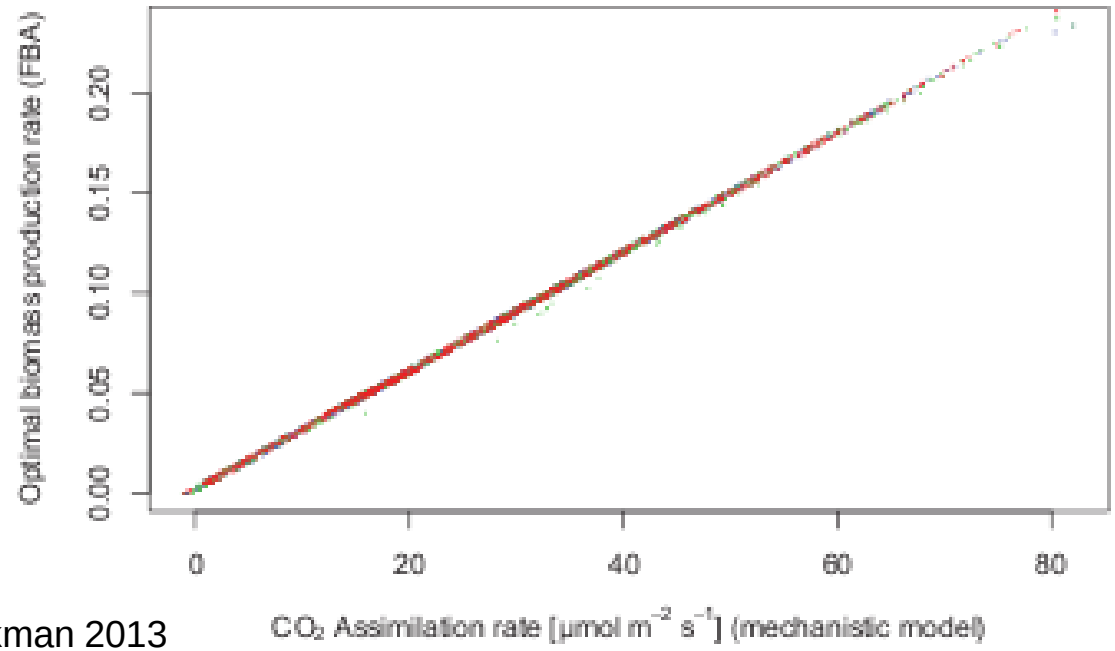
- The paper shows an excellent example of how modeling can explain convergent evolution
- It involves experimental verification besides modelling
- It also shows a good example of the application of flux balance analysis

References

1. Predicting C4 photosynthesis evolution: modular, individually adaptive steps on a Mount Fuji fitness landscape. Cell, Vol. 153, No. 7. (20 June 2013), pp. 1579-1588 by David Heckmann, Stefanie Schulze, Alisandra Denton, et al.
2. Photorespiration and the evolution of C4 photosynthesis. Annual review of plant biology, Vol. 63 (2012), pp. 19-47 by Rowan F. Sage, Tammy L. Sage, Ferit Kocacinar

MISC

Different C4 acid decarboxylation enzymes in BS cell: (NAD malic enzyme, NAD-ME; NADP malic enzyme, NADP-ME; or phosphoenolpyruvate carboxykinase, PEPCK)



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Dependence of CO2 / O2 specificity and Michaelis Menten constant with rubisco turnover rate

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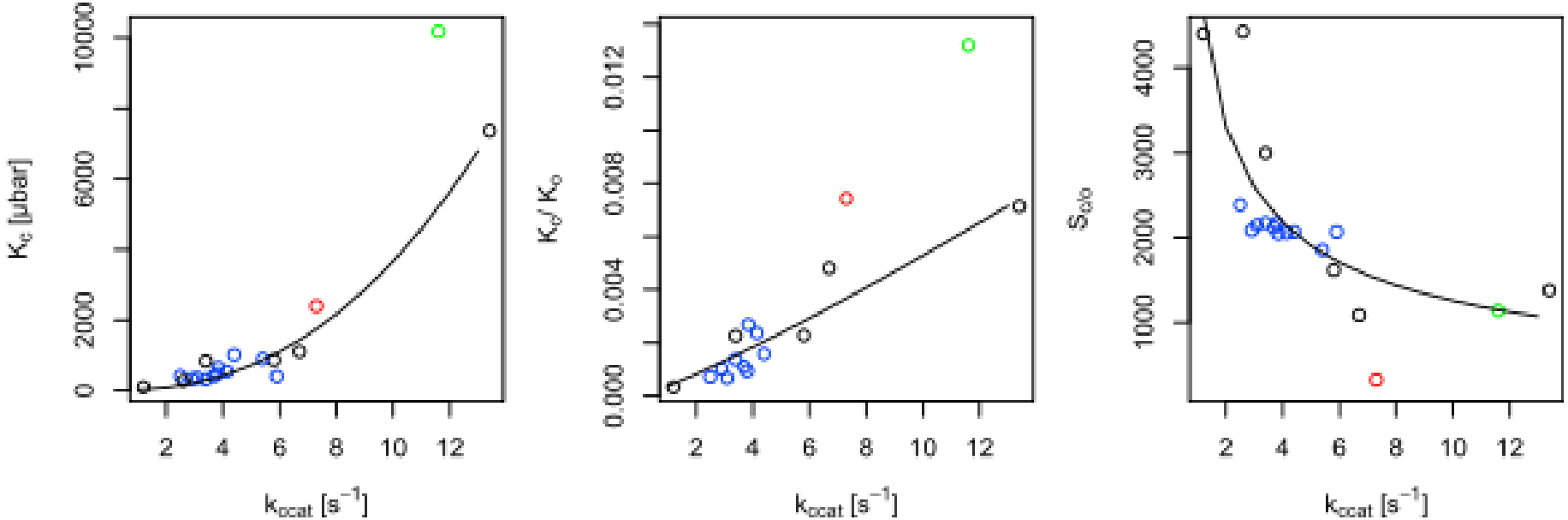

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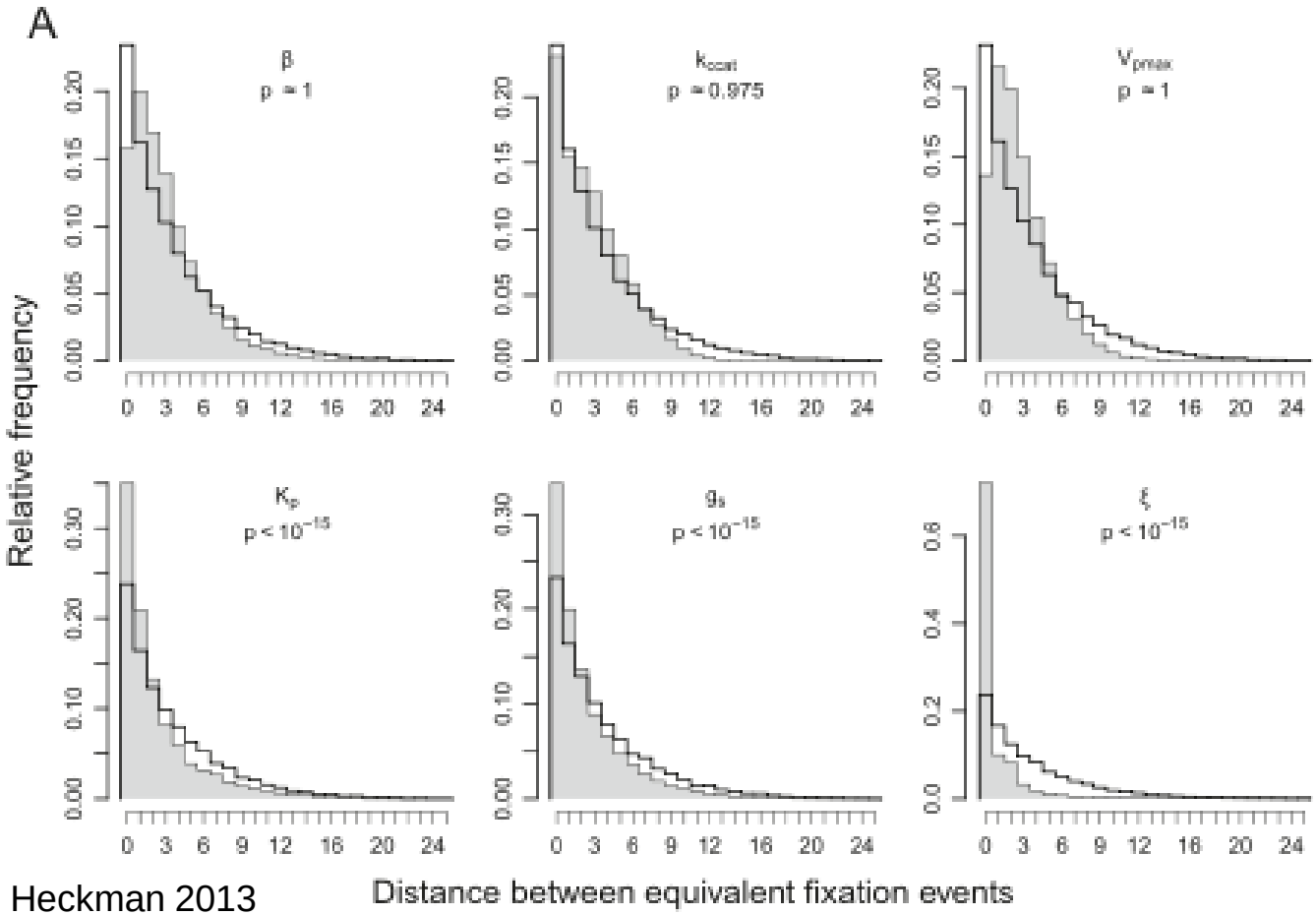
Distribution of distance between fixation events



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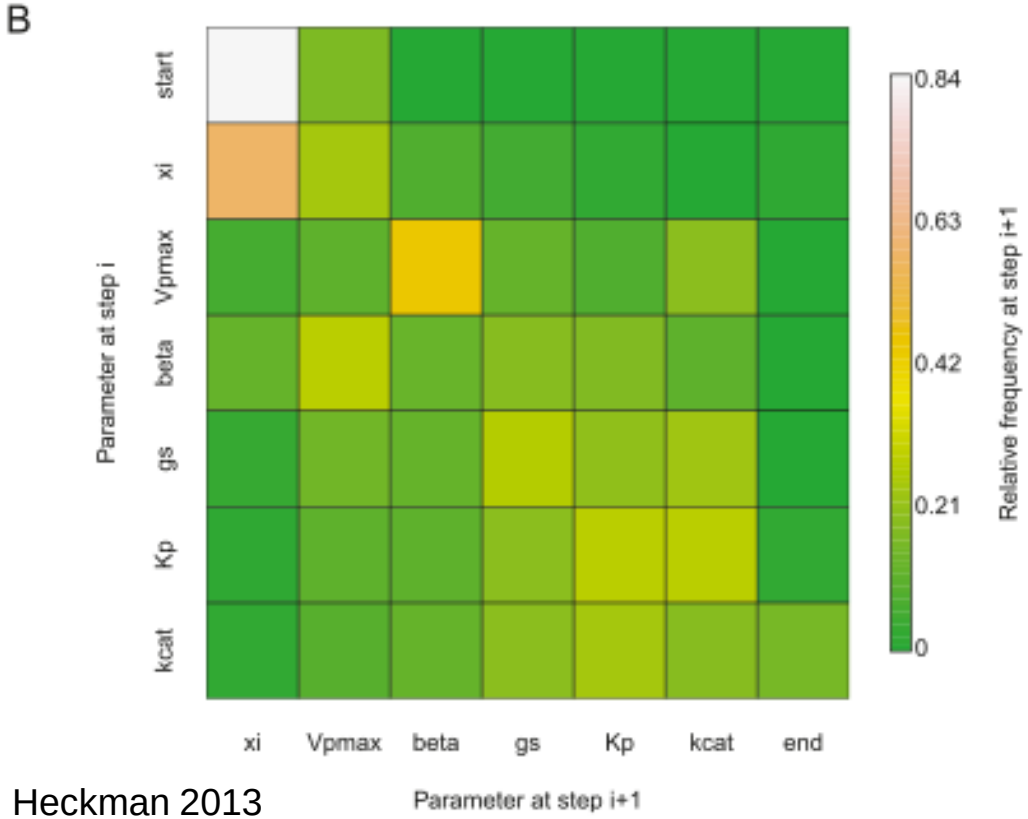
Distribution of frequency with which a change in Y at step i followed by a change in X in i+1



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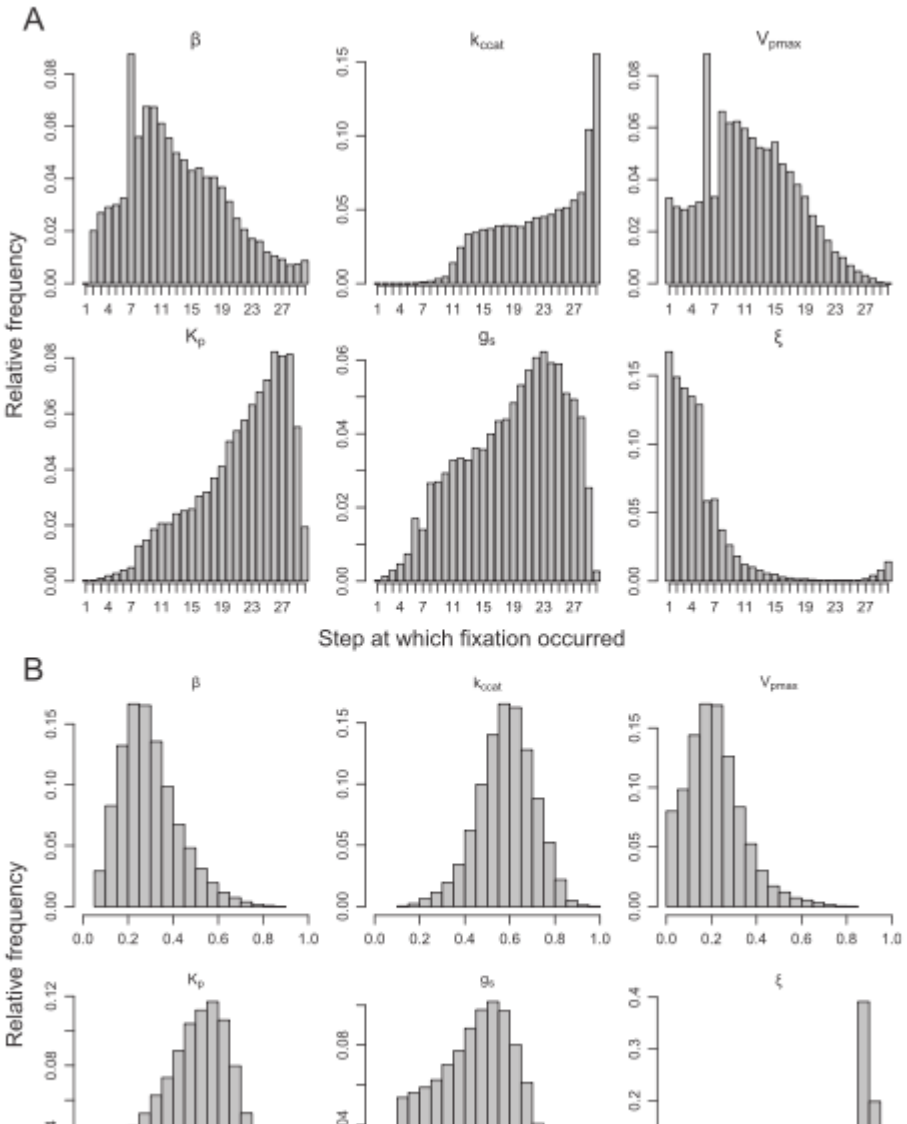

6 Dimensional Fitness Landscape

- Extensive epistasis, i.e., existence of preferential paths in the fitness landscape
- Shifting of GDC activity involves down regulation of 4 enzymes in M cell, but just variation of one parameter in the model
- Modular evolution of complex traits

Environmental Changes Correlated with C4 Evolution

Triggering factors:
- Low CO2 Concentration, Heat, Aridity, High Light, Salinity, Ecological Disturbance, Warm Climate...

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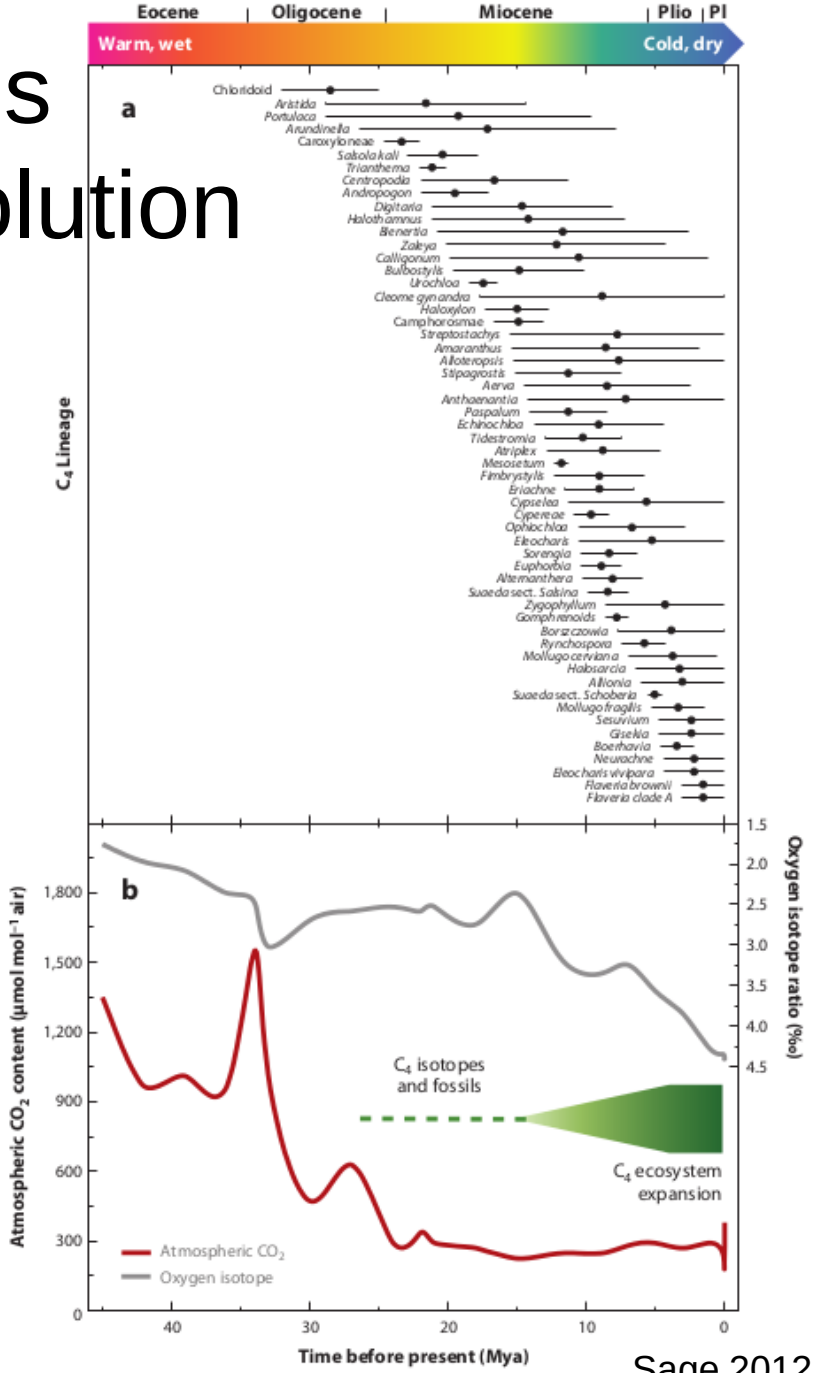
↑ Photorespiration, ↓ C3 Efficiency



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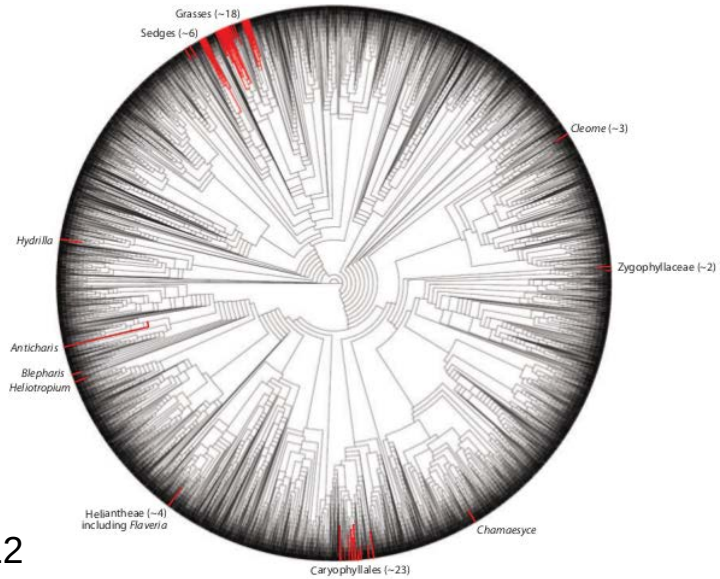
Roadmap to C4 - Preconditions

- Lack of C4 in most clades -> low potential for most to evolve C4
- Clustering of C4 species -> multiple steps evolution
- High vein density reduces distance BS and adjacent M cells, facilitating metabolites exchange
- Gene duplication allows sub-functionalize without harmful consequences



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Roadmap to C4 - Anatomic Change and C3-C4 Intermediate state

- Evolution of “Proto-Kranz” Anatomy
- Enlarged BS with increase organelle numbers, elongated shape has enable high exposure of the centrifugal wall to intercellular air space; this results in higher carbon assimilation
- Mitochondria concentrate at the centripetal wall of BS, along with a layer of chloroplasts

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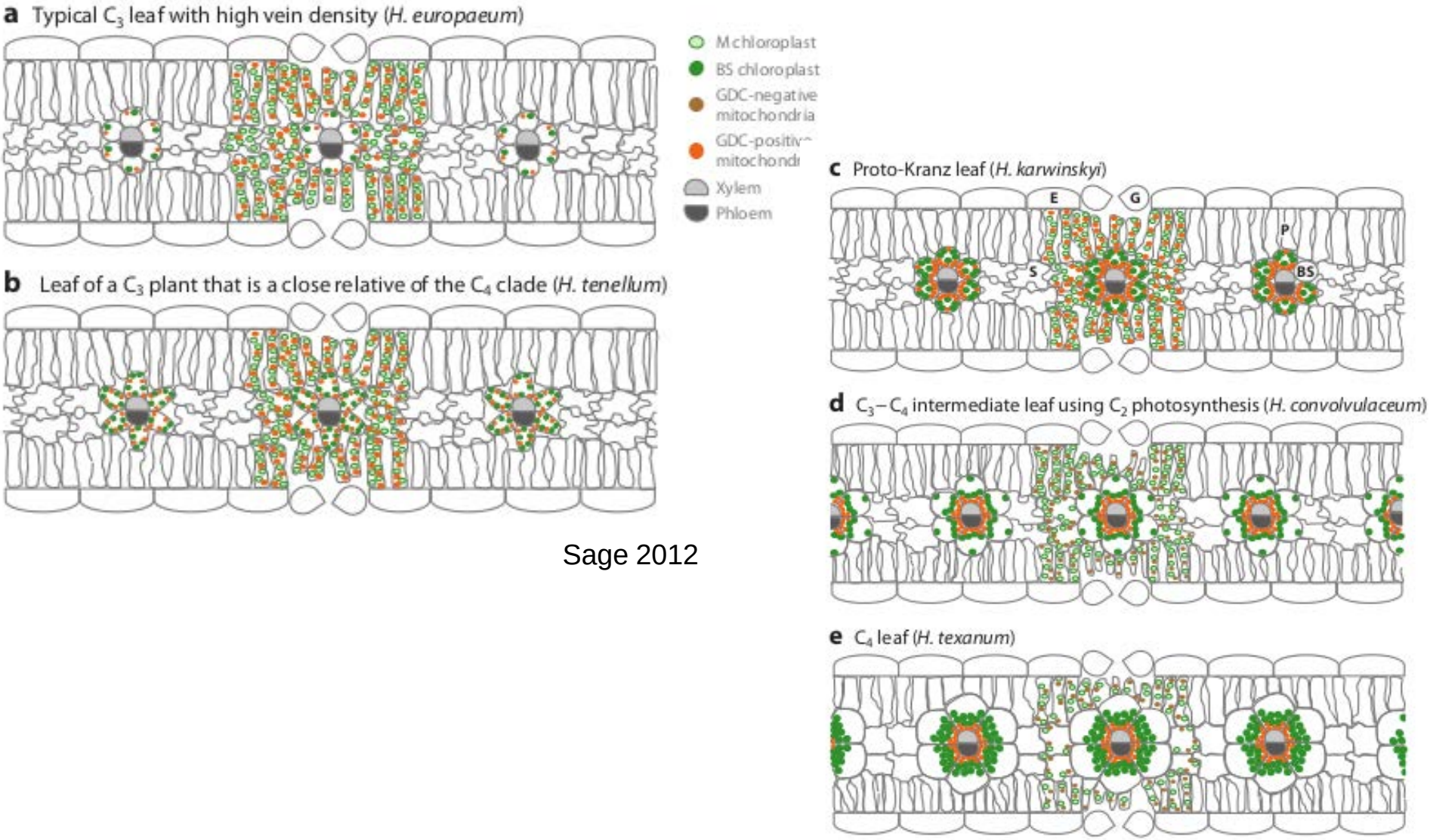
Roadmap to C4 - Anatomic Change and C3-C4 Intermediate state

- Photorespired CO2 is recovered at the centripetal mitochondria of BS; large BS vacuole helps CO2 build up, and allow rubisco of centripetal chloroplast to have higher efficiency
- M losses GDC activity, and that is shifted to BS
- Natural selection no longer inhibits photorespiration, instead it favours the capture of the photorespired CO2: further reduction of M cell volume, increase in BS organelle number, vein density, ...

Roadmap to C4 - Evolution towards full-fledged C4 Photosynthesis

- PEPC activity is boosted to shuttle CO2 from M to BS
- close association of M and BS minimize diffusion distances
- optimization of kcat and Kc of rubisco

Roadmap to C4



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