Finding genes responsible for evolution of complex 3D leaf anatomy using tomographic microscopy

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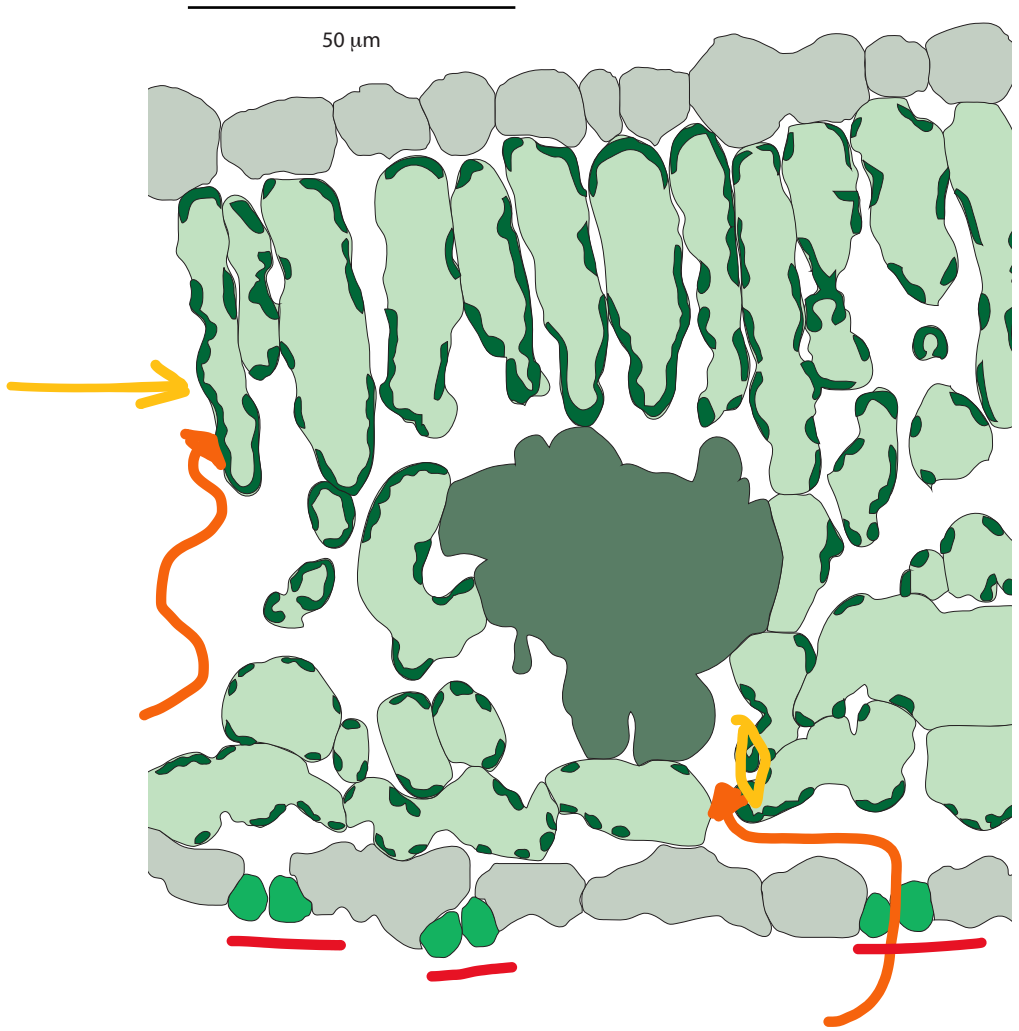
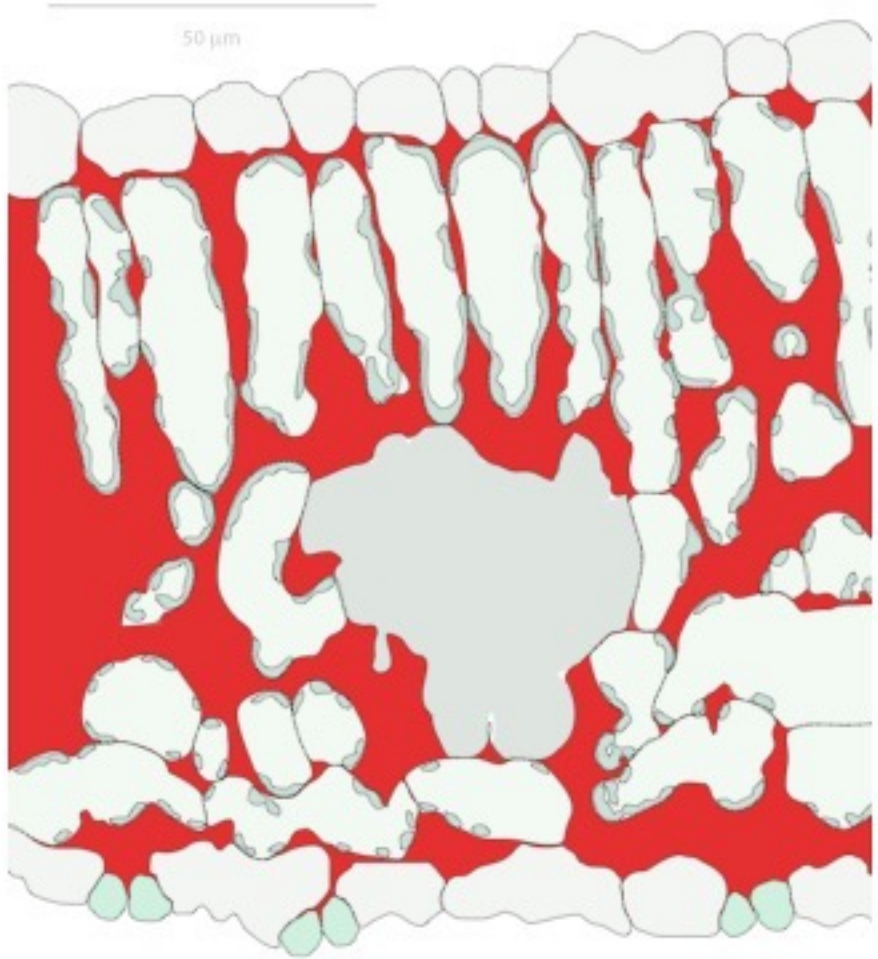


Position in pathway	Process
Reaction sites	
	Liquid-phase diffusion to chloroplasts
Cell Walls	
	Intercellular air-space diffusion
Substomatal cavity	
	Gaseous diffusion through stomata
Leaf surface	

Adapted from Parkhurst 1994



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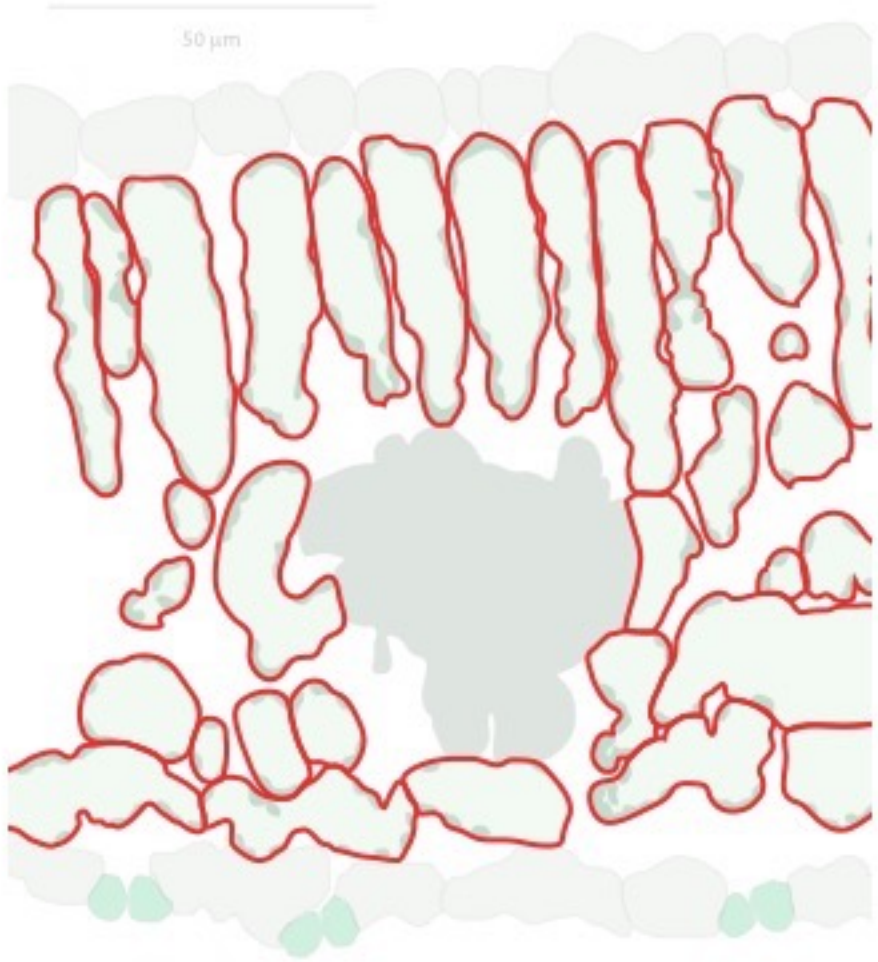

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	Liquid-phase diffusion to chloroplasts
Cell walls	
	Intercellular air-space diffusion
Substomatal cavity	
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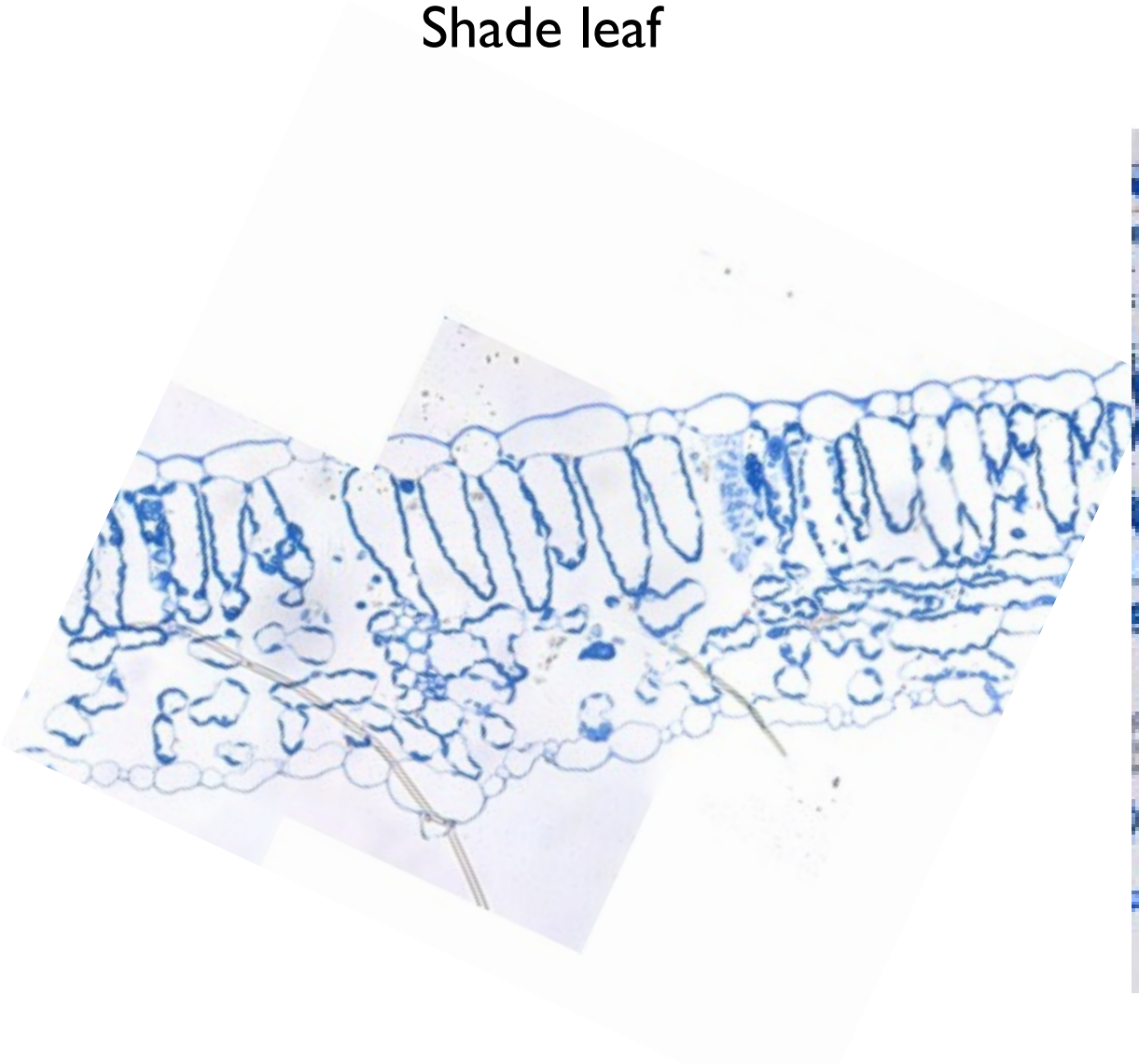
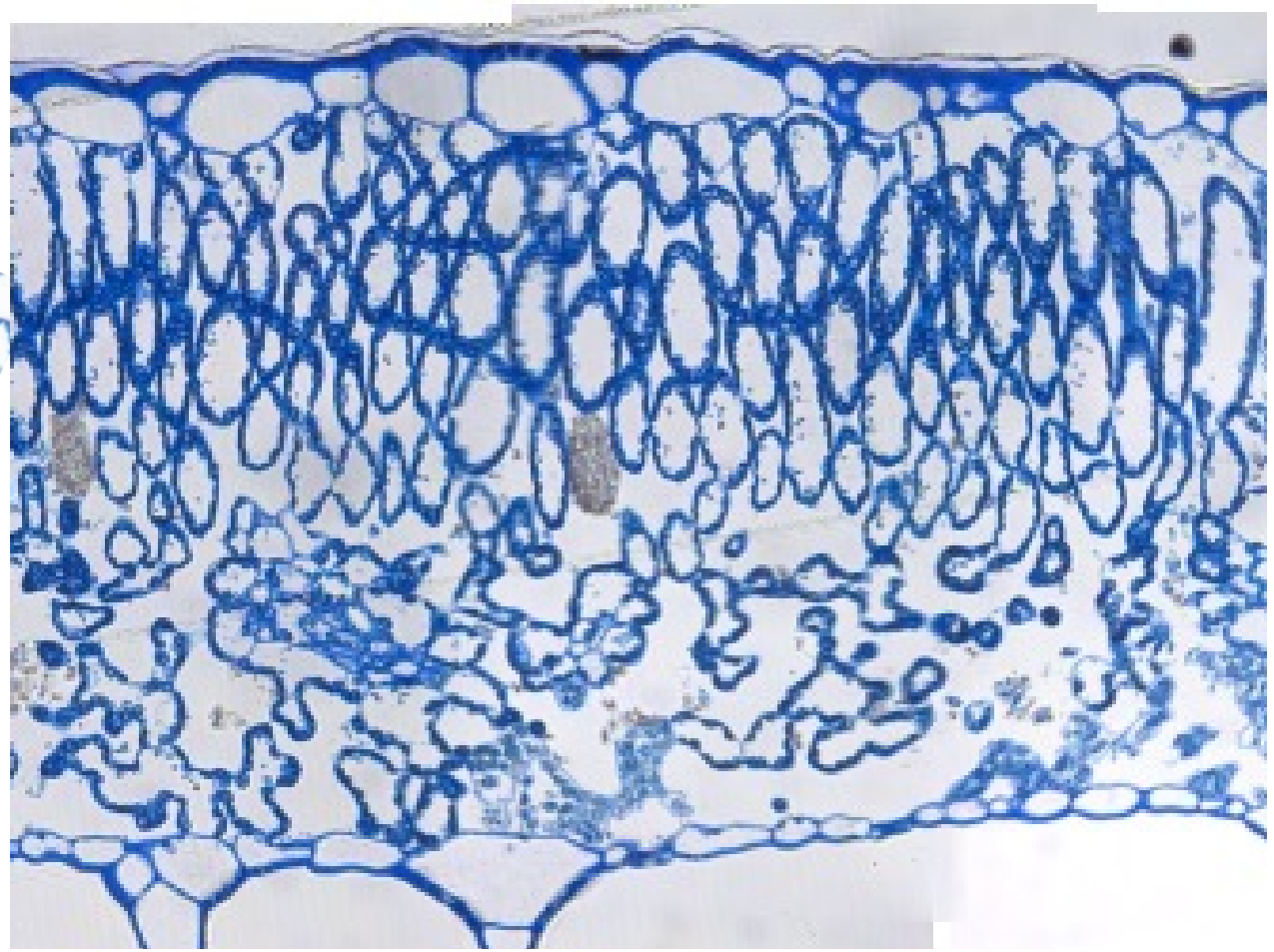
Adapted from Parkhurst 1994

Shade leaf



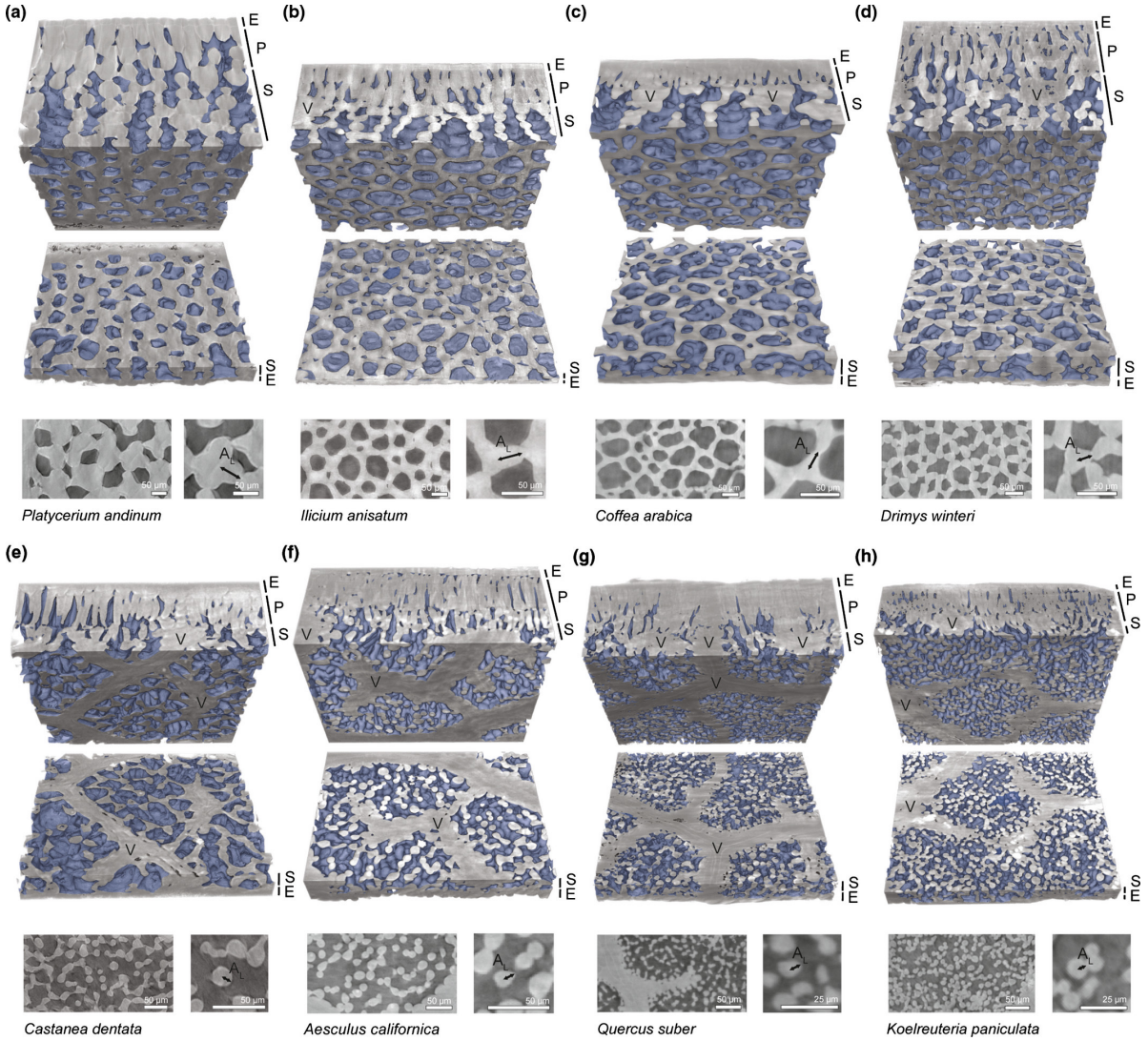
Sun leaf



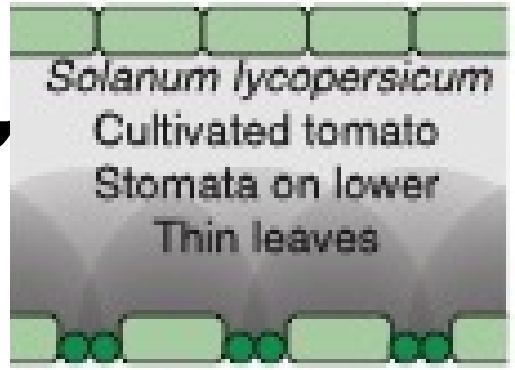
3D leaf anatomy using tomographic microscopy

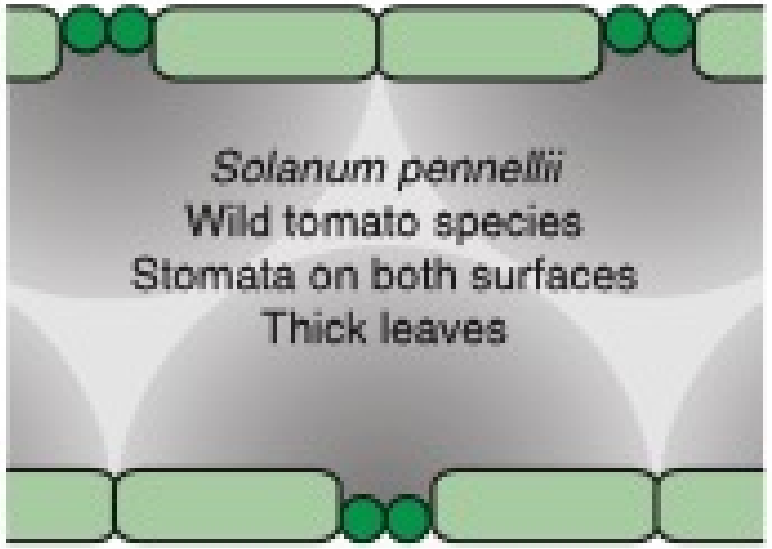
New discoveries

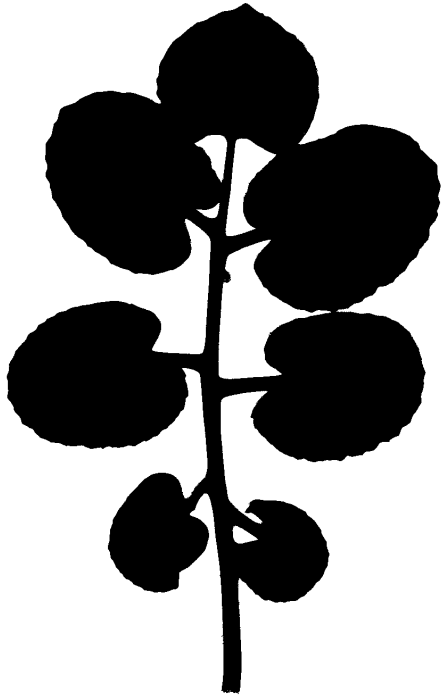
- 2D imaging can be biased (Théroux-Rancourt et al. 2017; Earles et al. 2018)
- Cell-packing constraints on CO_2 diffusion (Théroux-Rancourt et al. 2021)
- Honeycomb organization of spongy mesophyll (Borsuk et al. 2022)



Borsuk *et al.* (2022)

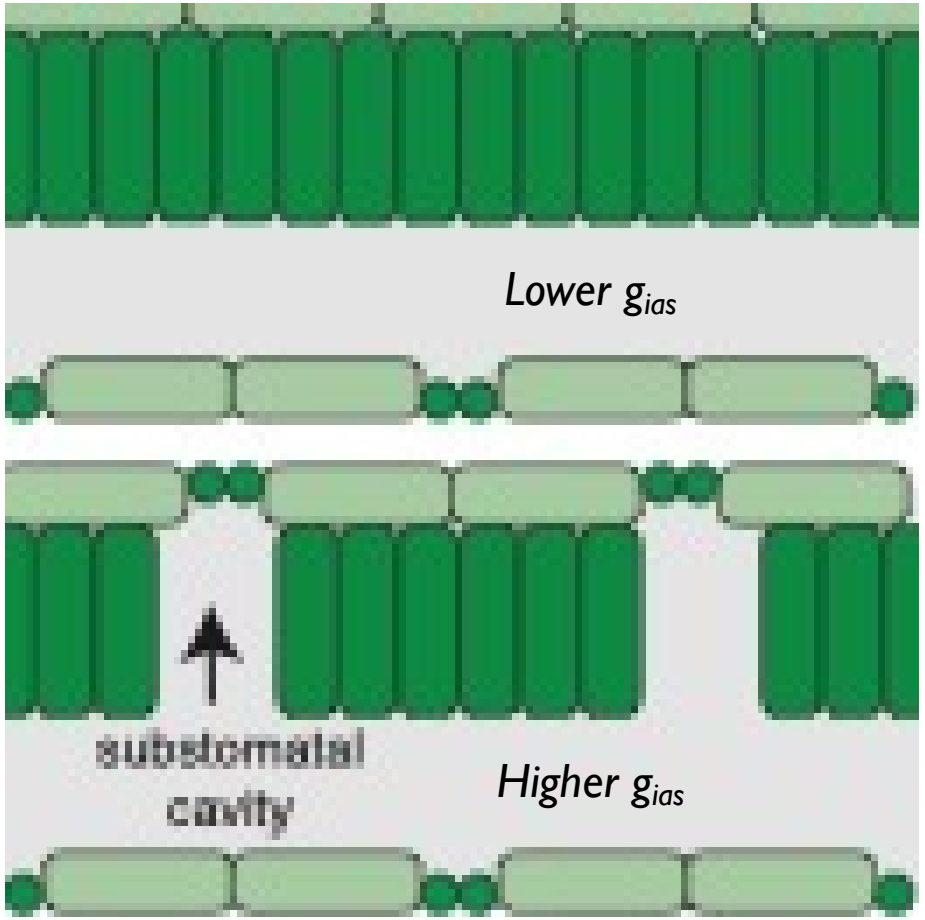

Solanum lycopersicum
Cultivated tomato
Stomata on lower
Thin leaves

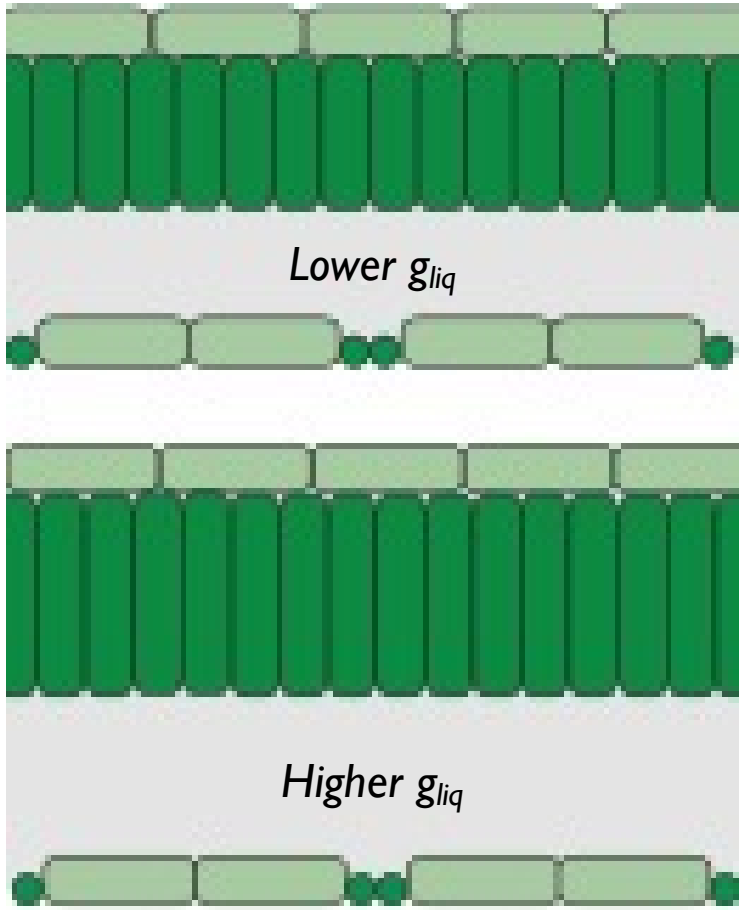

Solanum pennellii
Wild tomato species
Stomata on both surfaces
Thick leaves



Pleiotropy between stomata, leaf thickness, and CO_2 diffusion within the leaf

Adding stomata on the upper surface increases porosity (g_{ias})





A thicker leaf increases surface area exposed to mesophyll (g_{liq})

Predictions

Introgression lines	Porosity (g_{ias})	Surface area exposed to airspace (g_{liq})
Leaf thickness lt1 & lt2	?	increase
Stomatal ratio Upper:lower stomatal density sr1 & sr2	increase	?

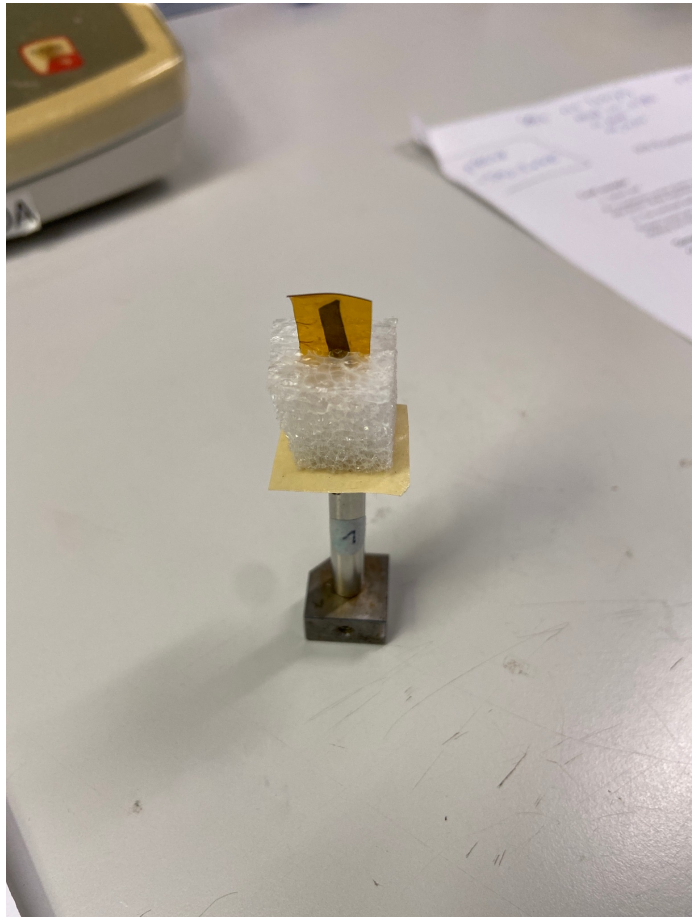
introgression lines:

Leaf thickness IL: Coneva *et al.* 2017
Stomatal ratio IL: Muir *et al.* 2014

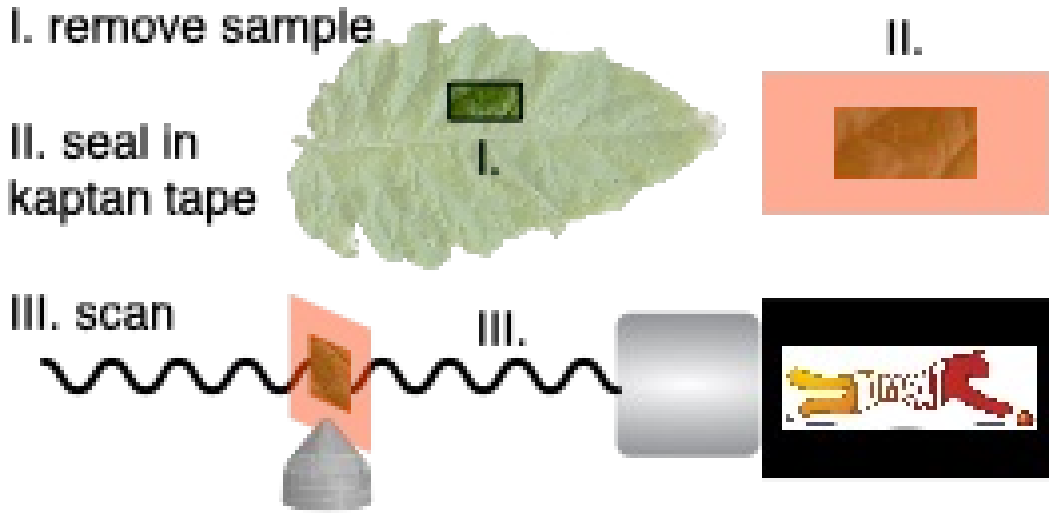
Solanum lycopersicum	5
lt1	6
lt2	5
sr1	5
sr2	6
Solanum pennellii	5

Grown at UIB by Miquel À Conesa and Jeroni Galmés

Tomographic microscopy at the Swiss Light Source

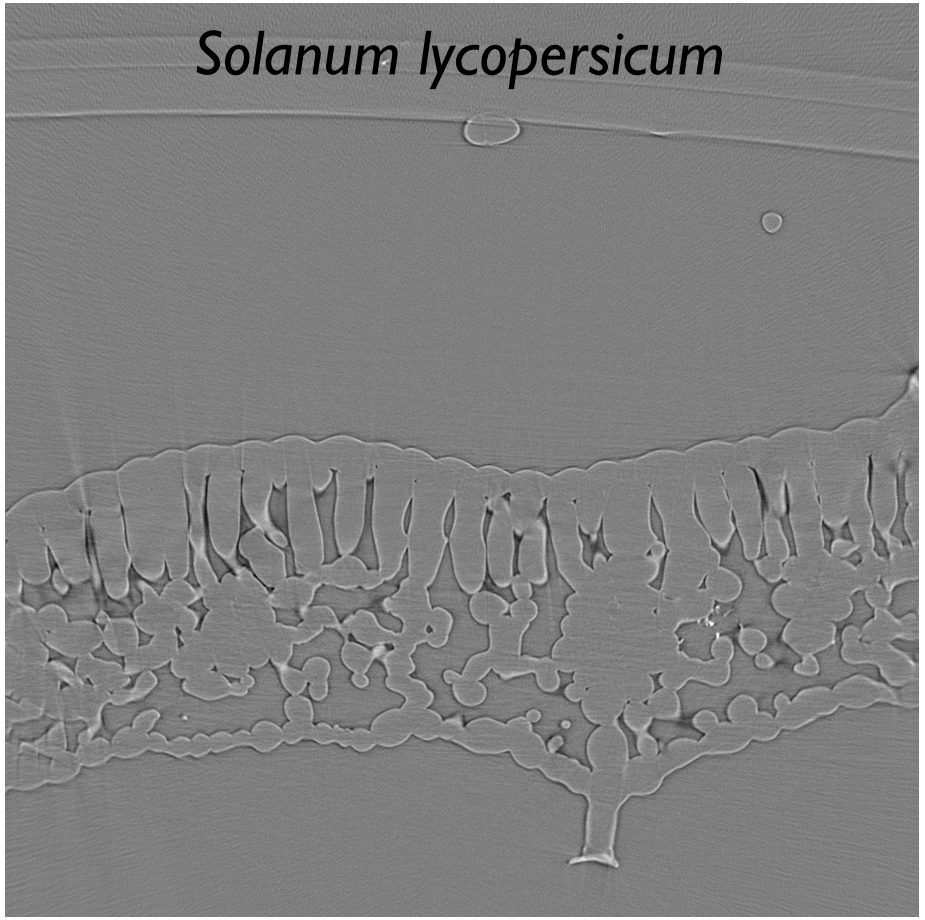


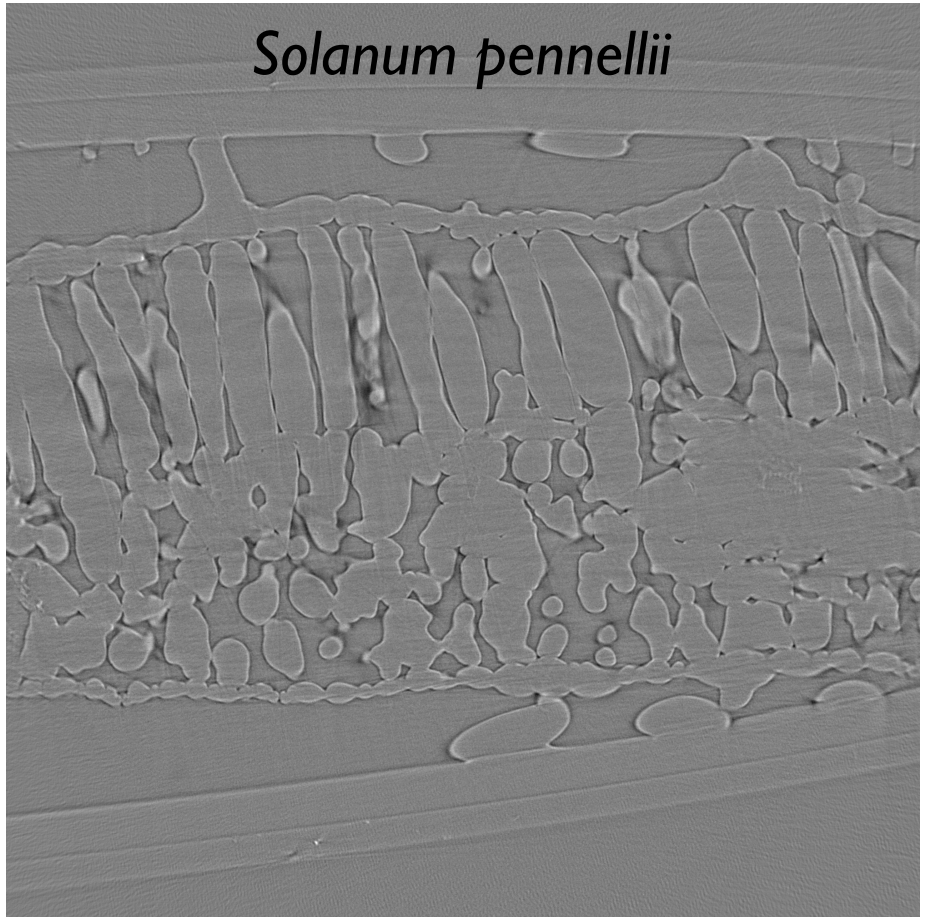
Anne Bonnin and Margaux Schmeltz



Syncrotron uCT
Monochromatic 21 keV, x20 (0.325um), 2min 15s per scan
Field of View: ~830um x 830um x 700um.
Phase Contrast

Raw data





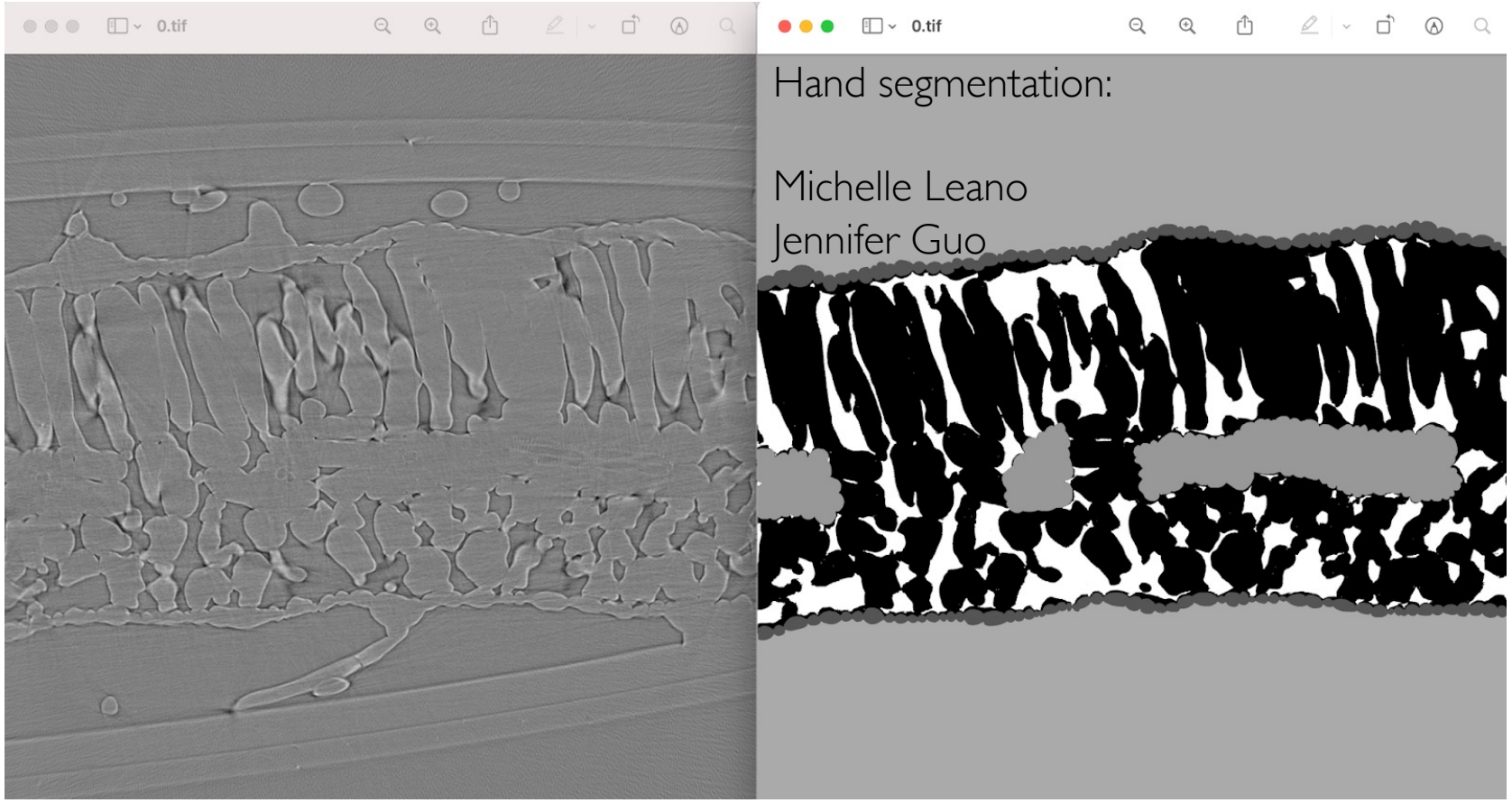
Segmentation using machine learning

Sam McKlin

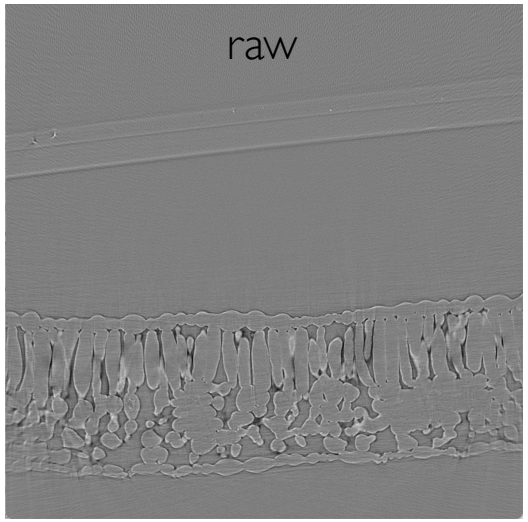
Devin Rippner

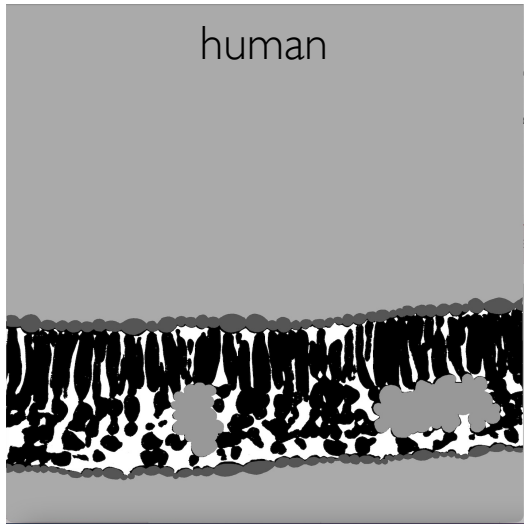
Guillaume Théroux-Rancourt

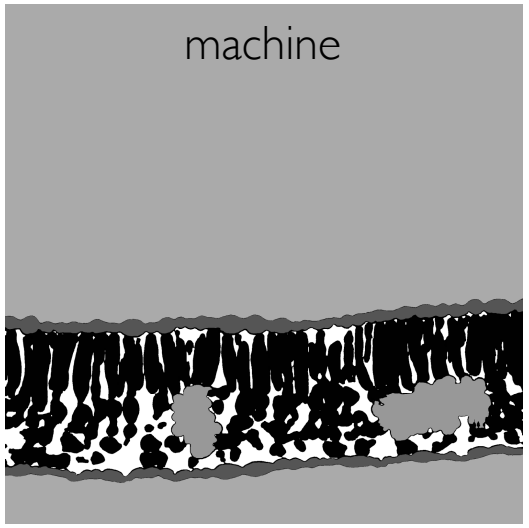
Deep learning with PyTorch implementation of a fully convolutional network with a res-net 101 backbone.



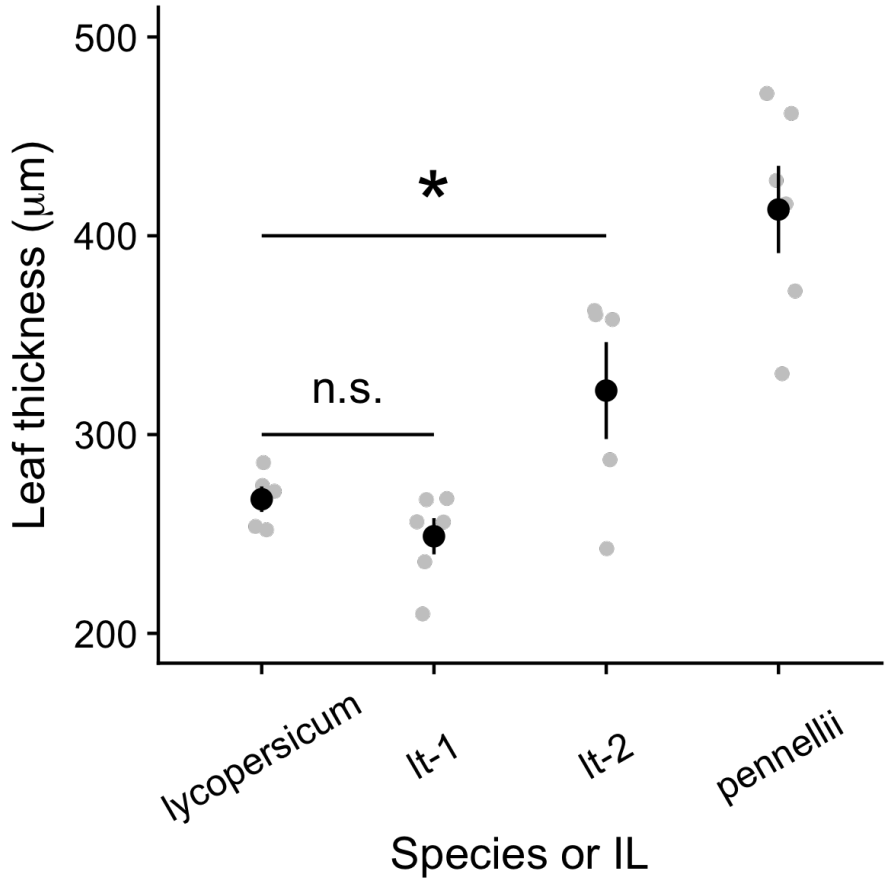
Machine segmentation was highly successful







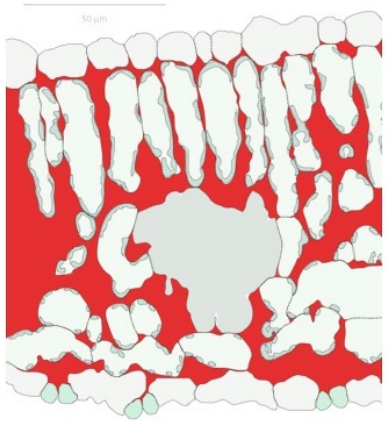
Results

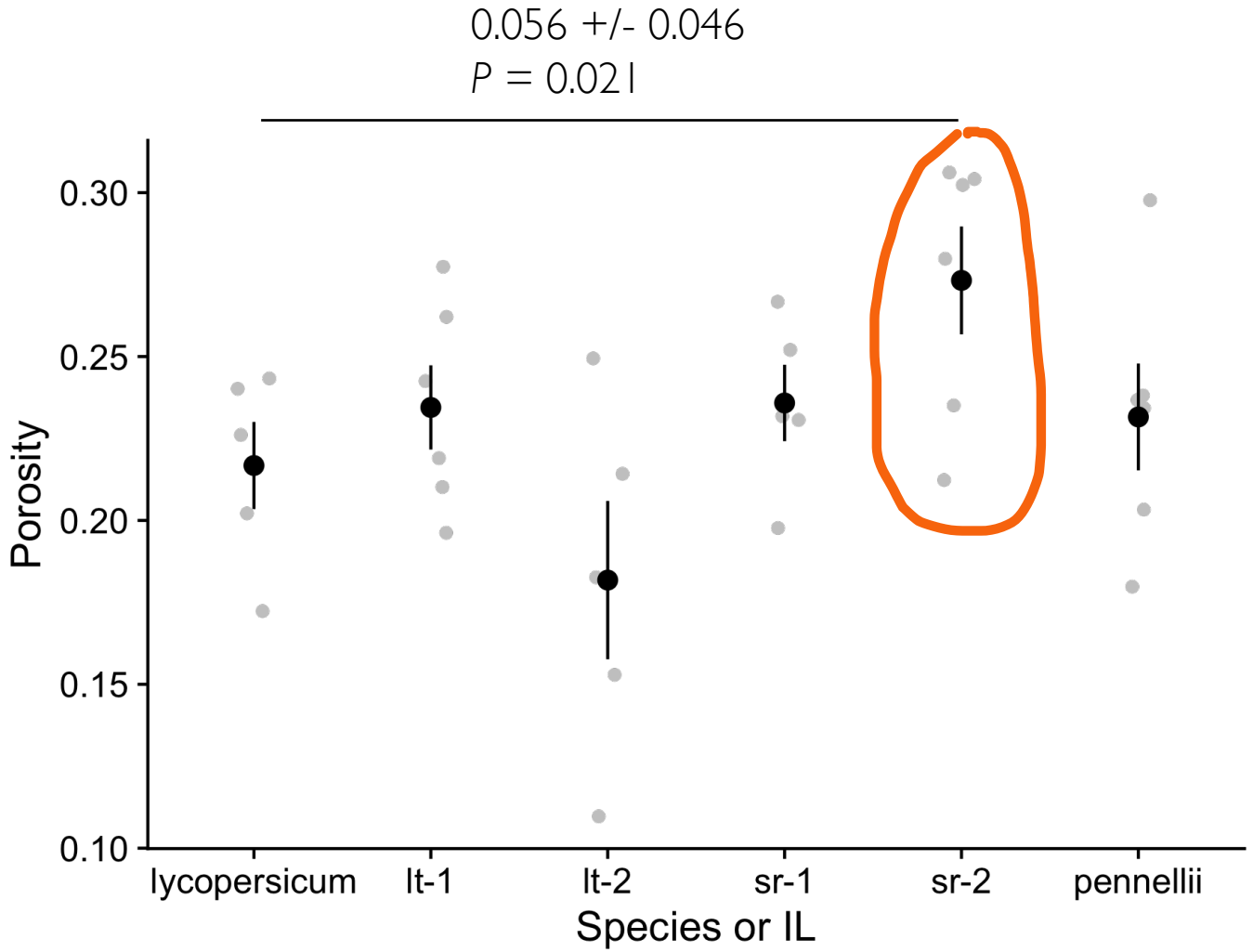


- *S. pennellii* has significantly thicker leaves
- Only one IL was significantly thicker than *S. lycopersicum*
- Stomatal ratio ILs did not affect thickness (not shown)

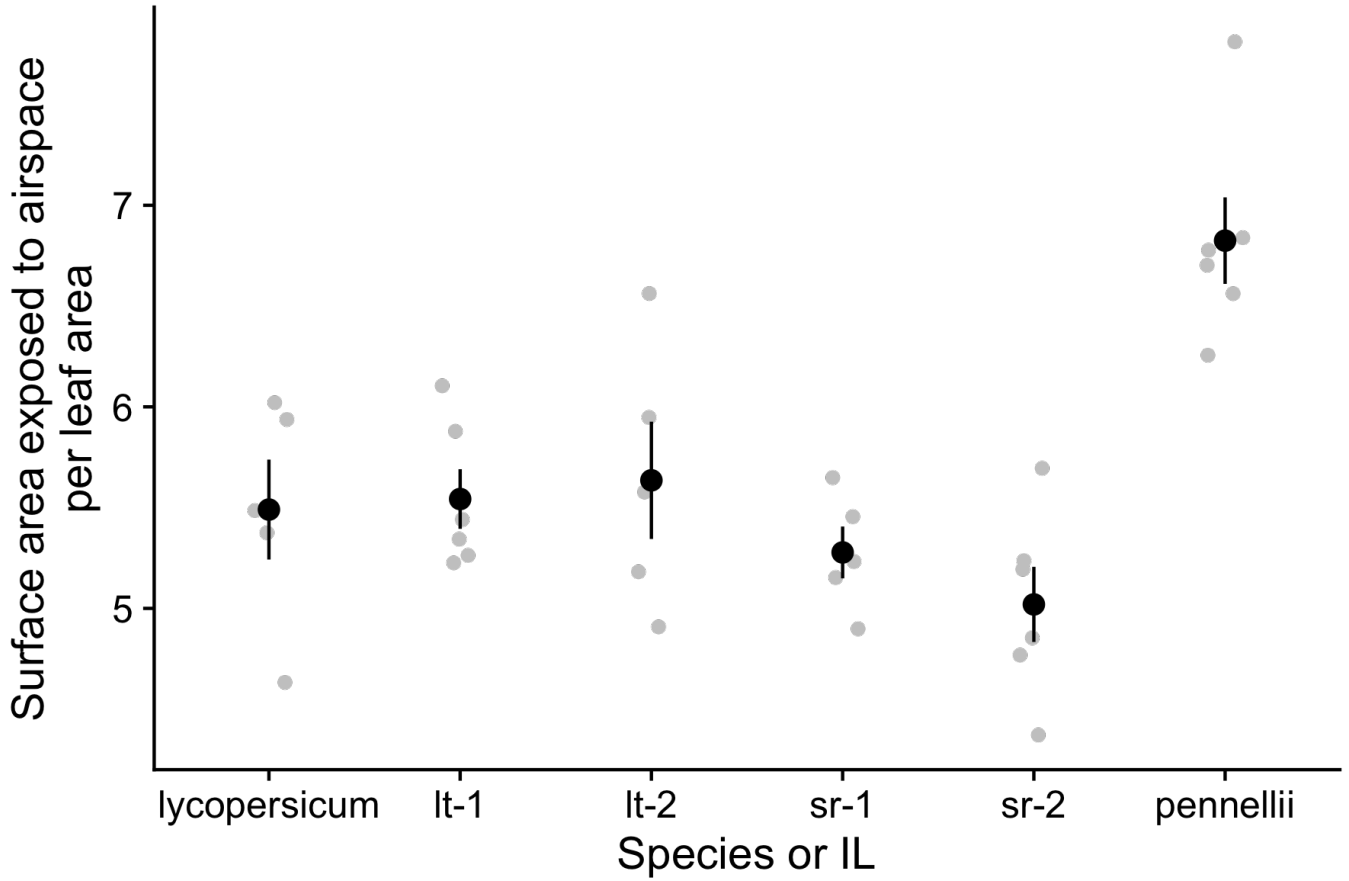
P-values based on planned comparisons

One stomatal IL increased porosity



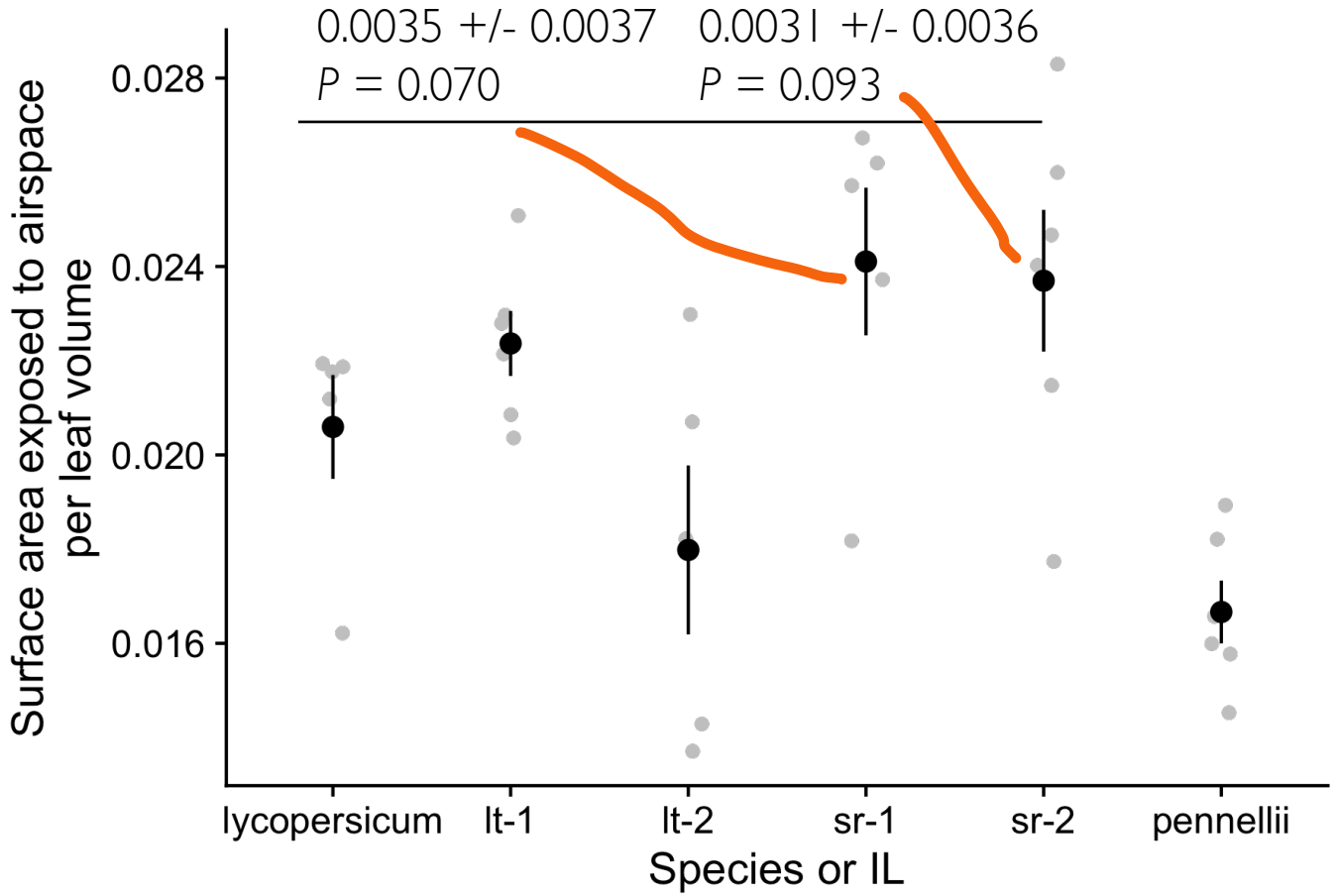


Little change in surface area exposed to mesophyll per leaf **area**



Units are $\mu m^2\ \mu m^{-2}$

Transgressive segregation in surface area exposed to mesophyll per leaf **volume**



Units are $\mu m^2\ \mu m^{-3}$

Predictions

Introgression lines	Porosity (g_{ias})	Surface area exposed to airspace (g_{liq})
Leaf thickness lt1 & lt2	?	increase
Stomatal ratio Upper:lower stomatal density sr1 & sr2	increase	?

Predictions

Introgression lines	Porosity (g_{ias})	Surface area exposed to airspace (g_{liq})
Leaf thickness lt1 & lt2	?	increase
Stomatal ratio Upper:lower stomatal density sr1 & sr2	**sr2 had slightly greater porosity**	?

Predictions

Introgression lines	Porosity (g_{ias})	Surface area exposed to airspace (g_{liq})
Leaf thickness lt1 & lt2	?	**No pleiotropy**
Stomatal ratio Upper:lower stomatal density sr1 & sr2	**sr2 had slightly greater porosity**	?

Predictions

Introgression lines	Porosity (g_{ias})	Surface area exposed to airspace (g_{liq})
Leaf thickness lt1 & lt2	**lt2 thicker and slightly greater lower porosity (n.s.)**	**No pleiotropy**
Stomatal ratio Upper:lower stomatal density sr1 & sr2	**sr2 had slightly greater porosity**	?

Predictions

Introgression lines	Porosity (g_{ias})	Surface area exposed to airspace (g_{liq})
Leaf thickness **lt1 & lt2**	**lt2 thicker and slightly greater lower porosity (n.s.)**	**No pleiotropy**
Stomatal ratio **Upper:lower stomatal density** **sr1 & sr2**	**sr2 had slightly greater porosity**	**Transgressive segregation?**

Summary

- Tomographic microscopy and segmentation using machine learning were highly successful
- Small sample sizes sufficient to distinguish 3D anatomy between closely related species (*lycopersicum* vs. *pennellii*)
- Stomatal ratio ILs had pleiotropic effects on porosity and surface area exposed to airspace per volume

Conclusions

Discovering the genetic basis of natural variation in 3D leaf anatomy using tomographic microscopy will be challenging

Large-effect loci for stomata and leaf thickness did not greatly alter 3D anatomy (not repeatable? small sample size?)

Tomographic microscopy may help confirm effects discovered using higher-throughput methods & discover unexpected pleiotropy

Acknowledgements

A long and winding path: the physical challenge of CO_2 transport within leaves

- C_3 plants use passive diffusion to transport CO_2 from atmosphere to chloroplast
- Diffusion is "free", but only effective over very short distances
- Diffusion through liquid phases is ~10^4 slower than air

High photosynthetic rates require physical solutions to these constraints

- Thin leaves with unobstructed airspace
  - Increases conductance through airspace
  - Symbolized as g_{ias}
- Large surface area of chlorophyll exposed to airspace
  - Increases conductance through liquid phase
  - Symbolized as g_{liq}

Questions that I care about

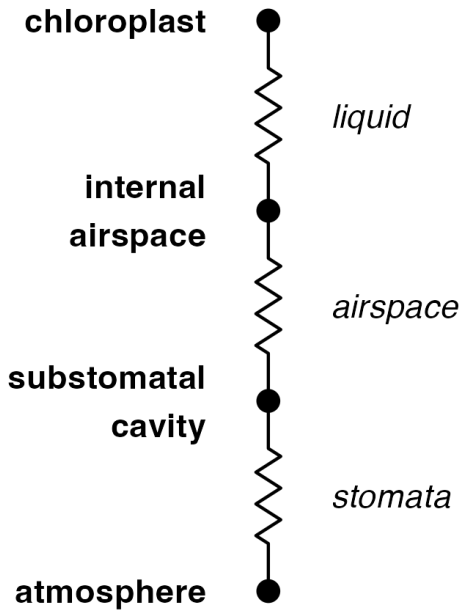
1. Why does internal leaf anatomy vary so much?
2. Can we increase CO_2 gain without increasing water loss?

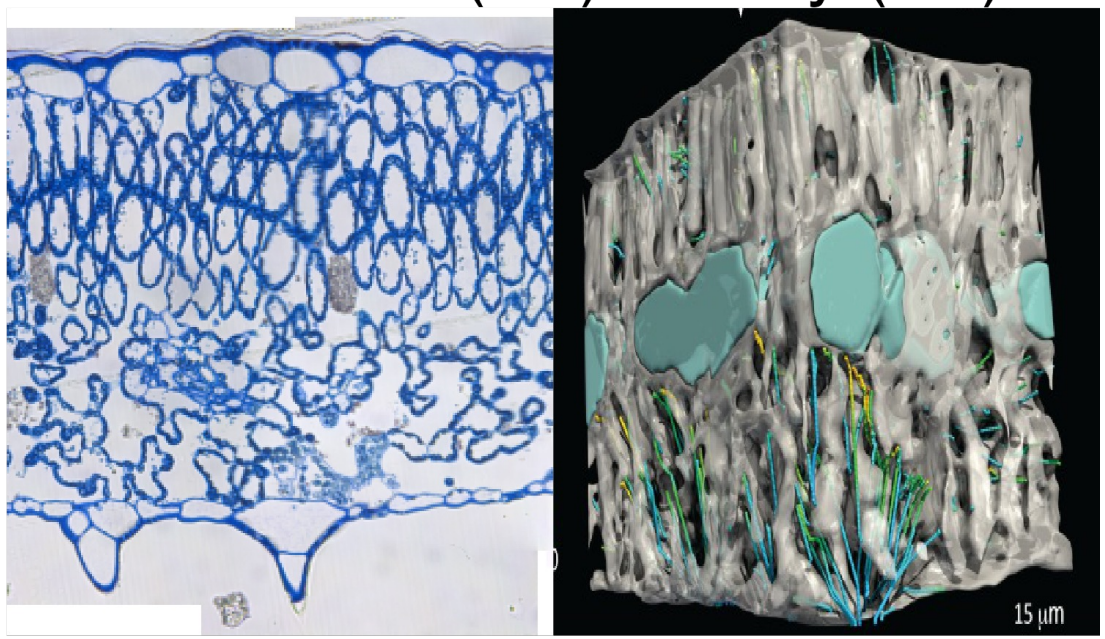
Two major limitations

1. Reality is 3-dimensional, our models and measurements of leaf anatomy are (mostly) not

Reality is 3-dimensional

model (1D) measurements (2D) reality (3D)





Earles *et al.* (2018)

Two major limitations

1. Reality is 3-dimensional, our models and measurements of leaf anatomy are (mostly) not
2. We do not know whether leaf anatomical traits can evolve independently