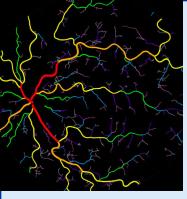
Microarray data analysis of space grown *Arabidopsis* leaves for genes important in vascular patterning. A. J. Weitzel, ^{1,2} S. E. Wyatt³, P. Parsons-Wingerter ¹.

¹Space Biosciences, NASA Ames Research Center, Mountain View, CA ²Biology Undergraduate, Grand Valley State University, Allendale, MI ³Environmental and Plant Biology, Ohio University, Athens, OH

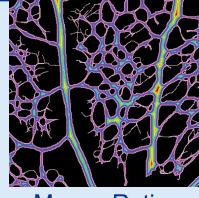
Venation patterning in leaves is a major determinant of photosynthesis efficiency because of its dependency on vascular transport of photoassimilates, water, and minerals. *Arabidopsis thaliana* grown in microgravity show delayed growth and leaf maturation. Gene expression data from the roots, hypocotyl, and leaves of *A. thaliana* grown during spaceflight vs. ground control analyzed by Affymetrix microarray are available through NASA's GeneLab (GLDS-7). We analyzed the data for differential expression of genes in leaves resulting from the effects of spaceflight on vascular patterning. Two genes were found by preliminary analysis to be upregulated during spaceflight that may be related to vascular formation. The genes are responsible for coding an ARGOS like protein (potentially affecting cell elongation in the leaves), and an F-box/kelch-repeat protein (possibly contributing to protoxylem specification). Further analysis that will focus on raw data quality assessment and a moderated t-test may further confirm upregulation of the two genes and/or identify other gene candidates. Plants defective in these genes will then be assessed for phenotype by the mapping and quantification of leaf vascular patterning by NASA's VESsel GENeration (VESGEN) software to model specific vascular differences of plants grown in spaceflight.

Research supported by a NASA Ames Center Innovation Fund Award.



NASA'S VESGEN Software

Research Discovery Tool for Fundamental and Translational Space Biology Research



Mouse Retina

Using NASA's GeneLab for VESGEN Systems Analysis of Vascular Phenotypes from Stress and Other Signaling Pathways

Patricia Parsons-Wingerter Space Biosciences Research Branch





VESGEN Patents Pending



Collaborators

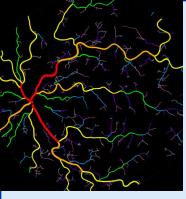
Alexander Weitzel, USRA Summer 2016 Internship, NASA Ames Research Center & Grand Valley State University,

Ruchi Vyas and Matthew Murray, MORI Associates and Blue Marble Space Institute, NASA Ames Research Center

Mary B. Vickerman, Software Systems, NASA Glenn Research Center

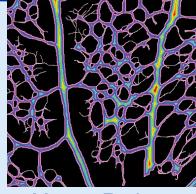
Sharmila Bhattacharya Space Biosciences Research Branch, NASA Ames Research Center

Sarah E. Wyatt, Environmental and Plant Biology, Ohio University, Athens OH



NASA'S VESsel GENeration Analysis [VESGEN] Software

Mapping and Quantification of Branching Vascular Patterns from Physiological Branching Rules



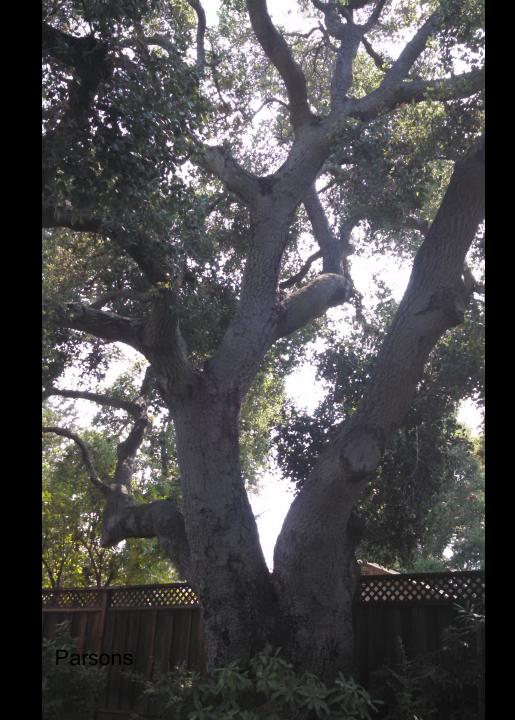
Mouse Retina

- Vascular trees, networks, and tree-network composites from set of weighted parameters for vessel connectivity, tapering and bifurcational branching
- <u>Requirements of fluid dynamics for laminar flow</u> Aqueous vascular transport by complex distributed system of fractal-based bifurcational branching
- Microvascular rules for fractal-based branching within humans, vertebrates, insects and dicot leaves therefore display many similarities



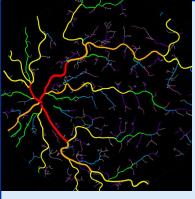


VESGEN Patents Pending

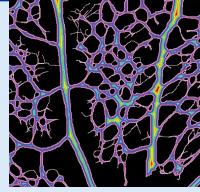








VESGEN Analysis for Fundamental Space Biology Research with Translational Applications to Astronaut Health and Countermeasures



Mouse Retina

Vascular Trees

Retinas of Astronauts and Human Bed Rest; Diabetic Retinopathy Mouse/Avian Coronary Vessels, Chorioallantoic Membrane (CAM), Yolksac

Vascular Networks

Mouse Intestinal Inflammation, CAM Lymphatic Vessels, Abnormal Mouse Corneal Angiogenesis, *Drosophila* (Fruitfly) Wing

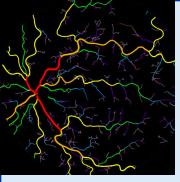
Vascular Tree-Network Composites

Mouse Postnatal Retina Early Embryonic Coronary Vessels, *Arabidopsis* Leaf Venation

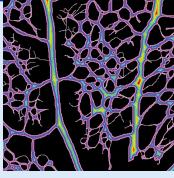




VESGEN Patent Pending



VESGEN Analysis of Vascular Patterns



Mouse Retina

Research Hypothesis

Vascular patterning offers useful readout of molecular signaling that necessarily integrates crosstalk among complex signaling pathways

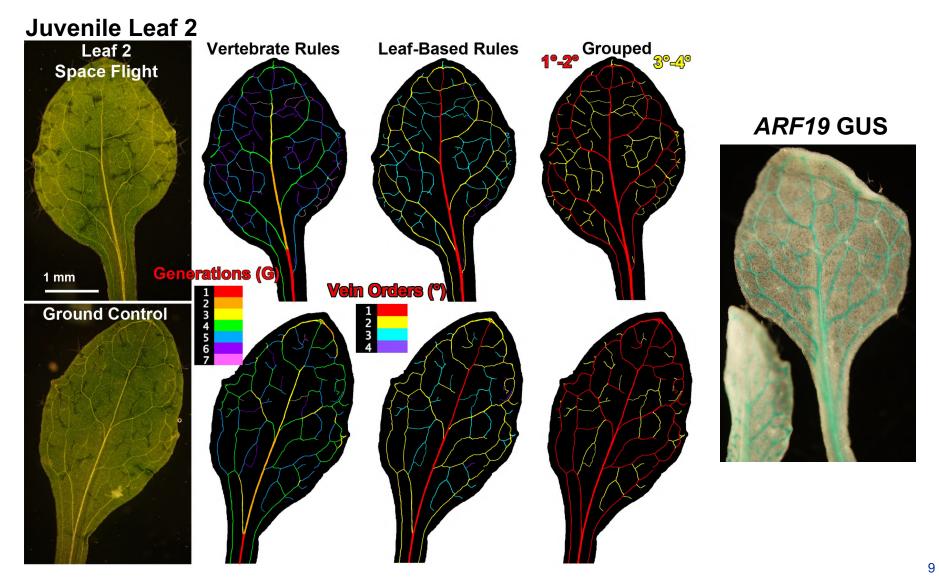
Fractal-Based Physiological Branching Rules

from fluid mechanics, anatomy, microscopic observations

Mapping and Quantification by Multiparametric Weighted Analysis

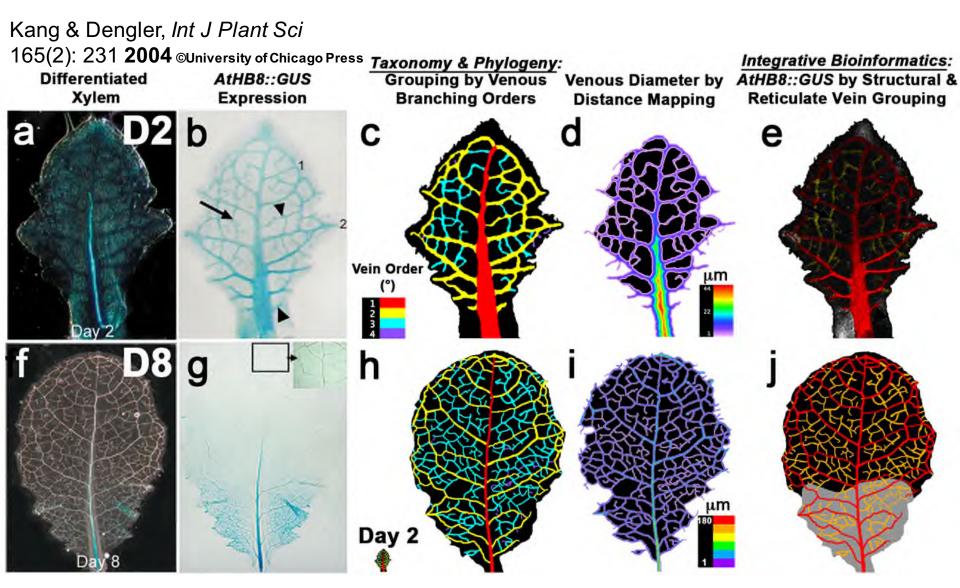
Fractal Dimension, D_f Vessel Number Density, N_v Vessel Length Density, L_v Vessel Diameter, D_v Branchpoint + Endpoint Densities, Br_v+E_v

Arabidopsis leaves from ISS: STS-130



Images of STS-130 leaves provided by A-L Paul & RJ Ferl, analyzed by P Parsons & M Vickerman P Parsons, M Vickerman, A-L Paul, R Ferl, ASGSR 2012 Grav and Space Research 2(1) 2014

VESGEN mapping of Arabidopsis leaf venation with bioinformatic analysis



P Parsons, M Vickerman, A-L Paul, R Ferl, ASGSR 2012 Grav and Space Research 2(1) 2014



The Arabidopsis spaceflight transcriptome: a comparison of whole plants to discrete root, hypocotyl and shoot responses to the orbital environment



2 datasets available for download here:

ISA-TAB Metadata file Raw Data File

GeneLab Accession Number	GLDS-7			
Source Accession Number	E-MTAB-1264			
Contacts	Name	Role	Organization	Email
	Robert Ferl	Investigator	University of Florida	robferl@ufl.edu
	Anna-Lisa Paul	Investigator	University of Florida	alp@ufl.edu
	Agata Zupanska	Submitter	University of Florida	zupanska@ufl.edu

Space Grown *Arabidopsis* with Microarray Data from GeneLab: Identification of Genes Important in Vascular Patterning

(A Weitzel, P Parsons, S Wyatt; ASGSR 2016)

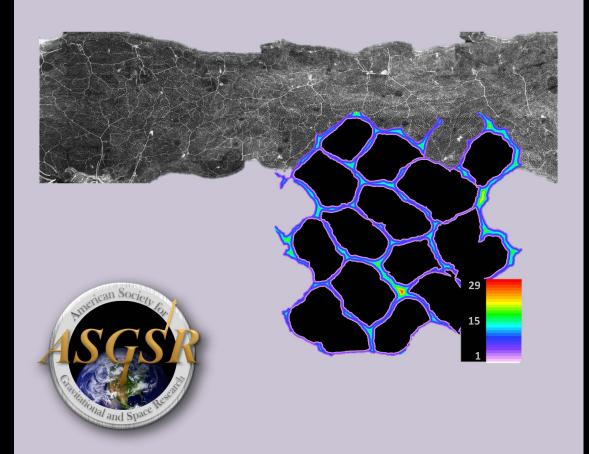
- Analysis of transcriptomic data from space flight and ground control leaves identified differential expression of 22 genes, of which seven may be related to plant vasculature
- Two gene clusters suggest there may be phenotypic changes in leaf venation resulting from development in microgravity
 KISS ME DEADLY [KMD] coding F-box genes
 NAM, ATAF1/2, and CUC2 [NAC] related genes
- Vascular-related changes in leaf gene expression can potentially be phenocopied by mutants in ground-based experiments and corroborated by VESGEN analysis
- Genetic, transcriptional and other molecular changes reported by GeneLab can be mapped to vascular phenotypes by VESGEN by bioinformatic co-localization of single molecular expression



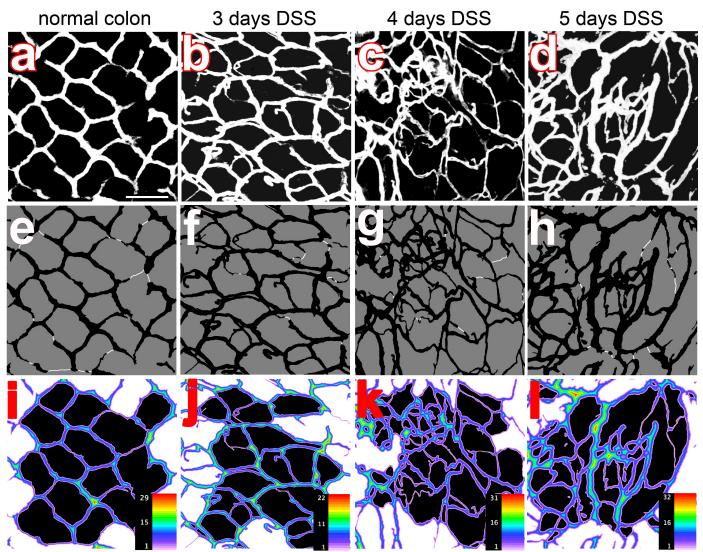


Gravitational and Space Biology

Publication of the American Society for Gravitational and Space Research

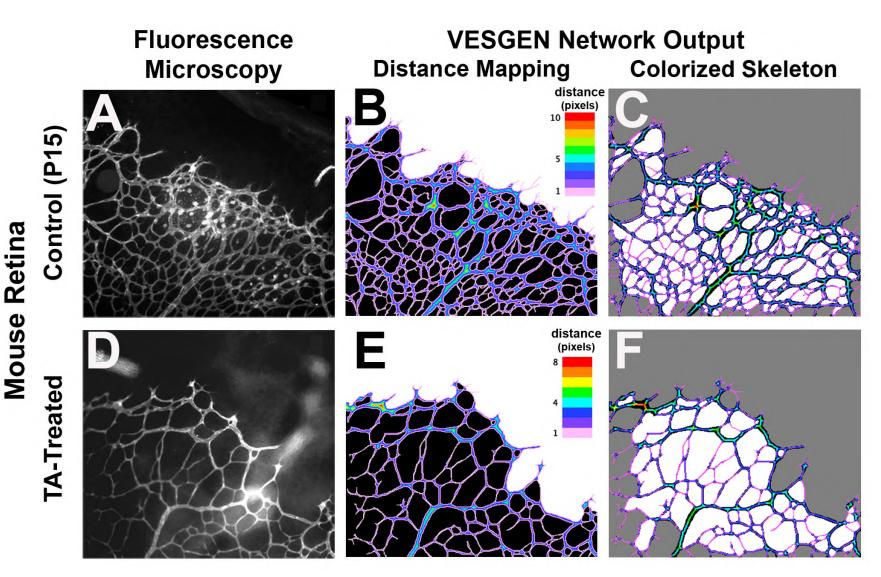


VESGEN mapping of vascular networks for progressive GI inflammation progression with mouse model



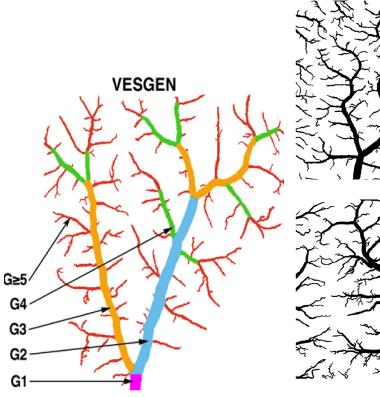
Parsons, Reinecker, Chen et al Gravitational Space Biology 25(1):69 2012; PlosONE 2013

Vascular Networks in Transgenic Mouse Retina



with J Sears & Q Ebrahem (Cole Eye Institute), from Vickerman et al, Anat Rec 292(3), 2009

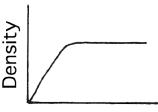
VESGEN Hypothesis *'Fingerprint'* or *'Signature'* Vascular Pattern as Useful Integrative Readout of Complex Molecular Signaling Pathways



he form of an object is a 'diagram of forces'

D'Arcy Thompson

bFGF as Simple Stimulator Arterio Thromb Vasc Biol 20 (2000)



VEGF as Vascular Complexity Factor: Phenotypic Readout with eNOS Signaling *Microvascular Research* 72 (2006)



TGF-β1 as Simple Inhibitor but Complex Potentiator *Microvascular Research 59 (2000)*

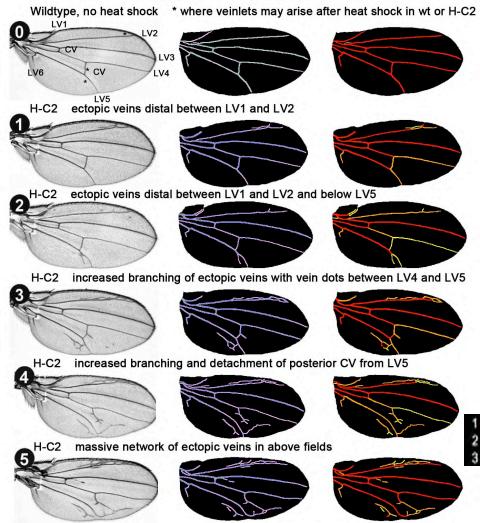


Concentration

Fruitfly (*Drosophila melanogaster*): Major Genetic Model Organism

Mapping by VESGEN: Hairless (H-C2) overexpression induces phenotypic series of increasing ectopic wing venation

6 Longitudinal Veins (LV) with anterior and posterior Cross Veins (CV)



Johannes B, Preiss A. Wing vein formation in Drosophila melanogaster: hairless is involved in the cross-talk between Notch and EGF signaling pathways. Mechanisms of development 2002; 115(1-2): 3-14

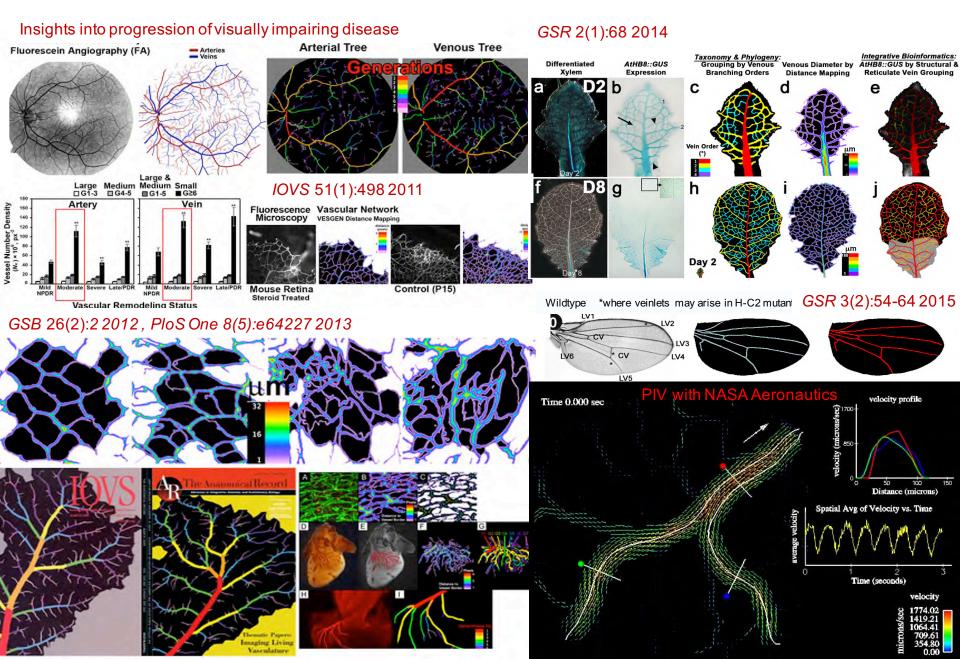




Expression of Genes Involved in Drosophila Wing Morphogenesis and Vein Patterning Are Altered by Spaceflight

Parsons, Hosamani, Vickerman, Bhattacharya Grav Space Res 3(2):54-64 2015, ASGSR 2012

VESGEN Analysis of Vascular Patterning for Fundamental Space Biology with Translational Applications to Astronaut and Terrestrial Health





Acknowledgments



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Cleveland Clinic Foundation Cole Eye Institute– Peter Kaiser, Jonathan Sears, Quteba Ebrahem Lerner Research Institute– Paul DiCorleto, Unni Chandrasekharan

Case Western Reserve University Michiko Watanabe, Monica Montano, Karunamuni G

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