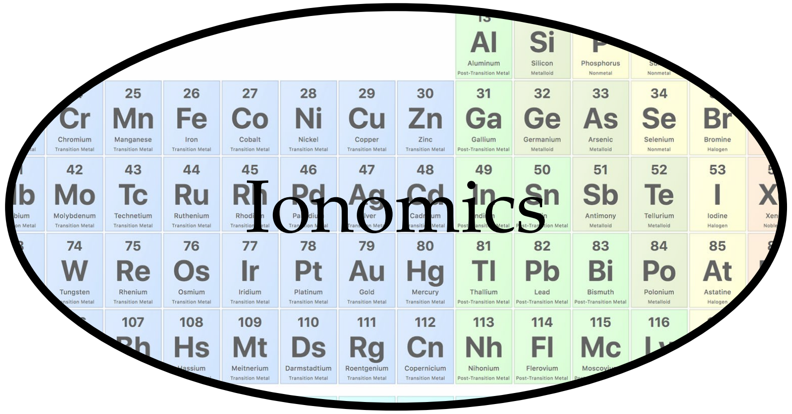
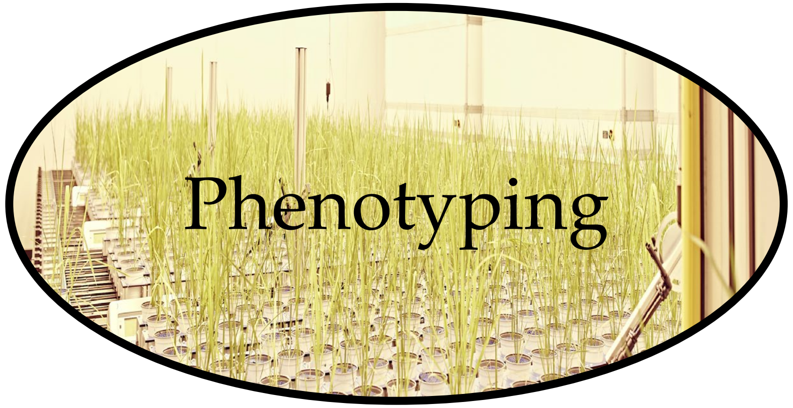
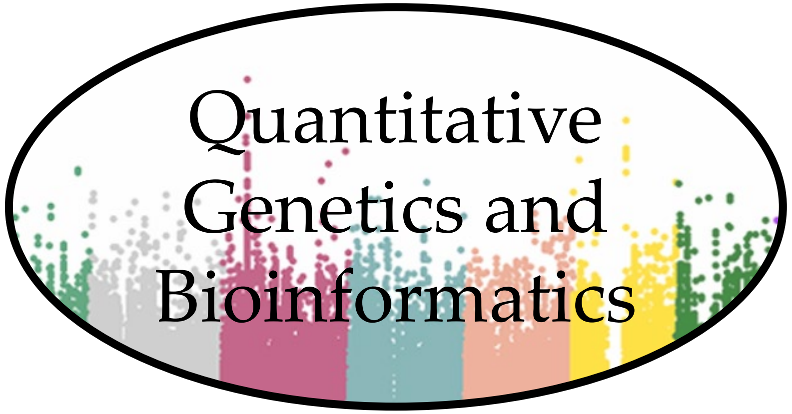
A Comparative Approach for Selecting Orthologous Candidate Genes Underlying Signal in Genome-Wide Association Studies across Multiple Species

Baxter Lab
Donald Danforth Plant Science Center

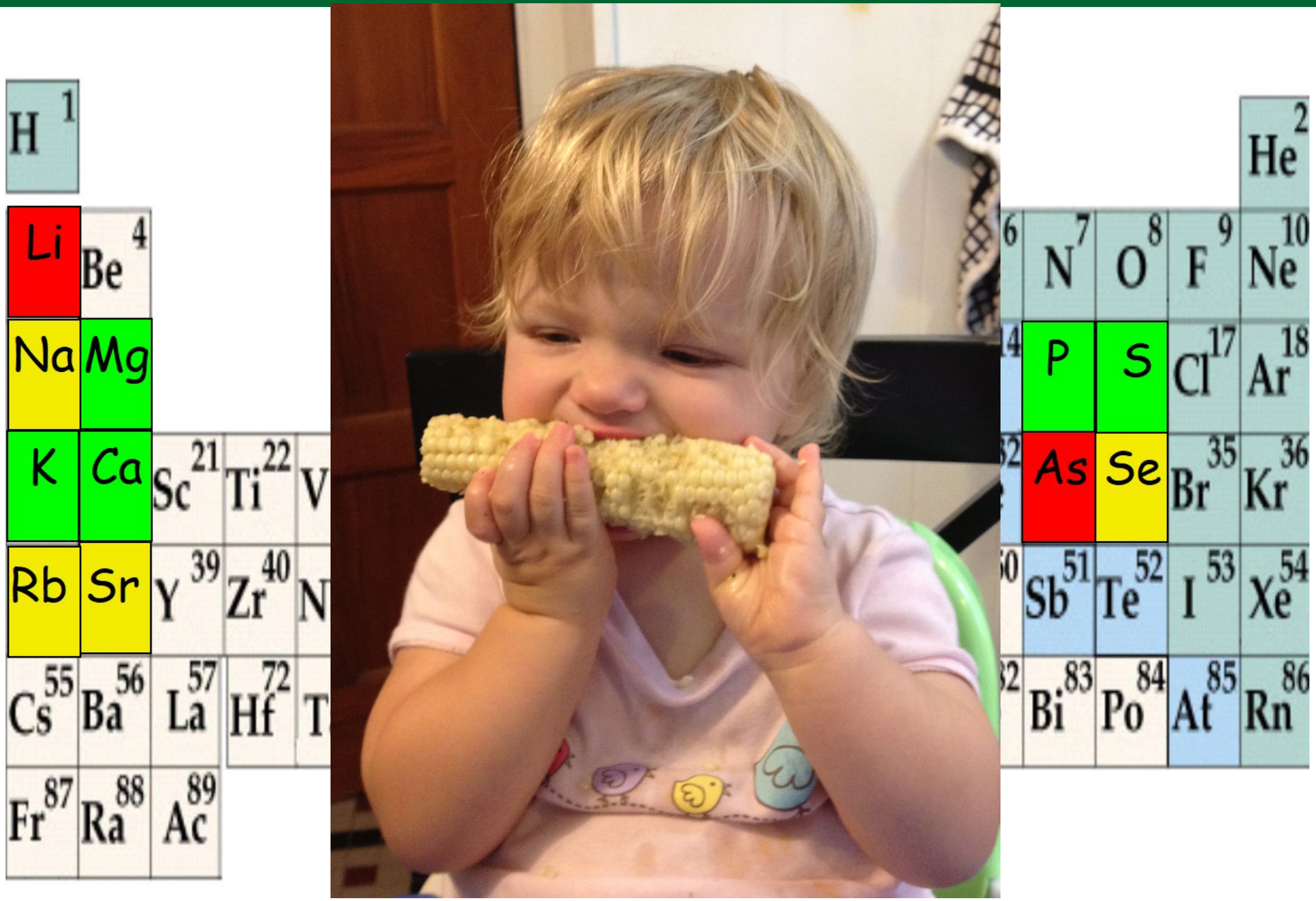
The Baxter Lab


Ionomics


Phenotyping

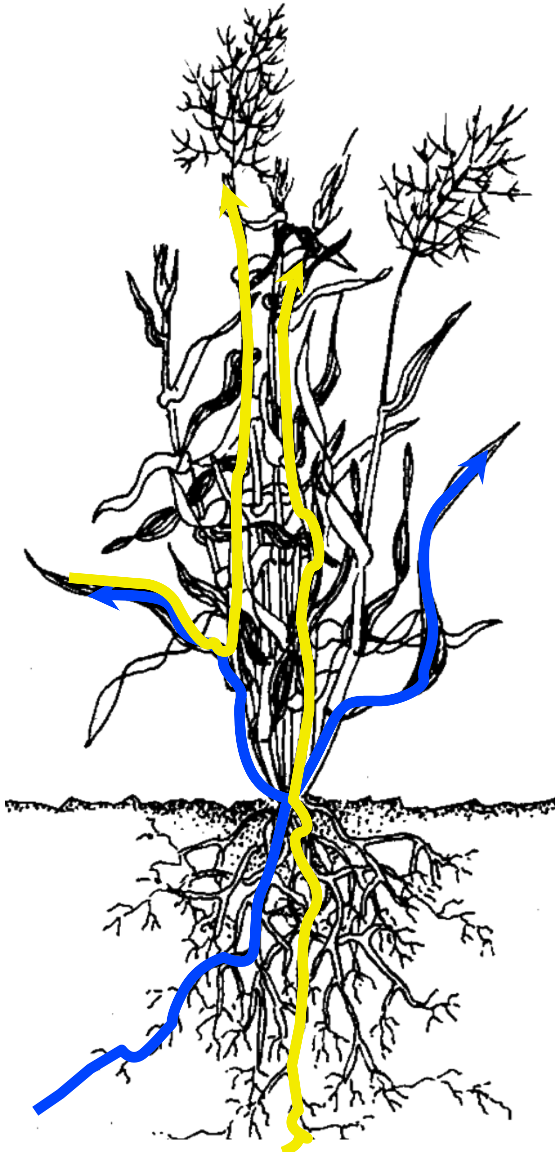

Quantitative Genetics and Bioinformatics

The Ionome: What Is It and Why Do We Care?

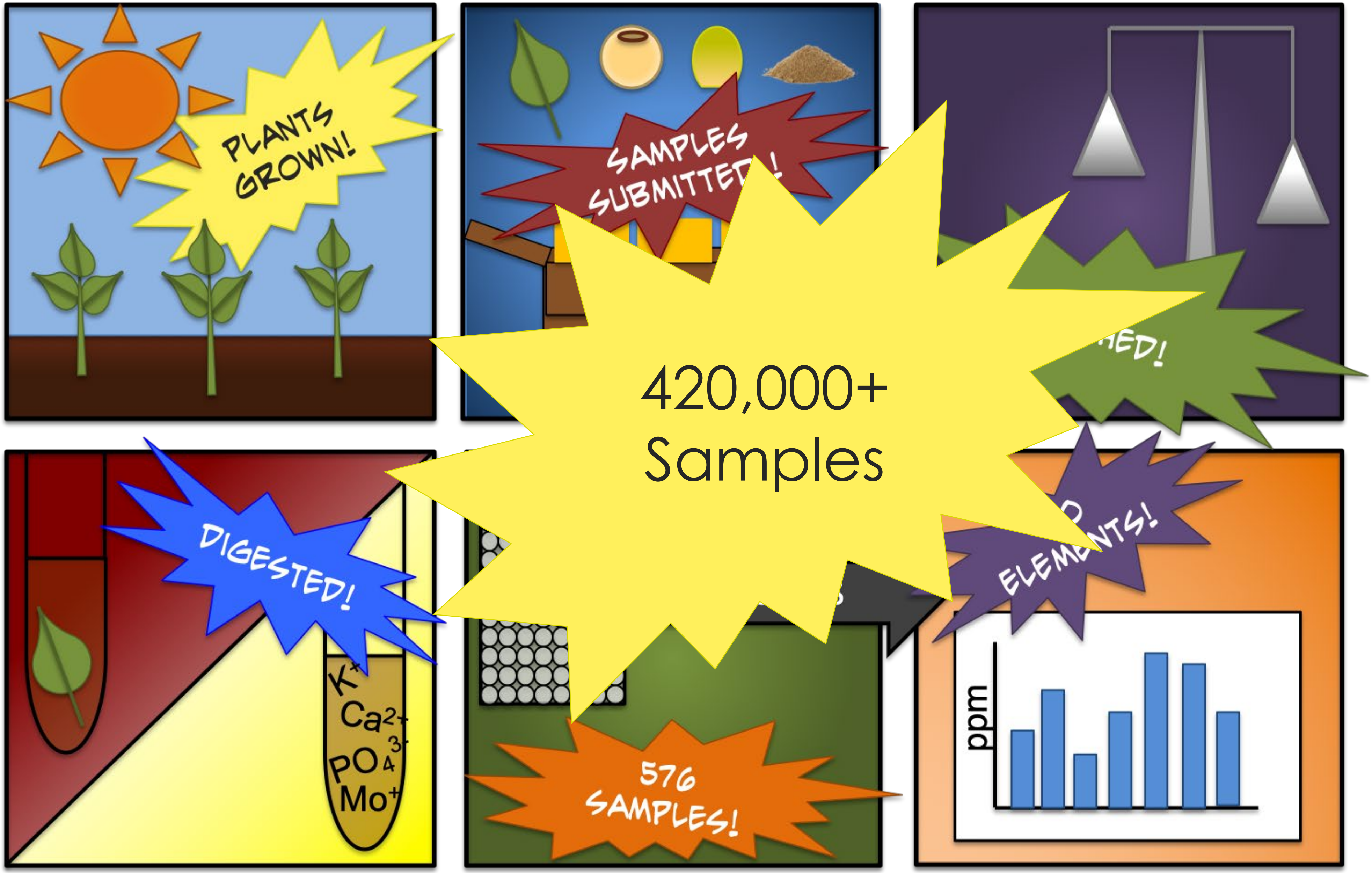


Ionome: “all” the inorganic ions within a cell, tissue, or organism

Elemental Accumulation Is Determined by Plant/Environment Interactions



The Ionomics Pipeline



420,000+
Samples


Jurkowski


Ziegler

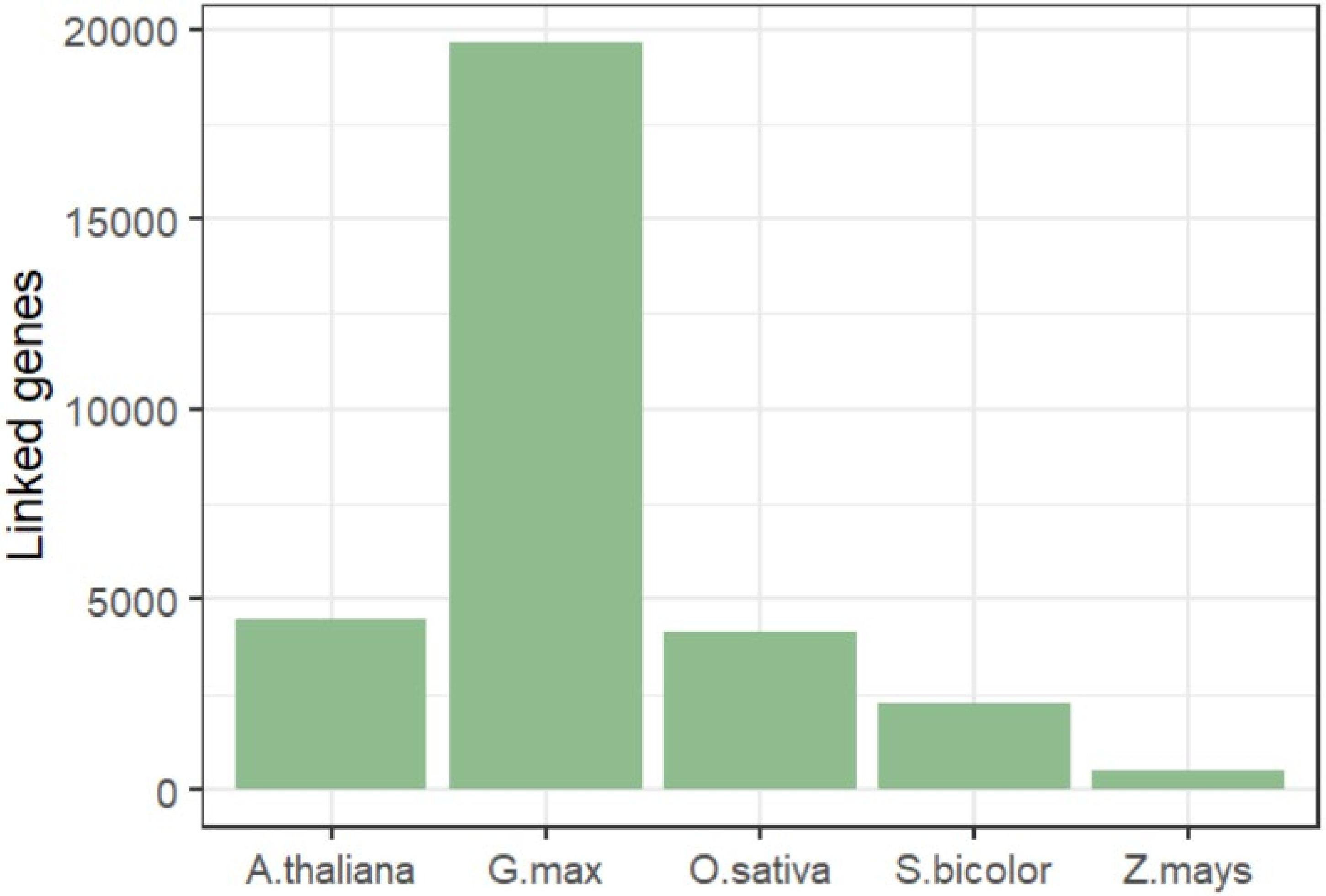
Seed ionome GWAS datasets are available for a many species.

	Arabidopsis thaliana	Glycine max (soybean)	Oryza sativa (rice)	Sorghum bicolor	Zea mays (corn)
Environments	Single	Multiple	Multiple	Multiple	Multiple
Loci across 19 traits	1404	7308	432	1768	905

Gene lists from GWAS can be quite large, so how do we narrow them down?

Genes linked to molybdenum uptake loci

Species	# GWAS loci
A. thaliana	109
G. max	362
O. sativa	70
S. bicolor	115
Z. mays	73



What Are the Genes Underlying Elemental Accumulation?

New York Times

By **Carl Zimmer**

Sept. 18, 2018

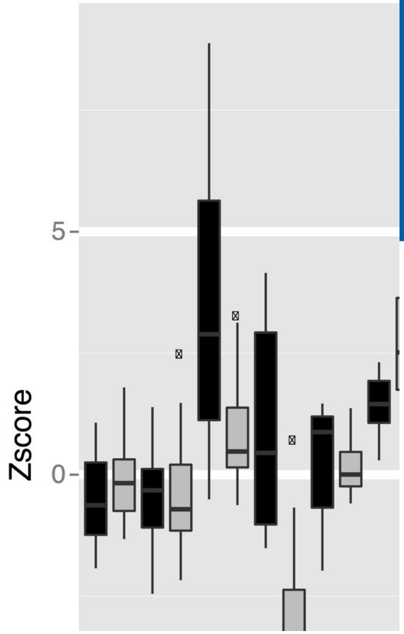
MATTER

Why Your DNA Is Still Uncharted Territory

Scientists are focusing on a relatively small number of human genes and neglecting thousands of others. The reasons have more to do with professional survival than genetics.

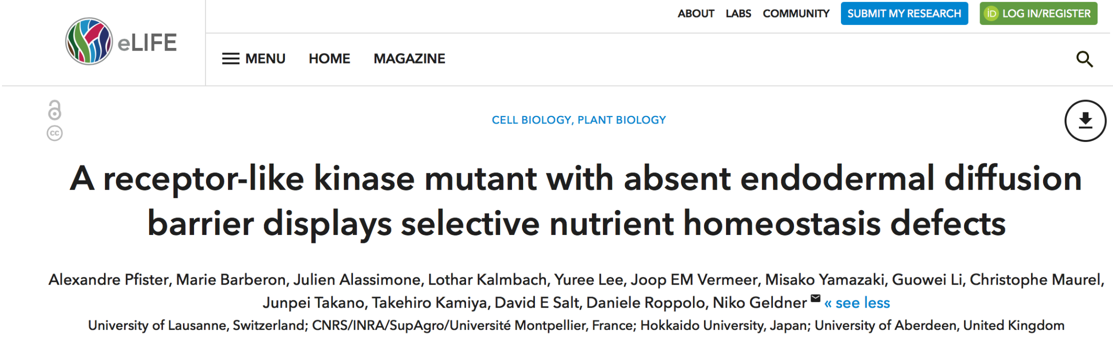
Just 15 explanations mostly accounted for how many papers have been published on a particular gene. The reasons have more to do with the working lives of scientists than the genes themselves.

What Does an Ionomic Candidate Gene Look Like?



Dirigent domain-containing protein is part of the machinery required for formation of the lignin-based Casparian strip in the root

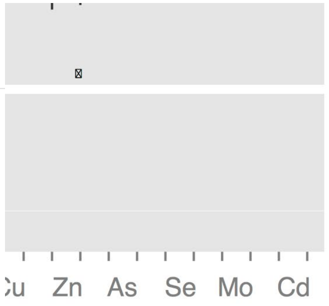
Prashant S. Hosmani, Takehiro Kamiya, John Danku, Sadaf Naseer, Niko Geldner, Mary Lou Guerinot and David E. Salt



A receptor-like kinase mutant with absent endodermal diffusion barrier displays selective nutrient homeostasis defects

Alexandre Pfister, Marie Barberon, Julien Alassimone, Lothar Kalmbach, Yuree Lee, Joop EM Vermeer, Misako Yamazaki, Guowei Li, Christophe Maurel, Junpei Takano, Takehiro Kamiya, David E Salt, Daniele Roppolo, Niko Geldner « see less

University of Lausanne, Switzerland; CNRS/INRA/SupAgro/Université Montpellier, France; Hokkaido University, Japan; University of Aberdeen, United Kingdom



esb1 mutant in two different trays

Baxter et al. *PLoS Genetics* 2009

Known Ionomic Genes: What do we know?

Plant Direct

Open Access

ORIGINAL RESEARCH | Open Access

A curated list of genes that affect the plant ionome

Lauren Whitt, Felipe Klein Ricachenevsky, Greg Ziegler Ziegler, Stephan Clemens, Elsbeth Walker, Frans J. M. Maathuis, Philip Kear, Ivan Baxter

First published: 21 October 2020 | https://doi.org/10.1002/pld3.272

216 primary KIGs – experimentally proven

2523 inferred KIGs – homologs to primary KIGs

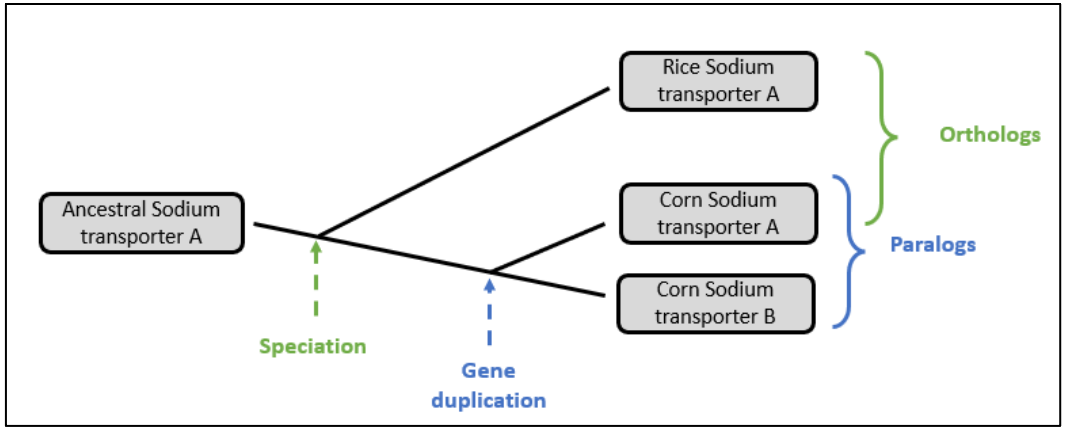
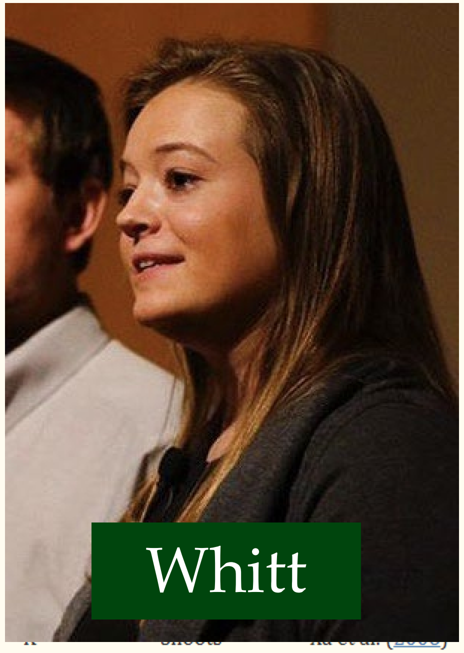


TABLE 1

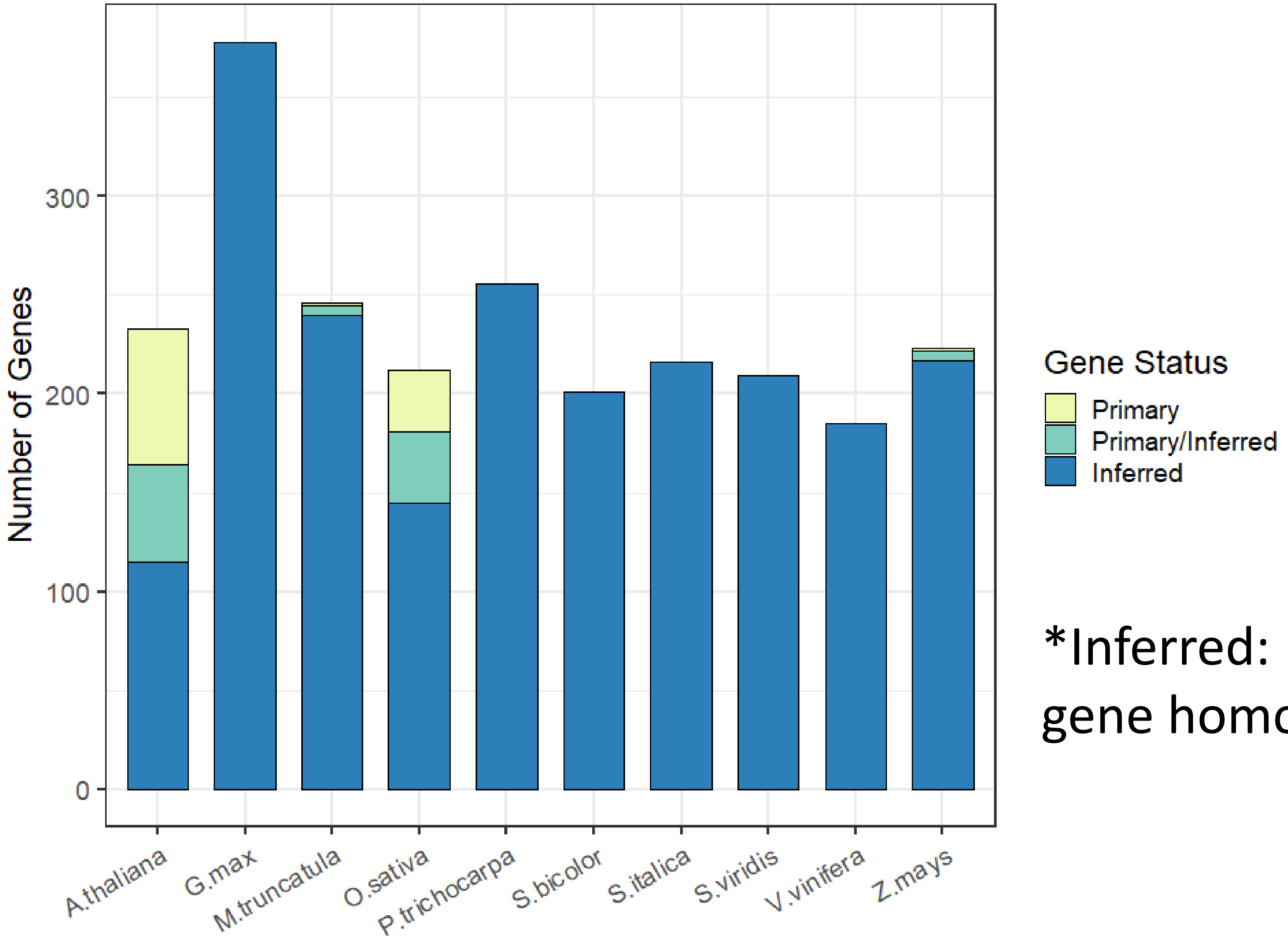
Primary known ionome genes

Species	GeneID	GeneName	Elements	Tissue	Citation(s)
A. thaliana	AT1G01340	CNGC10	K, Ca, Mg	Roots, shoots	Guo et al. (2010)
A. thaliana	AT1G01580	FRO2			
A. thaliana	AT1G07600	MT1A			
A. thaliana	AT1G08490	CPNIFS			
A. thaliana	AT1G12640	LPCAT1			
A. thaliana	AT1G14040	PHO1;H3			
A. thaliana	AT1G14870	PCR2			
A. thaliana	AT1G18910	BTSL2			
A. thaliana	AT1G20110	FYVE1			
A. thaliana	AT1G30270	CIPK23			
A. thaliana	AT1G30400	ABCC1	Cd	Shoots	Park et al. (2012)
A. thaliana	AT1G30450	CCC	Ca, K, Na,S	seeds	McDowell et al. (2013)



Whitt

We do Gene Discovery in Models



*Inferred: Primary gene homolog

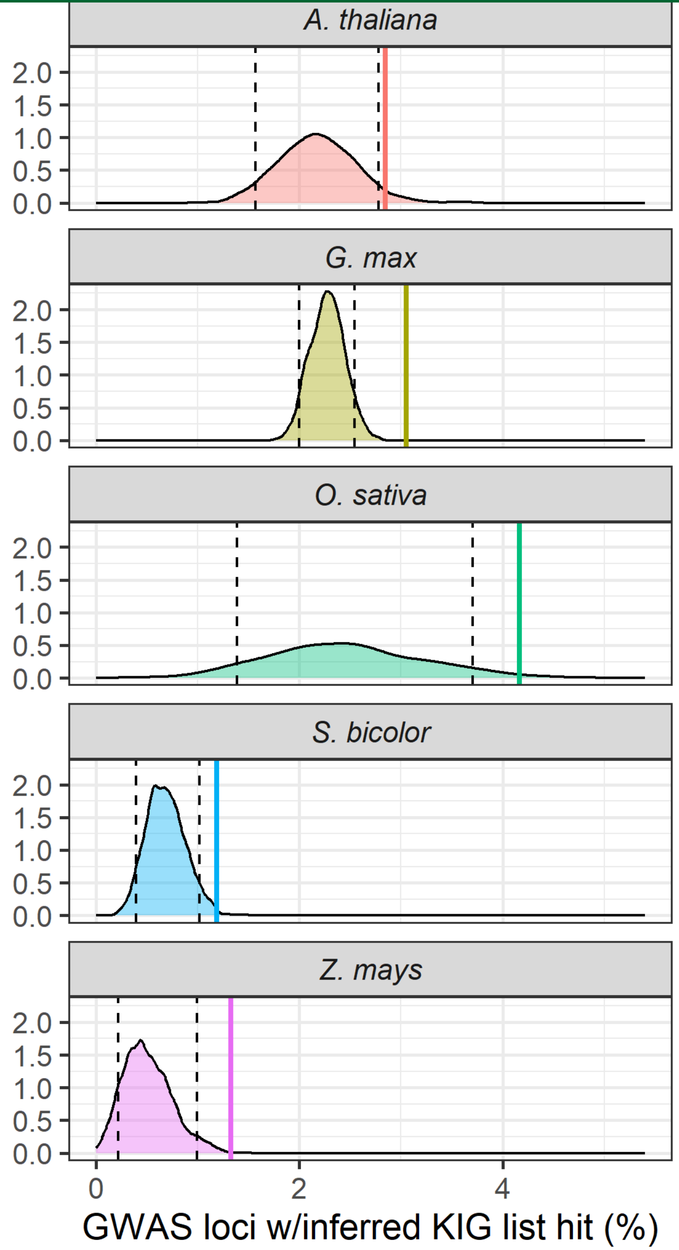
KIG ortholog groups overlapping GWAS loci in 3+ species

KIG family	Cited trait	GWAS trait	Orthologs
AtZIF1, AtZIFL2 OsZIFL12. ZmYS3	Cs, Zn, Fe	Fe	AtZIFL2 Glyma.06G313600 / 06G313700 Sobic.008G015700
AtPHT1;1, AtPHT1;4, AtPHT1;5 OsPT2	P, As, Se	P	AtPHT1;4 Glyma.10G186400 / 10G186500 LOC_Os08g45000
AtPEN3	Cd	Cd	AT3G16340 Glyma.07G015800 LOC_Os01g52560 / 06g36090
AtNRAMP3	Fe, Mn, Zn	Mn	AtNRAMP3 Glyma.04G044000 Sobic.001G170000 Zm00001d030846
AtMTP11	Mn	Mn	AtMTP11 Glyma.18G286100 Sobic.003G349200 Zm00001d042939
AtMOT2	Mo	Mo	AtMOT2 Glyma.08G208700 LOC_Os01g45830 Sobic.003G237000. Zm00001d044068
AtMOT1. MtMOT1.2, MtMOT1.3. OsMOT1;1	Mo	Mo	AtMOT1. Glyma.06G074100 / 17G203000 / 17G203200 / 17G203500. Zm00001d033053
AtBTS	Fe, Mn, Zn	Fe	Glyma.05G237500 / 08G044700 Sobic.009G222200 Zm00001d038916
AtESB1	Ca, Mn, Zn, Na, S, K, As, Se, Mo	Mn	AT1G07730, AT2G39430 Glyma.08G258300 / 12G067400 / 18G282600 Sobic.003G064800
AtHMA2, AtHMA3, AtHMA4 OsHMA3	Zn, Cd	Cd	AtHMA4, AtHMA2, AtHMA3 Glyma.09G055600 OsHMA3 Sobic.002G083000 / 002G083100 Zm00001d005189, Zm00001d005190

5/5 species

KIG/GWAS Overlap is Significant

Species	False discovery rate (FDR)
A. thaliana	0.73
G. max	0.64
O. sativa	0.53
S. bicolor	0.58
Z. mays	0.38



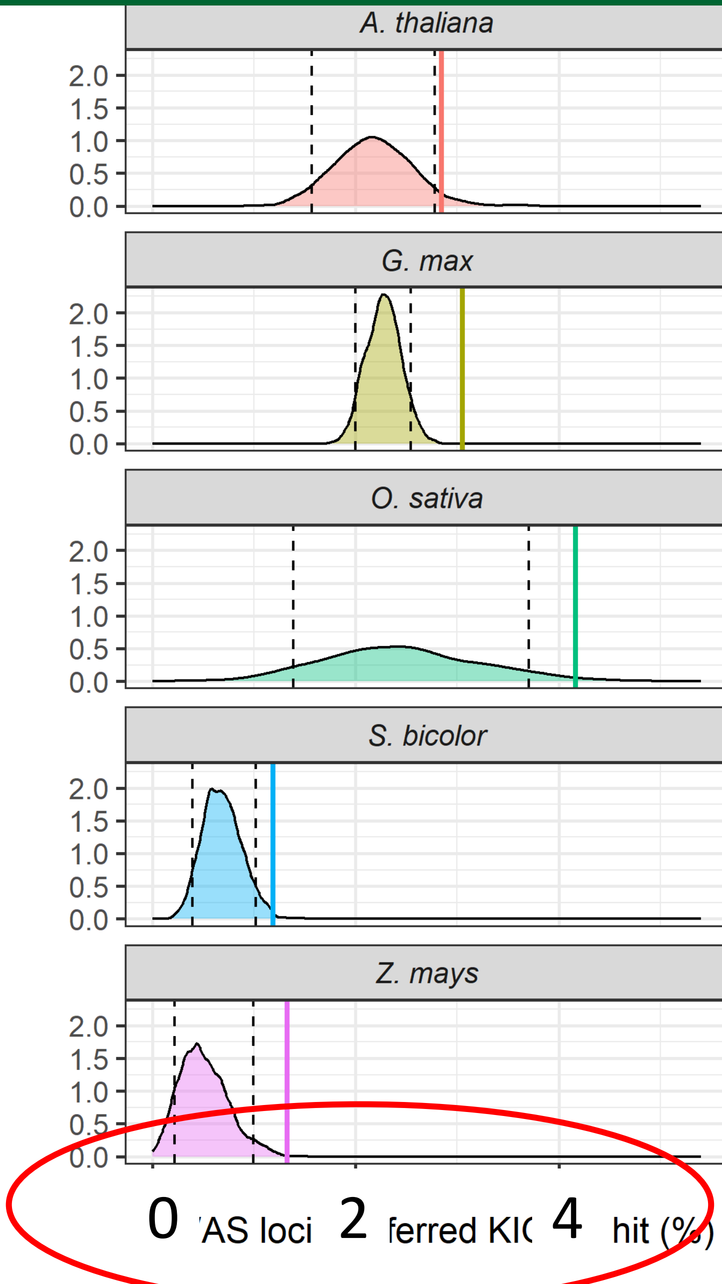
Solid lines mark the total number of loci

Curves show 1000 permutations of the data using random genome locations to replicate loci

Dashed lines mark the 5th/95th percentiles

KIG/GWAS Overlap is <5% of All Loci

Species	False discovery rate (FDR)
A. thaliana	0.73
G. max	0.64
O. sativa	0.53
S. bicolor	0.58
Z. mays	0.38



Solid lines mark the total number of loci

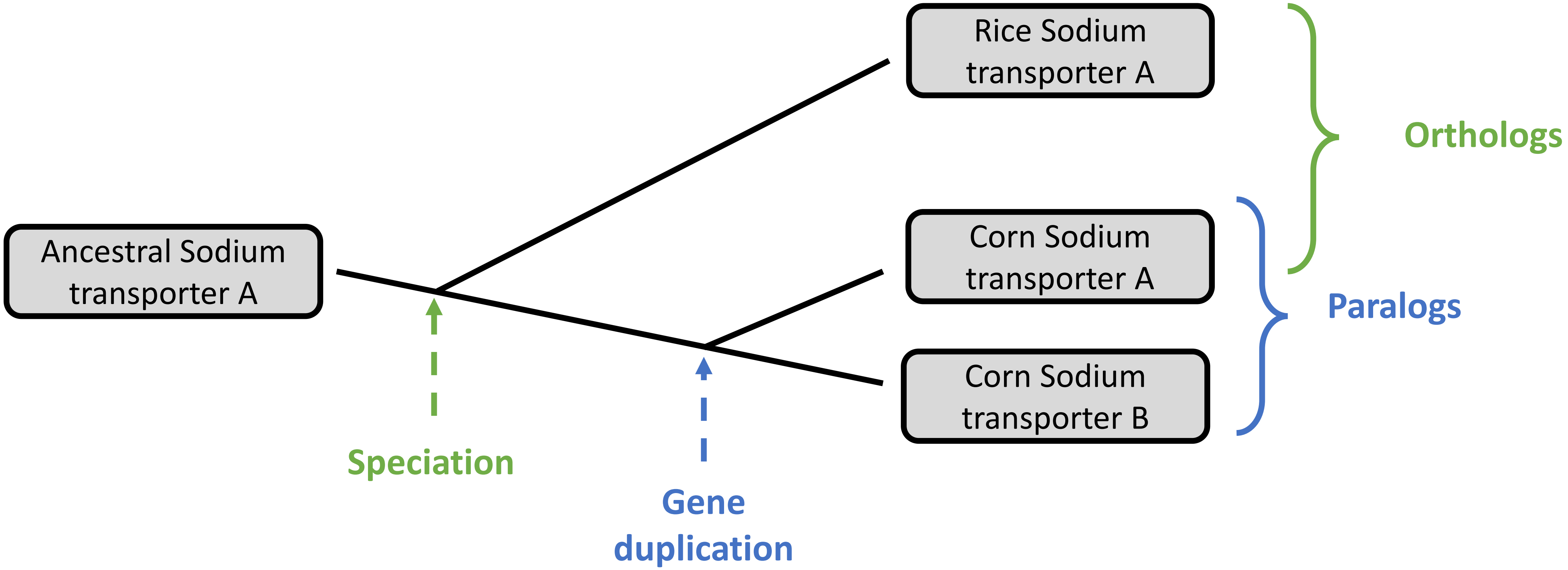
Curves show 1000 permutations of the data using random genome locations to replicate loci

Dashed lines mark the 5th/95th percentiles

How Do We Get to Unknown Genes?

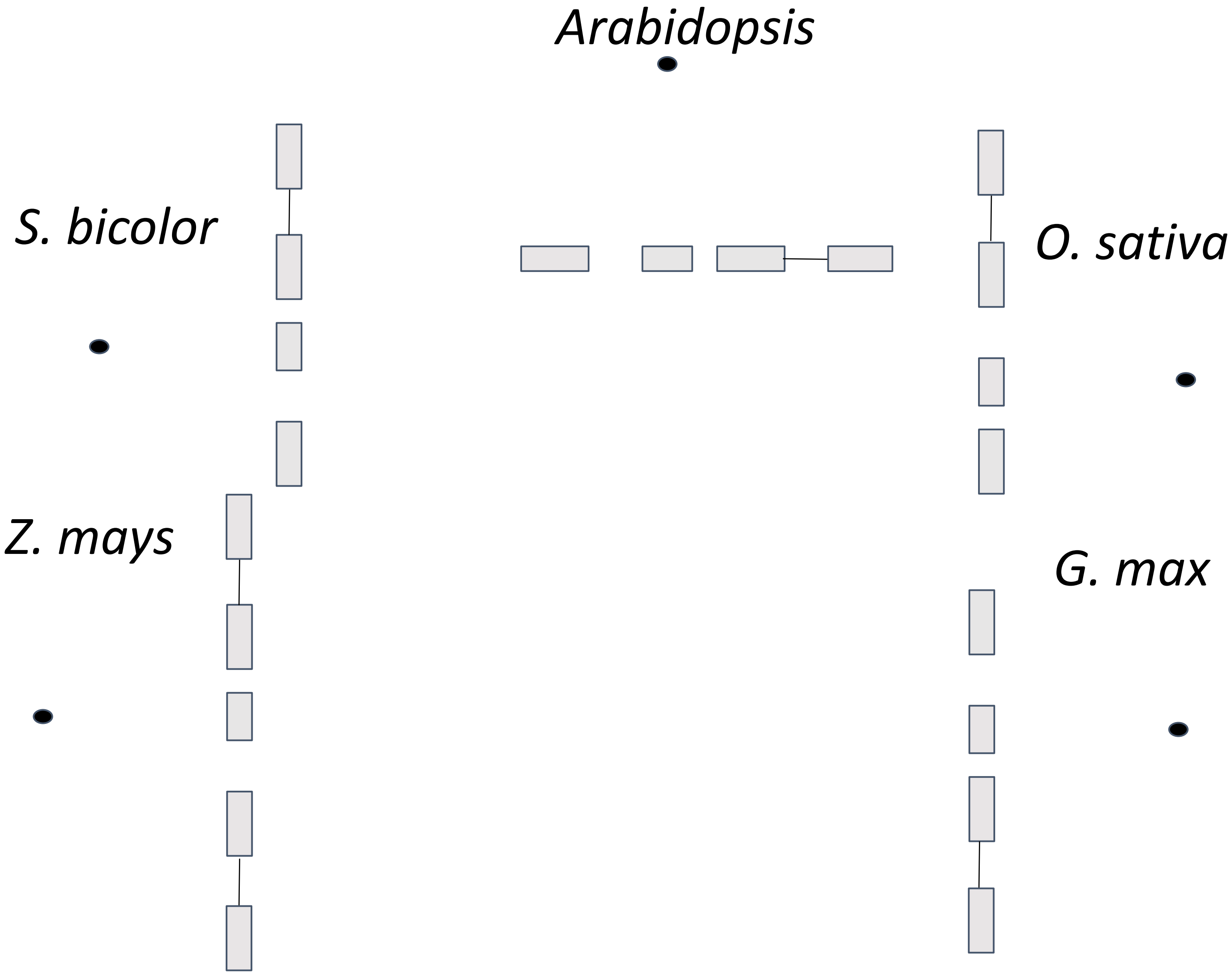
If known genes are conserved under peaks,

the unknown (un-KIG) are likely to follow the same pattern.



Filtering Results of Multispecies Analogous GWAS Experiments

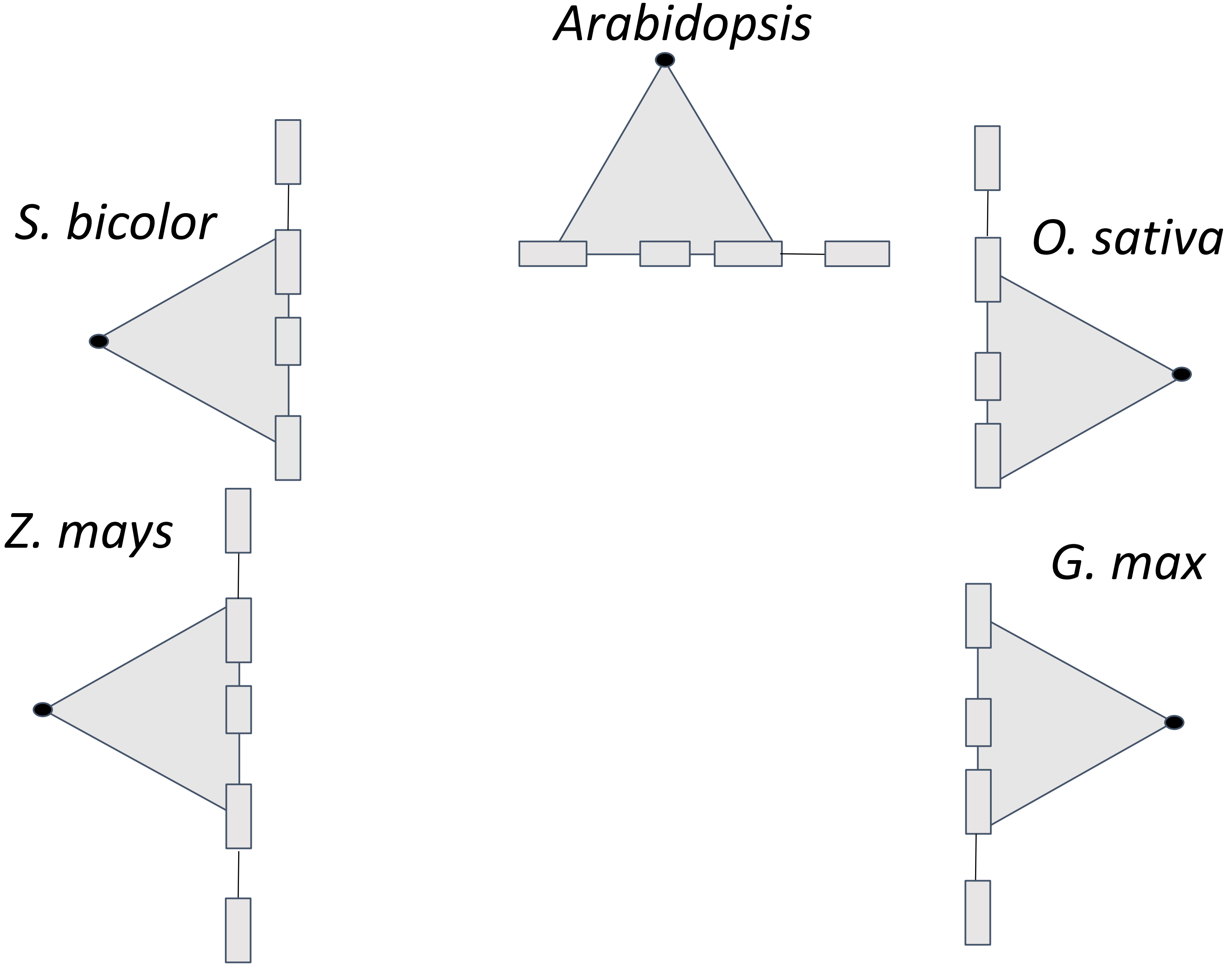
FiReMAGE Scheme



Legend

●	Marker/SNP
▭	Gene
◁	Linkage range
🟩	Orthologous gene

FiReMAGE Scheme



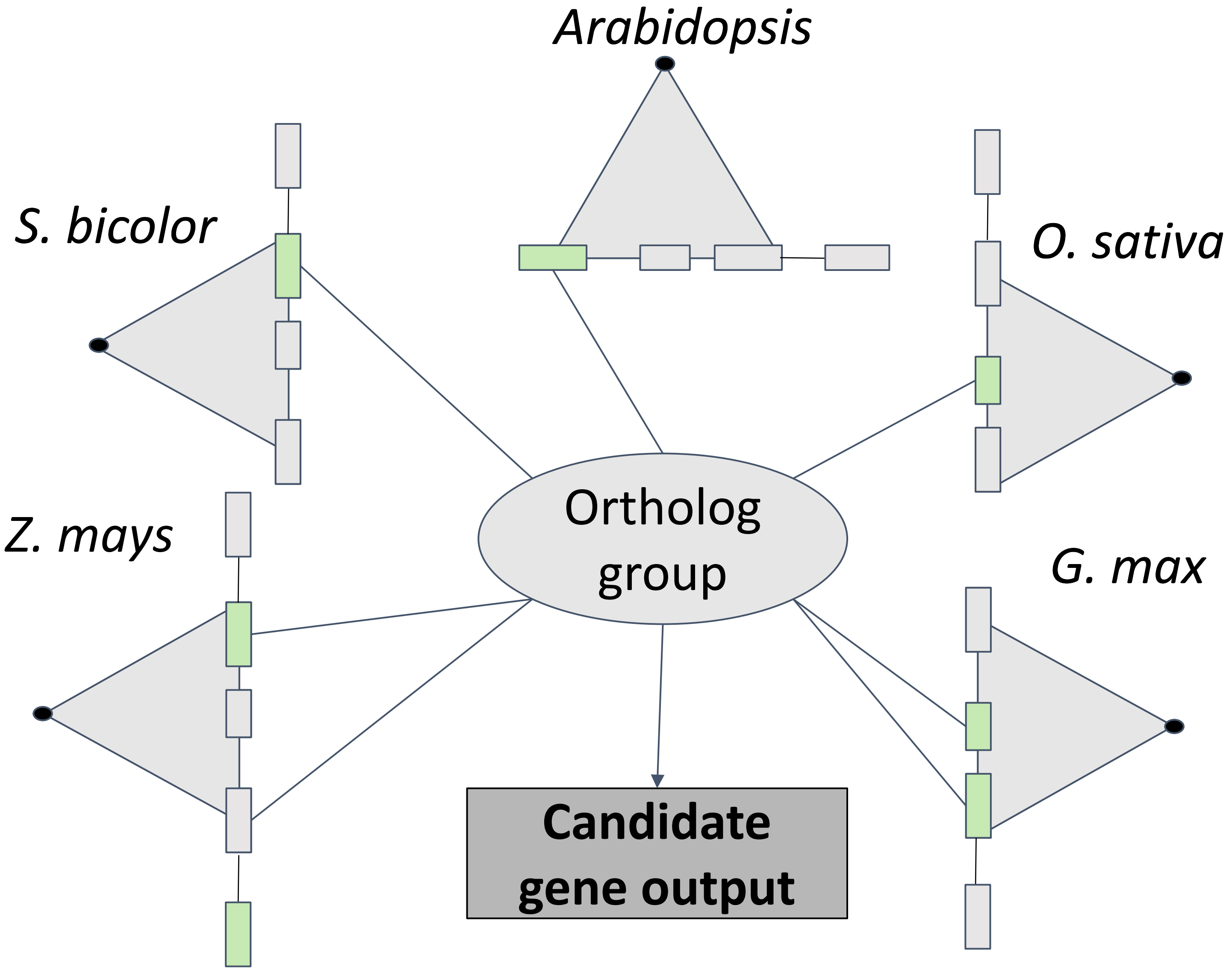
Legend

●	Marker/SNP
▭	Gene
◁	Linkage range
🟩	Orthologous gene

FiReMAGE Scheme

Legend

●	Marker/SNP
▭	Gene
◁	Linkage range
🟩	Orthologous gene



Arabidopsis

S. bicolor

O. sativa

Z. mays

G. max

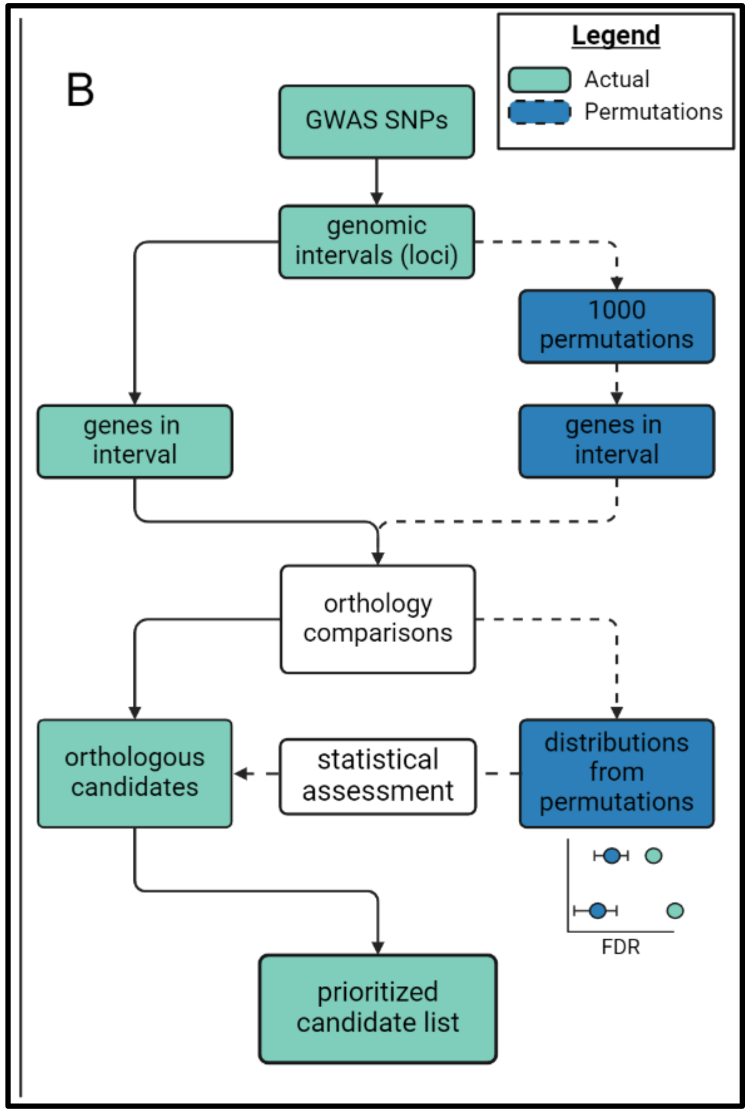
Ortholog group

Candidate gene output

Permutations allow us to estimate likelihoods without knowing the "correct" choice for each variable and parameter.

Variables to consider include (but are not limited to):

- Trait measurement error
- GWAS statistical power
- Differences between GWAS
- Species in the comparison
- Linkage ranges
- P-value thresholds

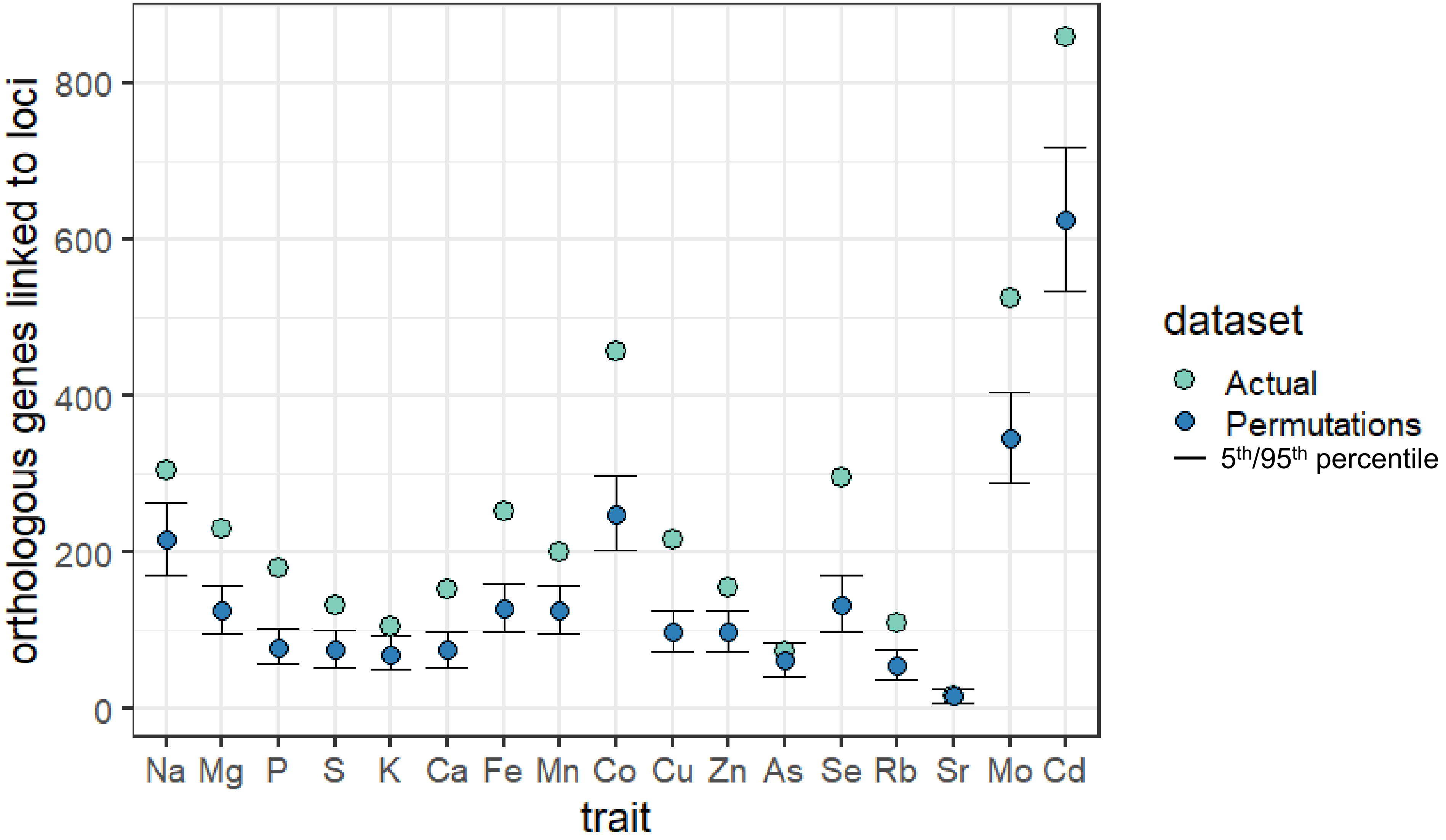


Permutations match the exact species and trait composition of the actual data

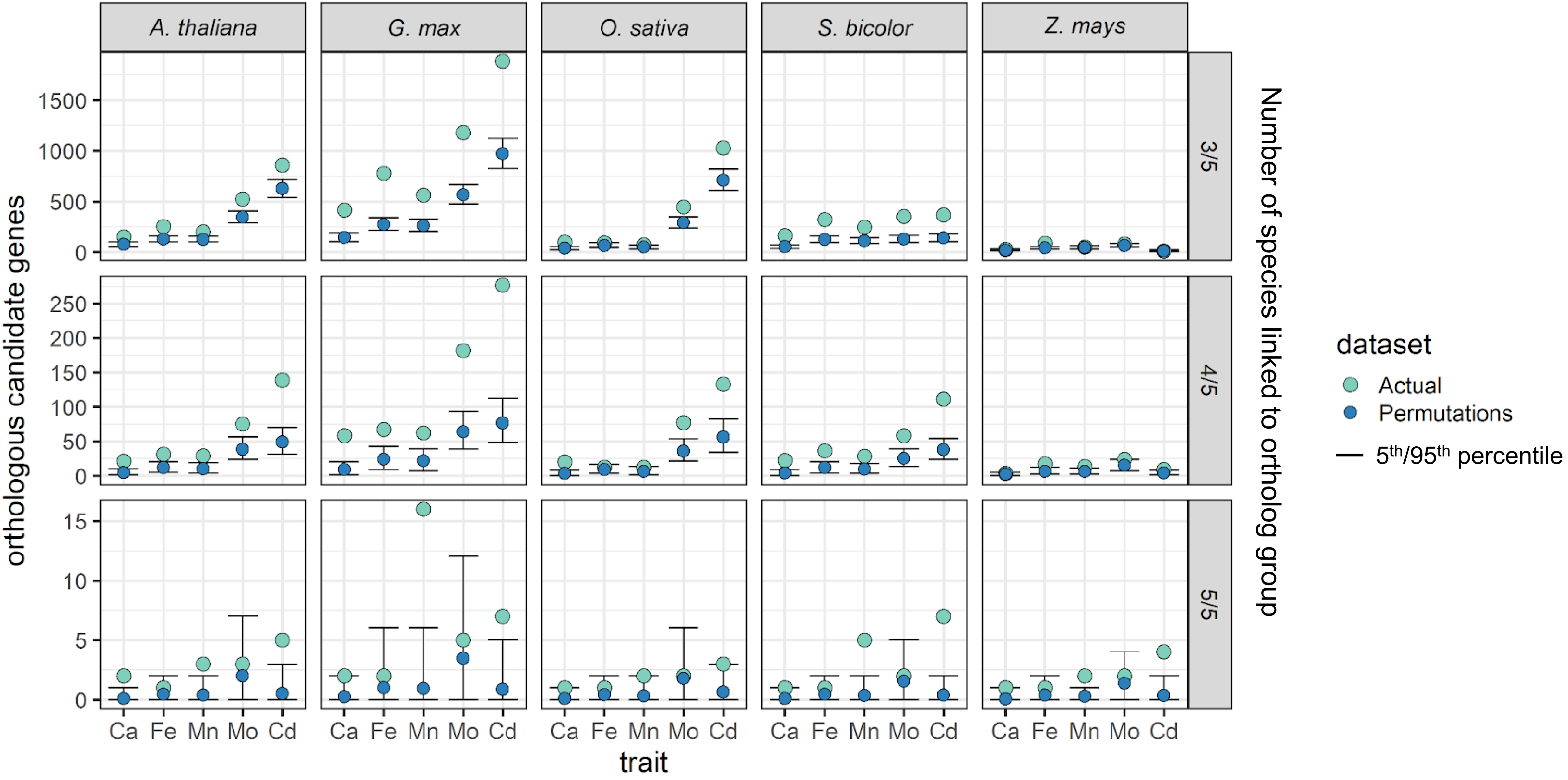
Loci locations are shuffled around randomly

Number of linked orthologs > than expected by random chance

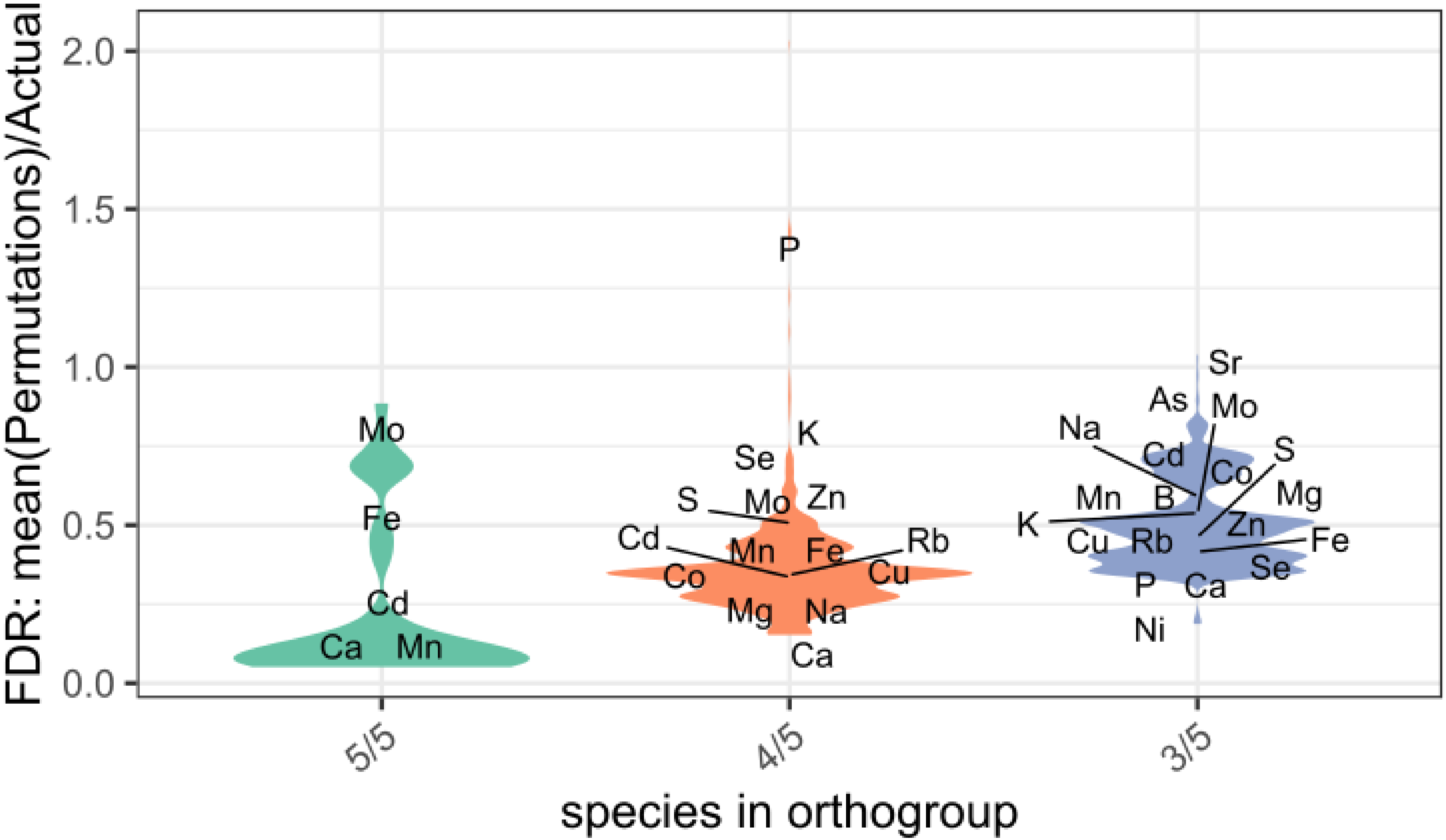
Arabidopsis candidate genes in ortholog groups linked to 3/5 species



Requiring ortholog groups to overlap loci in more species reduces gene list size



Requiring ortholog groups to overlap loci in more species reduces False Discovery Rate



5/5 Ortholog Groups: Known and Unknown

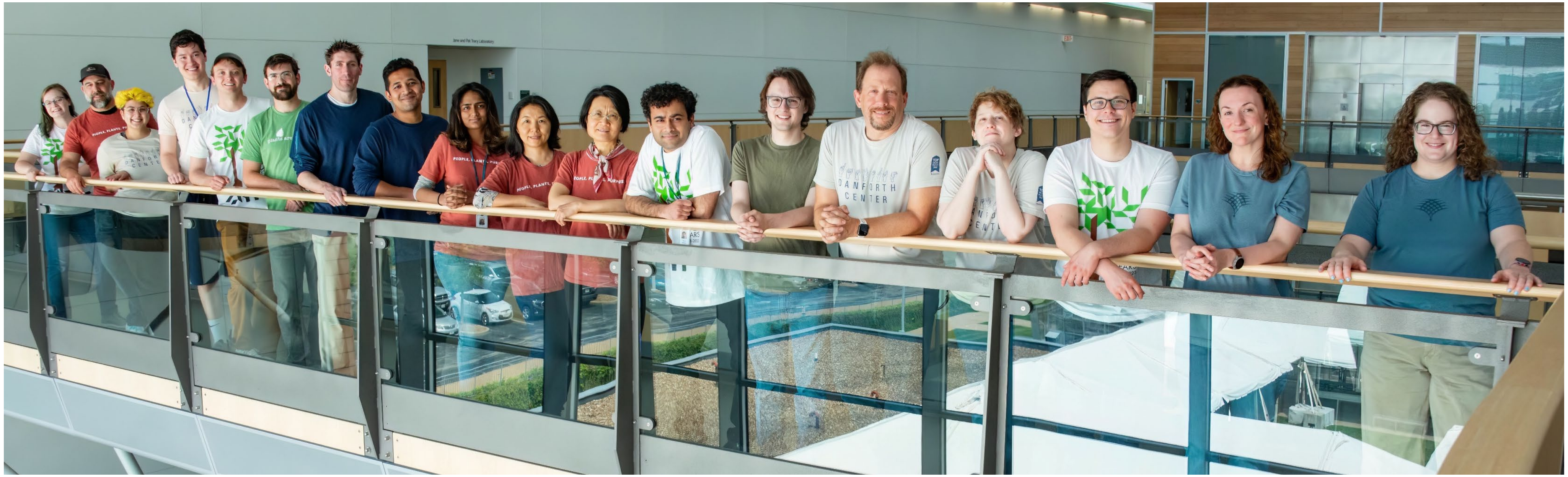
Ortholog group	trait	Phytozome description	Supporting Literature
OG0002041	Ca	Arabidopsis: RING/U-box superfamily	Currently unknown role
OG0005693	Fe	Mevalonate kinase	Currently unknown role
OG0000070	Mn	Histone proteins	Currently unknown role
OG0000306	Cd	RAB GTPase homolog A1	Currently unknown role
OG0002774	Cd	Cd/Zn heavy metal ATPases	**KIG list**
OG0013522	Mo	Molybdate transporter 2	**KIG list**
OG0000899	Cd	DUF567 – protein of unknown function	In a list of 129 *Arabidopsis halleri* genes associated **with metal hyperaccumulation** and expressed in roots (Filatov et al. 2006, Mol. Ecol.)
OG0002849	Mo	Adenine nucleotide transporters NTT1/NTT2	Molybdopterin is a **Mo containing cofactor** synthesized from nucleotide precursors (Hover et al. 2013, J. Amer. Chem. Soc.)
OG0000048	Mn	Cellulose synthase catalytic subunit	**Mn ions** are used to catalyze glycosidic bond formation during cellulose synthesis (Qiao et al. 2021, PNAS)

Baxter Lab

Lauren Whitt
Magdalena Janik
Liz Mahood
Greg Ziegler
Collin Luebbert
Jennie Barrett
Melissa Jurkowski

Slotkin Lab
Lauren McClain
Seth Edwards
Trey Klaas

Danforth Center
Noah Fahlgren
Josh Rothhaput
Armando Bravo
Toby Kellogg
Chris Topp
Topp Lab



Collaborators
Brian Dilkes, Purdue University
Jason Gillman, USDA-ARS / MU
David Salt, University of Nottingham
Grant Norton, University of Aberdeen

NSF

College of Agriculture, Food & Natural Resources
University of Missouri

FFAR Fellows
Future Leaders for Food & Agriculture

DONALD DANFORTH PLANT SCIENCE CENTER
DISCOVERY | COMMUNITY | IMPACT