Taming Variation:

Wild-types, Ecotypes and Phytometers of Arabidopsis thaliana and the UNPAK Project

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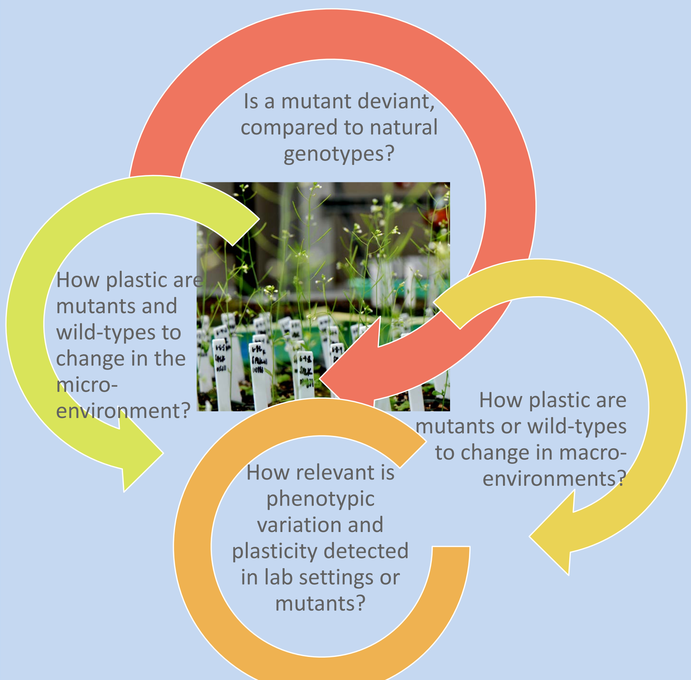
UNPAK : Quick Overview

Our collaborative network is phenotypically characterizing a large fraction of the extensive SALK knockout mutants library in Arabidopsis thaliana, emphasizing fitness-related traits and evolutionary questions, asking

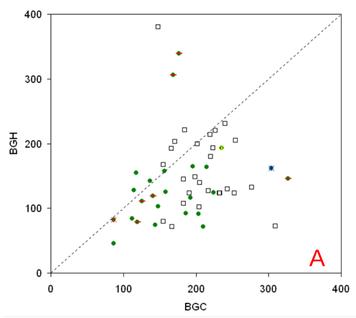
- How often and which mutations result in no change, an increase, or a decrease in fitness-related traits? *This information is a significant gap in our understanding of gene and genome evolution.*
- Are fitness-related phenotypes associate with variation in several gene attributes? *We can consider ontology, gene family size, many other attributes.*
- Can we maximize undergraduate engagement in authentic genomics research? *Beyond involvement in plant care and experiment implementation, they also design experiments, manage data, conceive and execute analyses, collaborate and communicate.*
- *Novel gene discovery or elucidation of gene function is not a central goal, but may be a by-product of UNPAK's efforts.*

A Few Key Terms and Concepts

Genotype	← →	Phenotype
"code"	← →	"function" (gain or loss)
"Normal" allele	← →	Wild-type function or trait
Mutation in allele	← →	Mutant phenotype
Natural polymorphic allele(s)	← →	Natural phenotypic variant(s)
"accessions" – sequences vary	← →	"ecotypes" – traits vary
DNA library	← →	Knockout mutant library
Gene pool (evolution)	← →	Population (functional ecology)
Genome	← →	Phenome



From Ecotype to Phytometer: Screening of Candidate Accessions



Genotype #	Ecotype
COL70000	Col-0
CS22596	HR-5
CS22597	HR-10
CS22599	NFA-10
CS22600	Sq-1
CS22601	Sq-1
CS22606	Kz-1
CS22611	Ren-11
CS22614	Cvi-0
CS22615	Lz-0
CS22617	Gu-0
CS22618	Ler-1
CS22619	Nd
CS22620	C24
CS22627	Van-0
CS22631	Gy-0
CS22633	Bay-0
CS22634	Ga-0
CS22635	Mrk-0
CS22636	Mz-0
CS22647	Ts-1
CS22649	Pro-0
CS22650	LL-0
CS22652	Sha
CS22653	Sorbo
CS22658	Oy-0
CS22659	WS-0

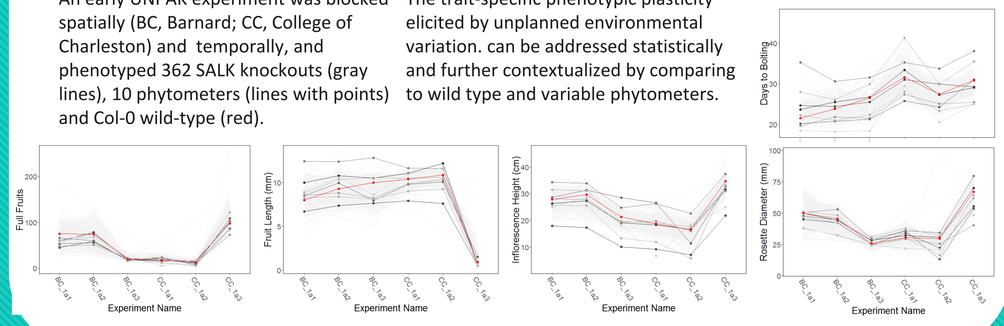
We screened 27 candidate phytometer lines (green dots) selected from an initial 97 lines. Pilot experiments included 27 SALK KO (open squares).

Phenotyping UNPAK traits was completed in growth chamber (BGC) and greenhouse (BGH) experiments, scoring means for fruit length, full and aborted fruit counts (and total fruits, A), inflorescence height and branching, rosette diameter, days to bolting (B) and germination. We selected the final panel of 10 phytometers (green in table), favoring ecotypes that bracketed variation in one or more traits expressed in SALK knockout library lines, created in a Col-0 wild-type background.

Beta Testing: Phytometer Performance Across Blocks in a Large-Scale Mutant Screen

An early UNPAK experiment was blocked spatially (BC, Barnard; CC, College of Charleston) and temporally, and phenotyped 362 SALK knockouts (gray lines), 10 phytometers (lines with points) and Col-0 wild-type (red).

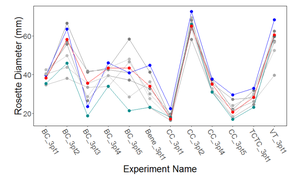
The trait-specific phenotypic plasticity elicited by unplanned environmental variation. can be addressed statistically and further contextualized by comparing to wild type and variable phytometers.

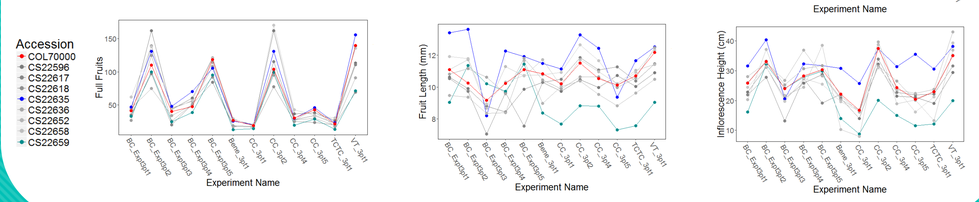


Here Come the Phytometers: Performance Across UNPAK Campuses

Plots documenting mean phytometer performance through time, before and after expansion of UNPAK to many more campuses.

Phytometers are helpful with any inconsistencies in phenotyping, important given UNPAK's reliance on multiple PIs and undergraduate interns.

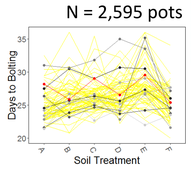


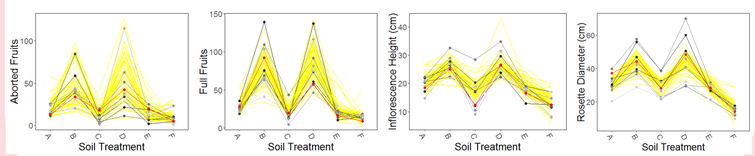


Soil Experiment

- Plasticity was elicited by soil treatments, and was similar in magnitude to that elicited by unplanned variation in previous studies that included campus-to-campus and temporal blocks
- Soil treatments revealed some trait deviation in KO lines, but not more often than prior experiments

6 soil treatments
phytometer + wt
62 SALK KO lines
N = 2,595 pots





A = normal Pro-Mix B = sand mix + 100% C = sand mix + 10%N D = sand mix + 10%P E = NaCl F = over-watering

Take-homes: On Mutants, Wild-types and Phytometers

- Concept of "a gene" in isolation is useless and meaningless
- Gene-trait-environment combinations are key
- Environmental variation can be neither controlled nor eliminated
- UNPAK's phytometers are not universal. They match our core traits and environments. A new set may be needed for other traits or environmental conditions.
- Compare to agri-business "bundles" –seed strain and accompanying fertilizer/herbicide/pesticide must match local climate and soil
- Key concept: distinguish "signal" from "noise" in data about phenotypes
- Phytometers, like any other well-characterized and useful biodiversity, are similar to intellectual property such as software

Key References

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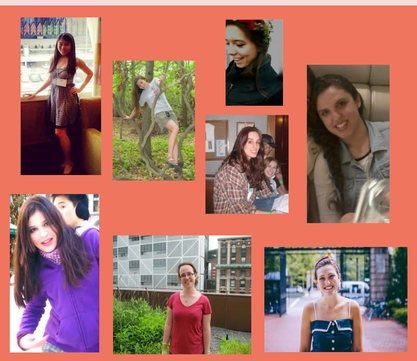

UNPAK: Plant genomics on the shoulders of undergraduates !!

25+ Barnard and CUNY's LaGuardia Community College students: D. Cassidy, K. Cronin, L. Fletcher, L. Flynn, L. Gomezdelatorre Clavel, K. Hanslits, S. Kern, K. Lake, K. Lam, A. McLamb, J. McCorkle, R. Nagpal, C. Nagy, M. Orozco, M. Pantalena, H. Peng, A. Sandoval, S. Schaeffer, M. Schwarz, J. Tan, N. Thulin, V. Varone, J. Wan, A. Wanar, G. Winship.

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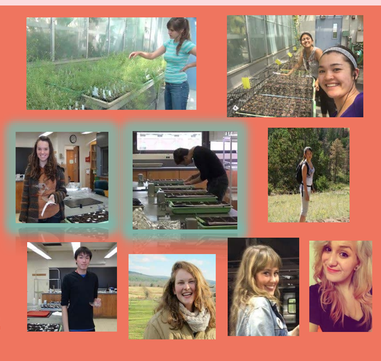

Come to Matt Rutter's talk, Tues @ 9:00 AM, Session 6B

Lessons from 200,000 *Arabidopsis* phenotypes: the power of UNPAK