

Association Mapping in Polyploid Perennial Grasses: Quality Traits in Switchgrass (*Panicum virgatum* L.)



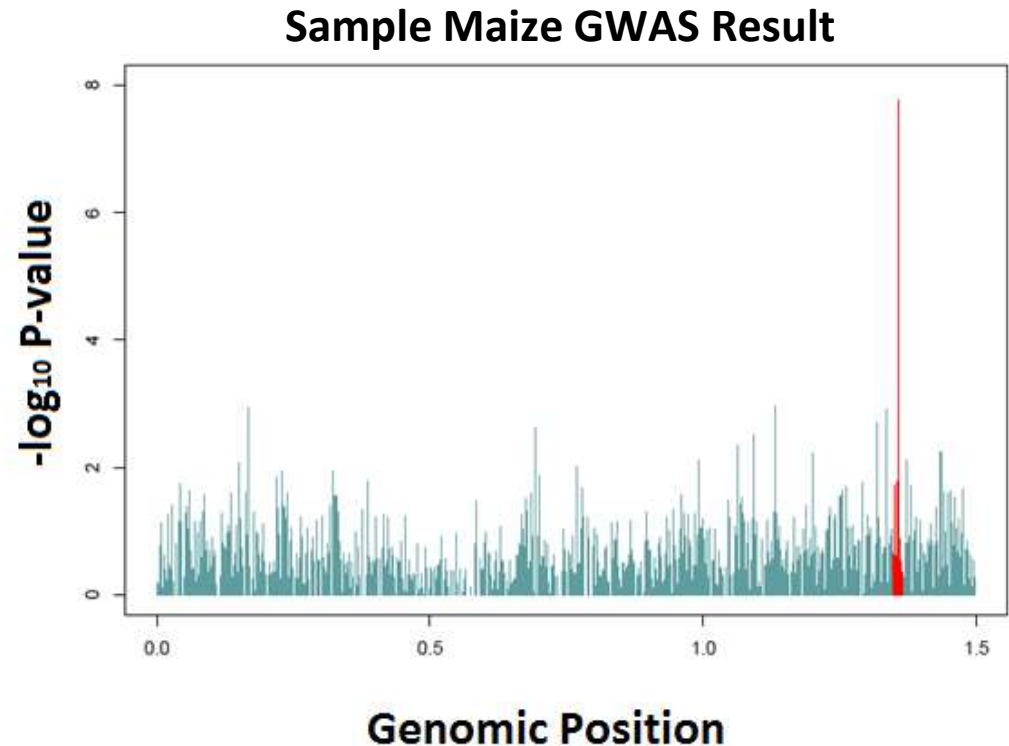
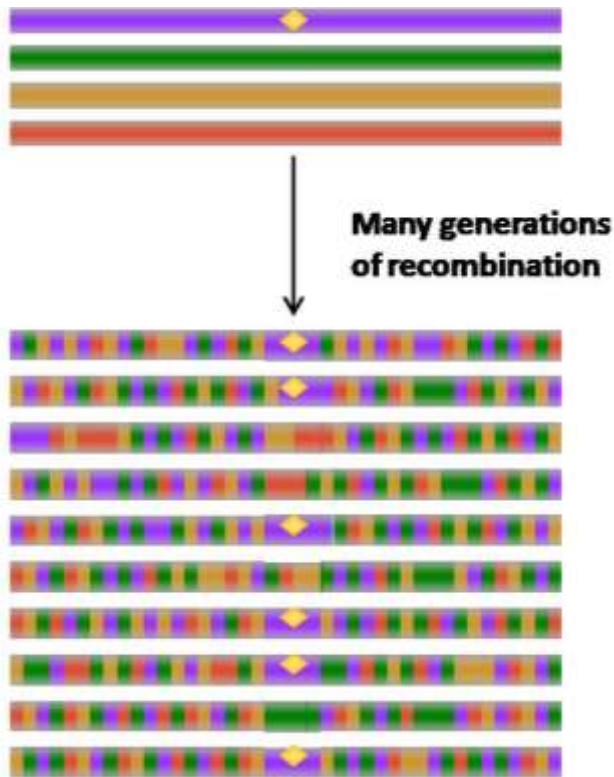
July 2009, Caldwell Field, Cornell University, Ithaca, NY

Denise E. Costich
USDA-ARS and Cornell University

Why Association Mapping?

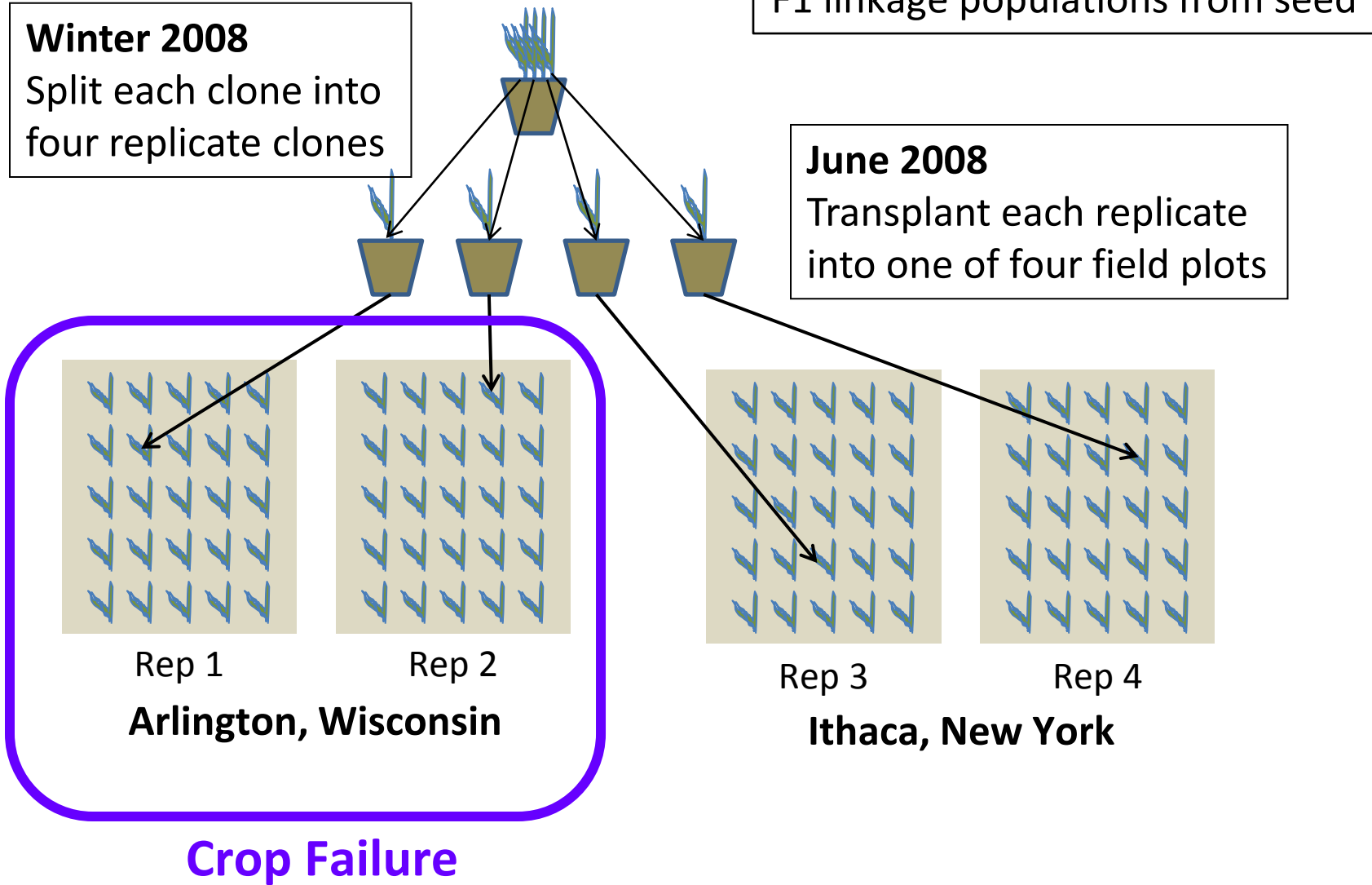
- Identification of molecular markers that are tightly linked to biomass-related trait loci
- Enables marker assisted selection
- Potential to accelerate the breeding programs for enhanced biomass production.
- In perennial polyploid species, effective marker assisted selection will be key to rapid selection for improved varieties and adaptation to environments across the US.

Association Panel



- Sample of diverse germplasm, short range LD
- Pinpoints gene(s) associated with a trait

Design of Field Experiment

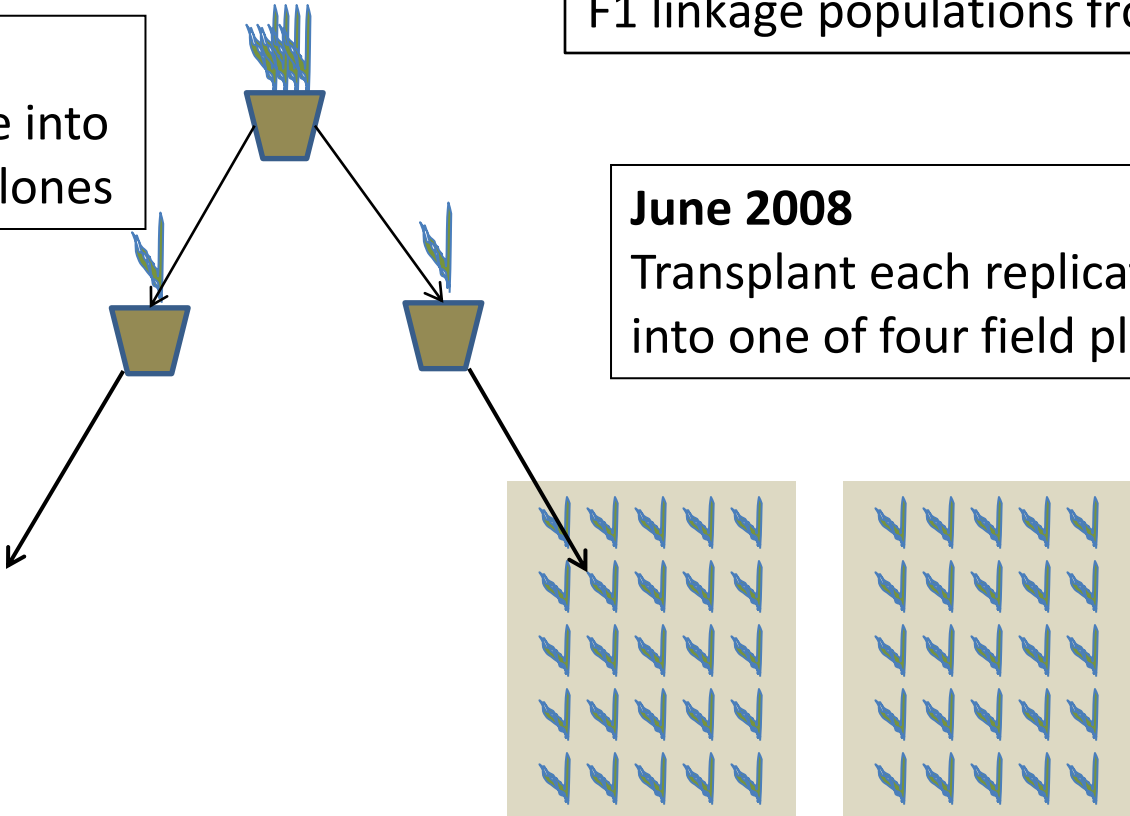


Design of Field Experiment

Winter 2008
Split each clone into four replicate clones

2007
Grow diverse germplasm and F1 linkage populations from seed

June 2008
Transplant each replicate into one of four field plots



2009-2011
Collect phenotypic and genotypic data

Rep 3 Rep 4
Ithaca, New York

Switchgrass Field Plots—Ithaca, NY

Linkage Populations

Association Populations



Rep 1

Rep 2

Rep 1

Rep 2

Experimental Design—Association Panel

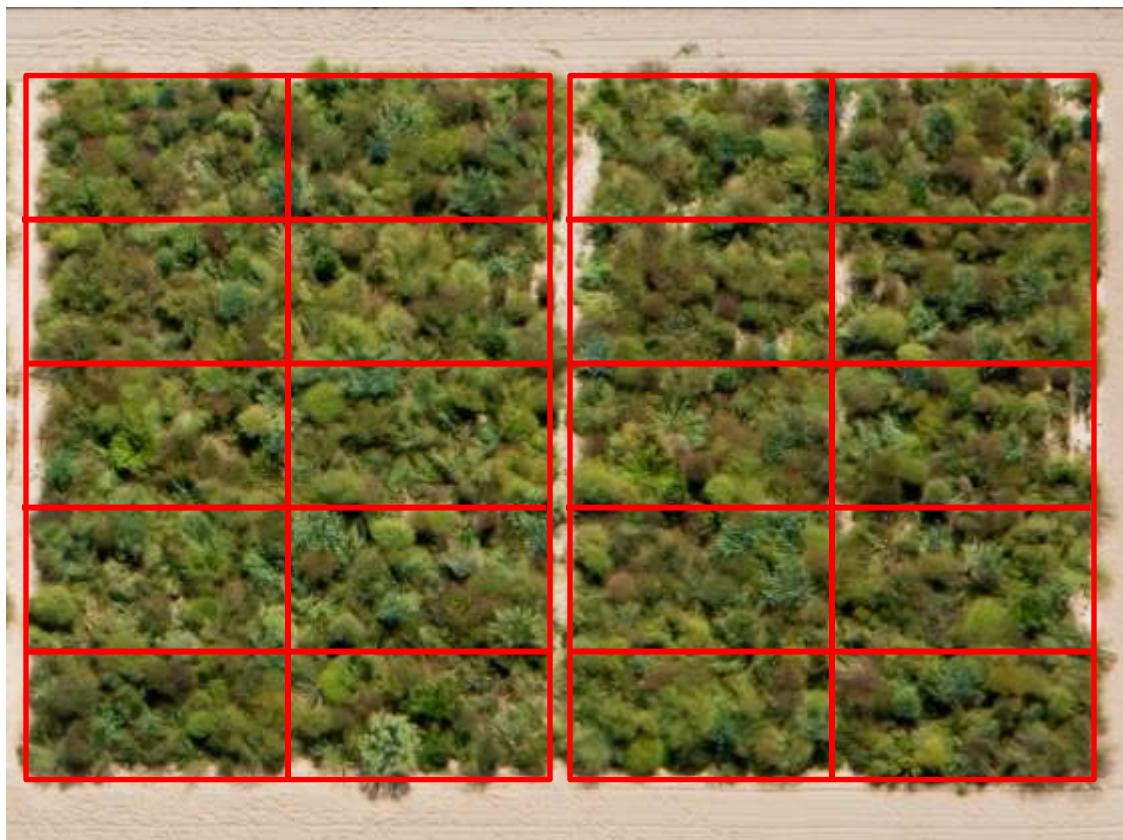


- 60 accessions x 10 plants/acc
- 2 full reps

Rep 1

Rep 2

Experimental Design—Association Panel



Rep 1

Rep 2

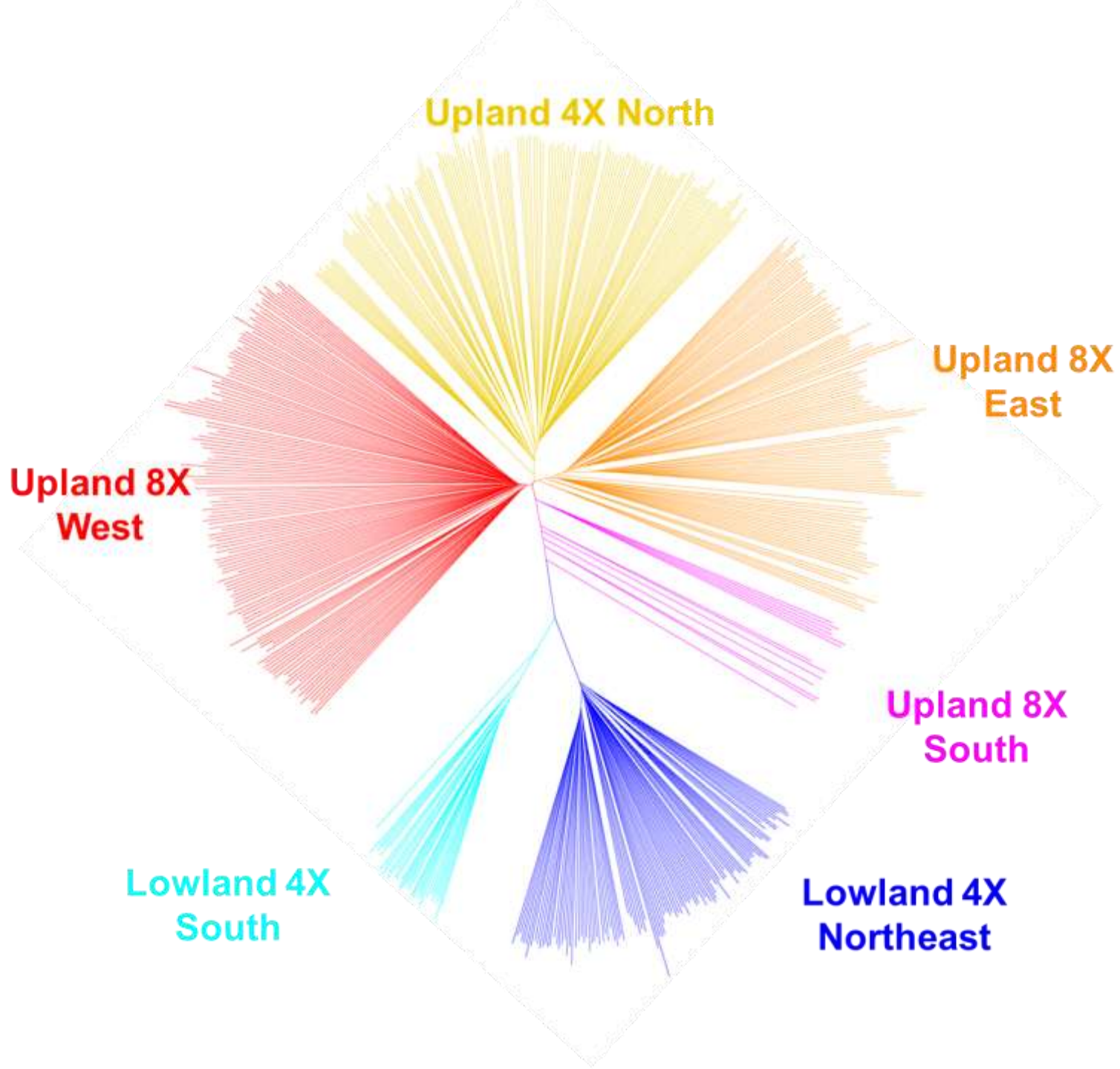
- 60 accessions x 10 plants/acc.
- 2 full reps
- 10 blocks of 60 plants/rep
- 1 plant per accession in each block
- 3' between all neighboring plants

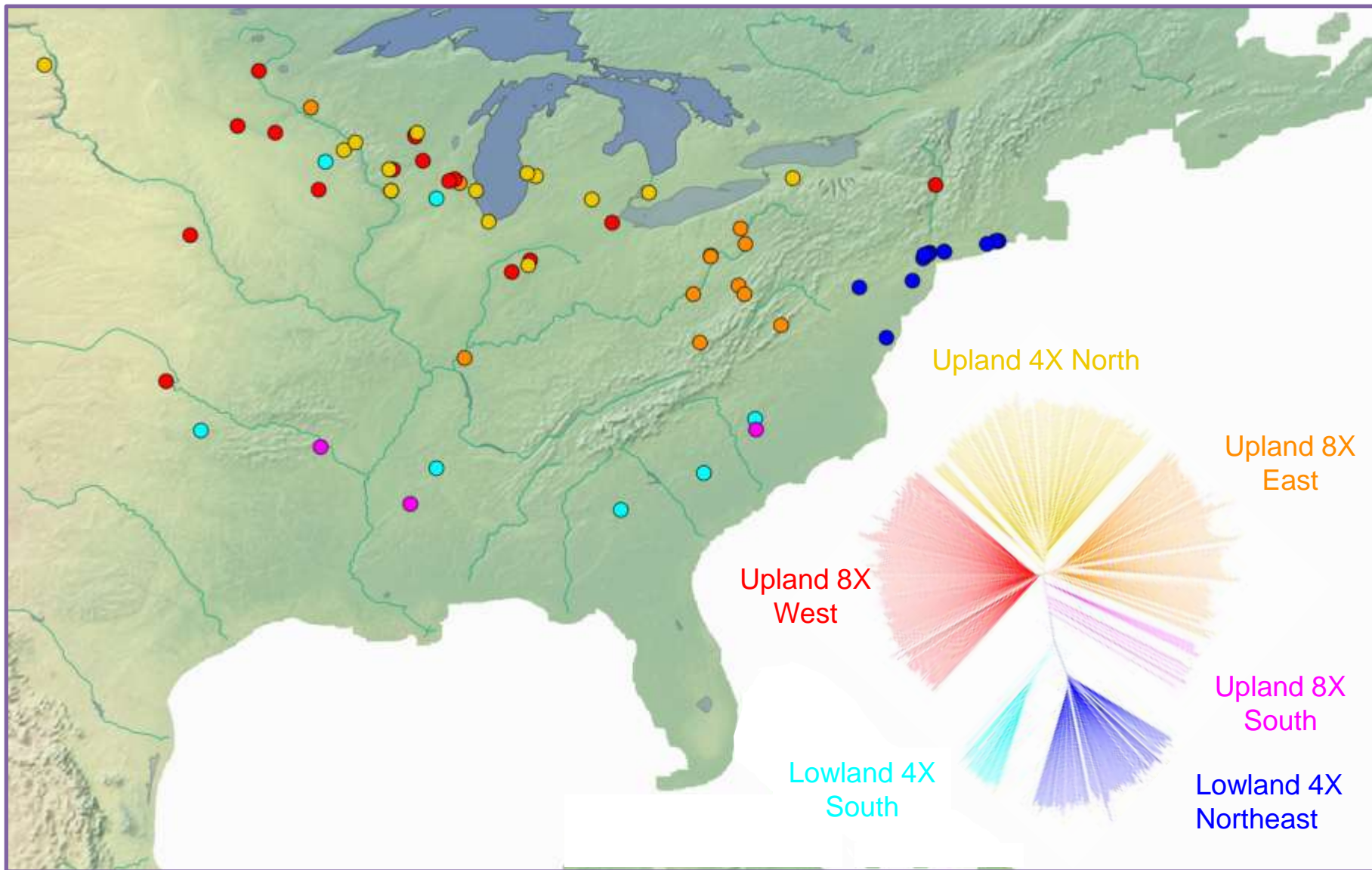
The genotypes

Fei Lu



- **Effective SNP calling pipeline developed—
UNEAK (Universal Network-Enabled Kit)**
- **It works well for non-reference, heterozygous, and polyploid species**
- **720,000 high density SNPs discovered for GWAS**
- **Robust phylogeny concurs well with ecotype, ploidy level and geographic distribution of switchgrass**





The phenotypes

Alex Lipka



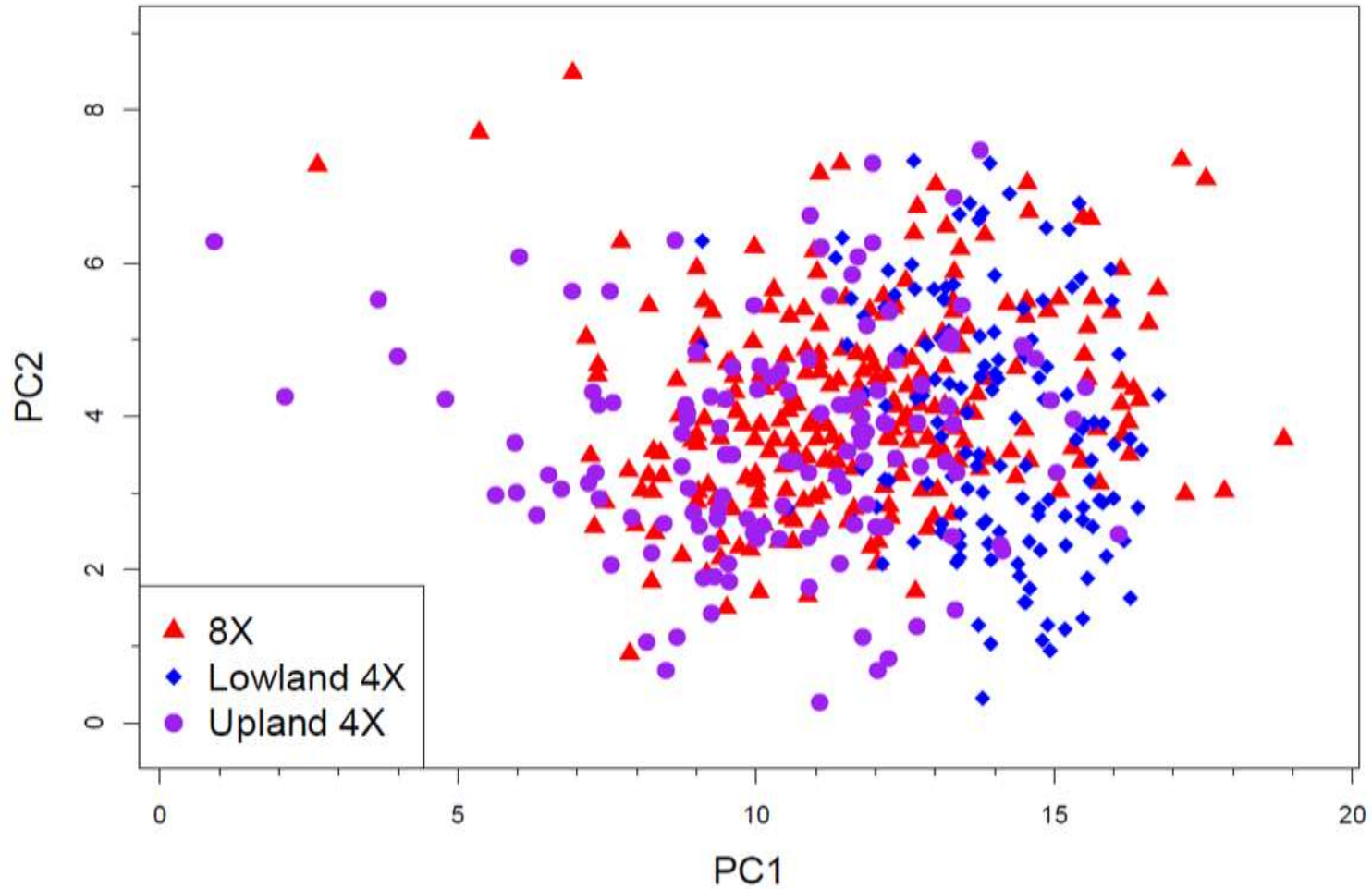
- 41 biomass quality traits measured using near-infrared reflectance spectroscopy (NIRS)*
- 1922 samples total, 961 in 2009 and 2010
- Leaves collected, dried and ground in Cherney lab
- All measurements carried out in Casler lab
- Principal Component Analysis (PCA) to summarize traits into orthogonal linear combinations

* Vogel, et al. 2011. Bioenergy Research 4:96-110

Four Principal Components (PCs) explain 77% of the variation in quality traits

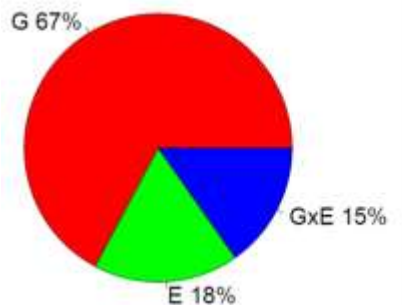
PC	Attributes
1	High digestibility & EtOH conversion Low fiber, glucose, xylose Low lignin and etherified ferulates
2	High sugar
3	Low lignin High cell-wall carbohydrates High EtOH conversion High pentose sugar release
4	High N and C High energy content Good for thermochemical conversion, pyrolysis, or gasification

Phenotypes Similar across Ecotype and Ploidy



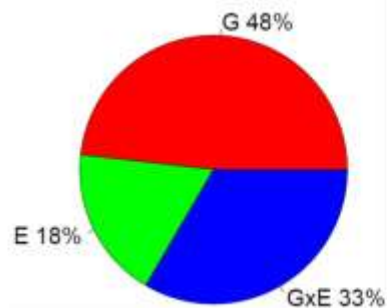
Quality PCs are Highly Heritable with Large Genetic Variance Components

PC 1



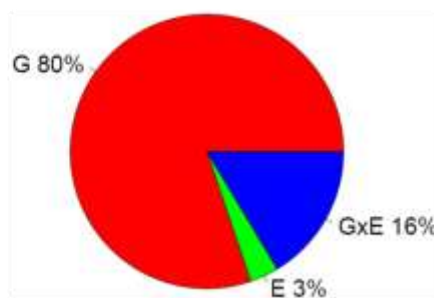
0.81*

PC 2



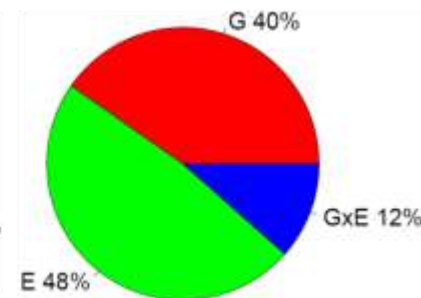
0.67*

PC 3



0.78*

PC 4



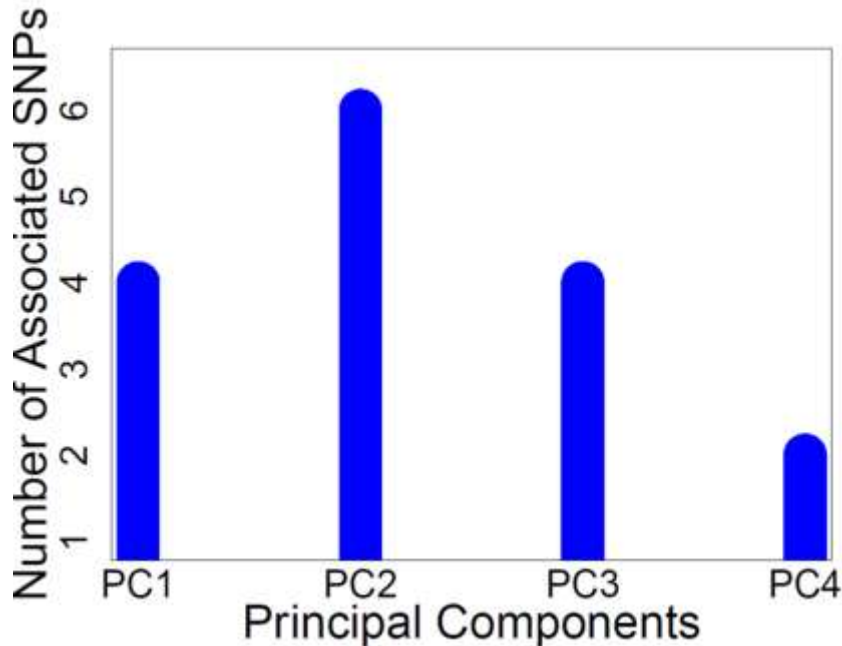
0.75*

* Heritability on a line mean basis

Variance Component Symbols:
G = Genotype
E = Environment [Year1 or Year2]
GxE = Genotype x Environment

Promising GWAS Results

Multiple SNPs with P -values $< 9.99 \times 10^{-5}$ for Each PC



Fitted Q+K models (Yu et al. 2006*) at 29,221 SNPs discovered from UNEAK.

QQ-plots suggest that these models sufficiently control for population structure and familial relatedness.

SNPs with low P -values are candidates for marker assisted selection.

*Yu, et al. 2006. Genetics 38: 203-208.

Acknowledgements

PIs:

Edward S. Buckler (USDA-ARS, Cornell)

Michael Casler (USDA-ARS, UW-Madison)

Jerry Cherney (Cornell University)

Field and Database:

Ken Paddock

Nick Lepak

Nick Baker

Nick Kaczmar

Dallas Kroon

Postdocs:

Ainong Shi

Elhan Ersoz

Moira Sheehan

Alex Lipka →

Fei Lu →



Undergraduates:

Michelle Denton

David Denton

Patrick Redmond

Andrew Keyser

Will Baldwin

Eric Cerretani

Sonam Sherpa

Brian Adler

Grisha Temnykh

Maite Iriondo

Eva Ge

Miles Crettien

Vivian Lin

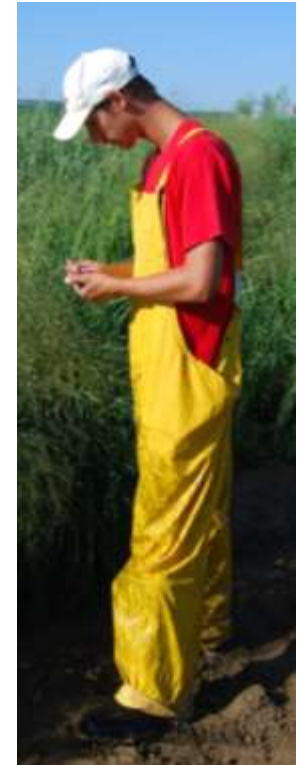
David Shin

Ryan Mitchell

Dan Miller

Caitlin Niederhofer

Graham Muzik



Supported by DOE (including JGI), USDA, and NSF