Dissecting Complex Traits in Sorghum with a Nested Association Mapping (NAM) Population

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QTL mapping: Strengths and weaknesses

**Biparental linkage**
- Mature and powerful technology
- Relatively quick to validate in near isogenic lines
- Captures very little genetic and allelic diversity of a trait with two parents
- Few recombination events, high LD, low mapping resolution

**Diverse association**
- Many recombination events, low LD, high mapping resolution
- Genotype once, phenotype many times
- Phenotypic covariates (e.g. flowering time) confound mapping efforts
- Synthetic associations (common alleles tag multiple rare variants) are positively misleading

KSU Sorghum Genetics team at PAG

Sophie Bouchet: Poster #563  
Genomic Analysis of a Sorghum NAM Population: High Resolution Mapping of Flowering Time and Plant Height

Marcus Olatoye: Poster #554  
Nested Association Mapping Of Agroclimatic Traits In Sorghum

Nested Association Mapping (NAM) population design

- Advantages of NAM for mapping complex traits:
  - More recombination events
  - Bring rare alleles to moderate frequency
  - Reduce effect of flowering time and other phenotypic covariates
  - Reduce sensitivity to population structure, genetic/allelic heterogeneity

Traits dissected with Maize NAM

- Buckler et al. 2009 (Science)  
  - Flowering time
- Kump et al. 2013 (Nature Genetics)  
  - Southern leaf blight
- Poland et al. 2011 (PNAS)  
  - Northern leaf blight
- Tian et al. 2011 (Nature Genetics)  
  - Leaf angle, width, length
- Brown et al. 2011 (Plant Genetics)  
  - Inflorcular architecture
- Cook et al. 2012 (Plant Physiology)  
  - Kernel starch, oil, proteins
- Chandler et al. 2012 (Crop Science)  
  - Kernel carotenoids
- Pfeiffer et al. 2013 (PLoS One)  
  - Stalk strength
- Pfeiffer et al. 2014 (Genetics)  
  - Plant height

Development of the Sorghum NAM

- Designed and bred by Bill Rooney (TAMU) and Mitch Turner (KSU)

<table>
<thead>
<tr>
<th>TAMU NAM Population</th>
<th>KSU NAM Population</th>
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<tbody>
<tr>
<td>BTx623 × Tx430</td>
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<td>F1s → SSD</td>
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<tr>
<td>NAM Founders (n=24)</td>
<td>NAM Population (n=2,500)</td>
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</table>
An introduction to the NAM founders

- **Ajabsido**
  Caudatum (Feterita) from Sudan
  Pre-flowering drought

- **Macia**
  Caudatum (Zera zera) from Zimbabwe
  Elite Improved African line

- **P898012**
  Caudatum from Purdue breeding
  Pre- and post-flowering drought

- **SC1103 (Ex-Mubi)**
  Guinean from Nigeria
  Grassy leaf and stem architecture

- **SC1345 (CSM-90)**
  Caudatum (Feterita) from Mali
  Underutilized caudatum germplasm

- **SC265 (Hadoui)**
  Guinean from Burkina Faso
  Higher yield potential guinea

- **SC283 (Msumbiji)**
  Guinean from Tanzania
  Aluminum tolerance (AltSB)

- **SC35 (Mashela Tinguish)**
  Durra from Ethiopia
  Post-flowering drought (Stay-green)

- **SC971 (Millo Blanco)**
  Farmer variety (Kafir) from Puerto Rico
  Limited transpiration, high yield (slow-wilting)

- **Segaolane**
  Kafir from Botswana
  High yield potential

Genetic diversity of the NAM founder lines

- All semi-dwarf (2-3 dwarf)
- All photoperiod insensitive
- All in the Sorghum Association Panel (Casa et al. 2008 Crop Sci)
- Captures 75% of global SNP diversity (Genotyping by sequencing)

Genetic structure of the NAM population

- Genotyping-by-sequencing (ApeKI) 2,500 F7 lines at ~500,000 SNPs
- TASSEL GBS pipeline (Glaubitz et al. 2014 PLOS One)

Segregation distortion due to selection

- Allele frequencies in RILs deviate greatly from 50% (random expectation)
- Selection due to viability, survival, flowering time
- Despite multiple locations substantial segregation distortion occurred (Buckler et al. 2009 Science)

Flowering time variation

Days to 50% anthesis in Manhattan

Flowering time GWAS

- High resolution mapping of several classic and newer maturity loci
- Chromosome 6 still tricky with low recombination and multiple loci
- Hypothesized florigens SbCN8, SbCN12 (Yang et al. 2014, Murphy et al. 2014)
  - Homologs of Arabidopsis FT and rice HD3A
Phenotyping the NAM across the Kansas precipitation gradient

Environmental effects on plant height

Precise mapping of classical dwarfing loci

Plant Height in Humid vs Semi-arid environment

Leaf and stem architecture

Leaf erectness

- Leaf erectness
  - Reduce competition with neighbors (Donald, 1968)
  - More light to lower leaves
  - Leaves erectness has increased dramatically in corn

- Leaf width
  - Component trait for Leaf Area Index (ecophysiological models)
  - Narrow in humid-adapted and wide in arid-adapted landraces

- Stem width
  - Component trait for standability
  - Non-structural carbohydrates and water
  - Narrow in humid-adapted and wide in arid-adapted landraces

- Genetic basis and adaptive role of these traits?
- Optimal architecture (ideotype) for yields under well-watered versus water-limited environments?
Stem Diameter

- Taken at 2nd internode from base
- Some shared QTL in Manhattan vs Hay, some unique

Sorghum NAM as a collaborative platform:

(1) Detailed phenotyping of NAM founders

Irrigated vs Dryland

Agronomic traits:

- Ram Perumal (Breeding)
  - Plant height (cm)
  - Panicle length (cm)
  - Panicle weight (g)
  - Seed weight (g, 1000-seed weight).

Ecophysiological traits:

- Vara Prasad (Ecophysiology)
  - Chlorophyll concentration
  - Photochemical efficiency of PSII (Fv/Fm)
  - Thylakoid membrane damage (F0/Fm)
  - Net photosynthesis (PA)
  - Stomatal conductance (gs)
  - Transpiration (E)
  - Leaf temperature

(2) Dissecting your favorite complex traits

- Use our plots:
  - Send a student, postdoc, or yourself to phenotype your favorite traits in Manhattan or Hays next summer
- Get plant materials:
  - Collaborations to phenotype collected panicle, grain, leaf, or stem tissue
- Get the seed:
  - Seed available for additional field trial locations
  - Eventually deposited in GRIN
- Support the development of the full sorghum NAM

KSU Sorghum Genetics (Morris Lab):

- Sophie Bouchet
  - Line development, genotyping, genomic analysis
- Marcus Olatoye
  - Flowering time, architecture traits
- Sandeep Marla
  - Harvest
- Zhenbin Hu, Brian Wempe, Bassirou Mbacke, Sam Knauss, Kelly Li, Ben Coomes, Brock Welch
  - Phenotyping, harvest, genotyping

Development of NAM:

- Bill Rooney, Mitch Tuinstra, Tesfaye Tesso, Jianming Yu

Growing the NAM (KSU Sorghum Breeding):

- Tesfaye Tesso (Daniel Hopper), Ram Perumal (Gerald Rohleder)

Genotyping facilities:

- Alina Ahkunova (KSU-IGF), Jesse Poland (KSU-Plant Path)

NAM Founder phenotyping:

- Ram Perumal, Vara Prasad (John Sun), Rob Alles

Thanks!