Dr. Michael Gore

CORNELL UNIVERSITY
ASSOCIATE PROFESSOR
The Integration of Nutritional Genomics and High-Throughput Phenotyping: Progress and Prospects for Climate-Resilient High Provitamin A Maize

Michael Gore
Plant Breeding and Genetics Section
School of Integrative Plant Science
Cornell University
The global challenge

Population ~7 Billion
Less developed ~5.8 Billion
Undernutrition ~1 Billion (1 in 7) (caloric)
Malnutrition ~2 Billion (1 in 3.5) (micronutrients: Fe, Zn, I, Vitamin A)

10.9 million children under five die in developing countries each year. Malnutrition and hunger-related diseases cause 60 percent of these deaths. UNICEF, 2007
What can be done to reduce micronutrient deficiency for people in developing nations?

Supplementation

Commercial Fortification

Dietary Diversity

“Biofortification”
Biofortification

- Biofortification is focused on the rural poor and has the potential to be sustainable and cost-effective.

- Identify genes associated with nutrient content in staple crops such as maize and cassava.

- Increase nutritional value of locally adapted crop varieties by selecting on favorable alleles of these identified genes in breeding populations.
Maize is a global staple crop

- Can account for more than 50% of total daily calories in African and Latin American countries with high nutritional deficiencies

- In these countries, maize varieties typically do not provide grain with adequate daily levels of essential nutrients – Fe, Zn, vitamin A, vitamin E, B vitamins

- The tremendous genetic diversity of maize can be potentially harnessed by biofortification to develop nutrient-dense grain

Source: Torbert Rocheford
Prevalence of vitamin A deficiency

- 7 million pregnant women and 127 million preschool-aged children are vitamin A deficient

Vitamin A deficiency causes 600,000 early childhood deaths and blindness in 500,000 children each year
Provitamin A from endosperm of maize grain is converted to vitamin A in the body.

Vitamin A (two retinyl groups)

β-Carotene

Cleaved by β,β-carotene 15,15'-monooxygenase in intestine

Lutein and zeaxanthin are non-provitamin A compounds but important for vision.
Grain carotenoids in a diverse maize panel: gradient of light yellow to dark orange color

Biofortification: HarvestPlus has an initial target of 17 μg/g β-carotene

Average: 1.7 β-carotene μg/g
Grain color and carotenoid content

Total carotenoids
37.36 μg/g

Total carotenoids
8.48 μg/g

Harjes et al. 2008 Science
Nested Association Mapping panel-centered design to identify key genes and alleles

**Phenotypic Data**
- HPLC Quantification
  - Mature Grain (2 yrs)
  - Carotenoids
  - Tocochromanols (vitamin E)
  - ~17,000 NAM runs

**Maize Germplasm**

**Expression Profiling**
- Developing Kernel Libraries
  - NAM Founder Inbreds
  - Six dev. stages
- Illumina Sequencing
  - ~40,000 genes profiled

**Genotypic Data**
- RNAseq
  - ~1 million SNPs
- Hapmap v1
  - ~1.6 million SNPs
- HapMap v2
  - ~26 million SNPs

Alex Lipka

Cathy Kandianis
Statistical analysis of NAM panel

Joint Linkage (JL) Analysis
- Identify QTL controlling metabolites
- Stepwise Model Selection (14k SNPs)

Genome Wide Association Study (GWAS)
- Identify genes controlling metabolites
- Model Selection (30 million SNPs)
Identified 103 QTL for 8 carotenoids and total carotenoids in maize NAM grain

Heritability range of the 9 traits: 0.75-0.95
Fewer QTL identified for carotenoid traits relative to tocochromanol traits

Heritability range of the 18 traits: 0.71-0.95

Flowering time (29-39)  
Plant height (35)  
Leaf architecture (30-36)

Carotenoids: Endosperm  
Tocochromanols: Endosperm and Embryo

Number of QTL per trait

Number of traits

0  0.5  1  1.5  2  2.5  3
Carotenoid traits have larger relative QTL effects than tocochromanol traits.

α–Tocopherol (vitamin E) 13 QTL
β–Carotene (provitamin A) 7 QTL
Grain endosperm carotenoid traits: fewer QTL and larger effect sizes because of shorter length of time for variation to accumulate.

Grain endosperm carotenoid traits came under selection after domestication.

Modified from Wallace et al. 2013
Intersection of candidate genes and QTL support intervals
JL-GWAS: Resolving the 16 identified QTL associated with level of total carotenoids
JL-GWAS: Resolving the 7 identified QTL associated with level of beta-carotene
Pathway-level breeding for beta-carotene

Isoprenoids

- G3P + pyruvate
  - $\text{DXS2/3}$
  - deoxyxylulose-5-phosphate
  - geranylgeranyl diphosphate

Carotenoids

- PSY1 Phytoene synthase
  - phytoene
  - lycopene
  - phytofluene
  - lycopene $\varepsilon$ cyclase
  - LYCE
  - a-carotene
  - zeinoxanthin
  - lutein
  - zeaxanthin
  - b-cryptoxanthin
  - CRTRB1/3 $\beta$-carotene hydroxylase
  - b-carotene

Carotenoids that accumulate in cereal endosperm tissue are synthesized in amyloplasts

Xanthophyll Cycle

ABA Synthesis
GWAS signals proximal to candidate genes are within putative regulatory regions.

Does differential expression underlie the genetic basis of these loci?

lycopene ε-cyclase (lycE1)

β-carotene hydroxylase (crtRB1)
Consistent correlation: 
crtRB1 expression, beta-carotene QTL effects

beta-carotene effects at QTL near crtRB1

**12 Days After Pollination**
- \( r = -0.7 \)

**20 Days After Pollination**
- \( r = -0.86 \)

**Graph Details:**
- The graph shows the correlation between crtRB1 expression and beta-carotene levels at specific QTLs near the crtRB1 gene.
- The logarithm of FPKM (fragments per kilobase of transcript per million mapped fragments) is plotted on the y-axis.
- The x-axis represents the expression levels, with a strong negative correlation indicated by the line of best fit and the correlation coefficients.

**Gene Details:**
- beta-carotene hydroxylase1 (crtRB1)
- Exons and 3' UTR (untranslated region) are indicated on the x-axis.
Stage-specific correlation: *lycE* expression, lutein QTL effects

**12 Days After Pollination**
- *lycE* expression, lutein QTL effects: $r = 0.33$

**20 Days After Pollination**
- *lycE* expression, lutein QTL effects: $r = 0.7$

Exons and UTR of lycopene ε-cyclase (*lycE1*)

<table>
<thead>
<tr>
<th>Exons</th>
<th>UTR</th>
</tr>
</thead>
<tbody>
<tr>
<td>138.881</td>
<td></td>
</tr>
<tr>
<td>138.886</td>
<td></td>
</tr>
<tr>
<td>138.891</td>
<td></td>
</tr>
</tbody>
</table>
Markers within ± 250kb of 8 QTL associated with carotenoid levels in prior linkage studies are as predictive as genome-wide markers.

Panel: 201 diverse inbred lines

Standardized mean corr. resulting from the fivefold cross-validation and S.D.
We Collaborate with

Our research results flow to CIMMYT, IITA in Africa, successfully using MAS for lycE and crtRB1 In Zambia for high provitamin A maize
Sustainable maize production is threatened by increasingly variable weather patterns and diminishing fresh water resources.

Tanzanian farmer with drought-affected maize

https://www.flickr.com/photos/cimmyt/5190627819/
Genomic selection: faster development of nutrient-dense, stress tolerant maize varieties

Research needs: train prediction models, accelerate recombination, manage the population, identify informative lines, and improved phenotyping capacity.

Contrasting evolutionary rates between genotyping and phenotyping platforms

1997 – Capillary DNA sequencer

1974 – Steel measuring tape

2015 – Single molecule DNA sequencer

2015 – Barcoded measuring tape
**HTP: Proximal sensors, platform, and vehicle**

- **Infrared thermometer**
- **Ultrasonic Transducer**
- **Multi-spectral crop canopy sensor**
- **GPS-RTK (antenna)**
- **GPS-RTK (receiver and radio)**
- **Data loggers (CR1000 + CR3000)**

**Sensors and Equipment**:

- Apogee ACS-470 3-band
- Pulsar db 3-m
- Plant height
- Vegetation Indices

**Vehicle Specifications**:

- **High-clearance tractor**
  - Average speed of 2.82 km/h
  - 1 data point/meter (1 Hz)

**References**:

Andrade-Sanchez, P., Gore, M. A. et al. 2014 Functional Plant Biology

Duke Pauli
Central Arizona: clear skies, very limited rain, high temperatures

TM-1×NM24016 population: 94 RILs (Gossypium hirsutum; Upland cotton)

Treatments: 100 and 50% ET (2 reps) by drip irrigation

1pm MST on day 224 (12-Aug)

Wet Rep2: 28-36°C
Dry Rep2: 33-49°C
Wet Rep1: 29-36°C
Dry Rep1: 31-46°C
Significant time-by-treatment interaction for canopy temperature

Treatment: $P<0.05$
Time: $P<0.0001$
Treatment*Time: $P<0.0001$
Phenotypic variation: Canopy Tm

Wet and Dry Plots at 1 pm on Day 224 (Aug 12)

$H^2 = 0.52 \pm 0.11$

$H^2 = 0.58 \pm 0.09$
Repeatability of Dry/Wet Canopy Tm

Day 217: 1 pm Dry/Wet Canopy Tm (ºC)

Day 224: 1 pm Dry/Wet Canopy Tm (ºC)

$R^2 = 0.47$
### GBS-QTL Analysis: Dry/Wet Canopy Tm

<table>
<thead>
<tr>
<th>Chr_LG_cM</th>
<th>c10.38.5</th>
<th>c12.51.18</th>
<th>c17.68.14</th>
<th>c20.83.18</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-0.006</td>
<td>0.003</td>
<td>-0.005</td>
<td>-0.001</td>
</tr>
<tr>
<td></td>
<td><strong>-0.019</strong></td>
<td>0.008</td>
<td><strong>-0.016</strong></td>
<td><strong>0.015</strong></td>
</tr>
<tr>
<td></td>
<td>-0.004</td>
<td>0.005</td>
<td>-0.004</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>-0.011</td>
<td>0.010</td>
<td>-0.009</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td><strong>-0.020</strong></td>
<td><strong>0.018</strong></td>
<td><strong>-0.010</strong></td>
<td><strong>0.020</strong></td>
</tr>
</tbody>
</table>

| Day 217 7 am | -0.019 | 0.008 | -0.016 | 0.015 |
| Day 217 1 pm | -0.004 | 0.005 | -0.004 | 0.002 |
| Day 224 7 am | -0.011 | 0.010 | -0.009 | 0.009 |
| Day 224 10 am | -0.020 | 0.018 | -0.010 | 0.020 |
| Day 224 1 pm | -0.019 | 0.008 | -0.016 | 0.015 |

**Negative** – decrease °C  **Positive** – increase °C

All statistically significant allelic effects are indicated by a black rectangle border.

Considered identical QTL if support intervals overlapped.
Increased leaf transpiration rate under drought stress contributes to higher lint yield

Higher leaf transpiration provides more of an adaptive advantage in terms of yield when grown under drought
Small unmanned aircraft systems: potential to combine the throughput of aerial imaging with the precision of a ground-based system

Margaret Krause
Duke Pauli
Nicholas Kaczmar
James Clohessy
Plant phenotyping: hand-held for breeders

- Sensors: temperature, height, infrared imaging
- Hardware: intelligent real time processing
- Flexible: functional with or without RTK-GPS

<table>
<thead>
<tr>
<th>Component</th>
<th>Prototype Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raspberry Pi</td>
<td>$35</td>
</tr>
<tr>
<td>Model B+, ARM1178, 512MB RAM</td>
<td></td>
</tr>
<tr>
<td>5MP Camera</td>
<td>$25</td>
</tr>
<tr>
<td>IR filter removed</td>
<td></td>
</tr>
<tr>
<td>IR Temperature Sensor</td>
<td>$16</td>
</tr>
<tr>
<td>MLX90614</td>
<td></td>
</tr>
<tr>
<td>IR Range Sensor</td>
<td>$13</td>
</tr>
<tr>
<td>GP2Y0A21</td>
<td></td>
</tr>
<tr>
<td>High Resolution Sonar</td>
<td>$30</td>
</tr>
<tr>
<td>MB1023</td>
<td></td>
</tr>
<tr>
<td>Battery &amp; Solar Panel</td>
<td>$28</td>
</tr>
<tr>
<td>5000mAh capacity, 1W charging</td>
<td></td>
</tr>
<tr>
<td>3 Axis Accelerometer</td>
<td>$6</td>
</tr>
<tr>
<td>ADXL335</td>
<td></td>
</tr>
<tr>
<td>MicroSD Storage</td>
<td>$9</td>
</tr>
<tr>
<td>16GB</td>
<td></td>
</tr>
<tr>
<td>Additional Hardware</td>
<td>$30</td>
</tr>
<tr>
<td>LCD screen, buttons, case, etc</td>
<td></td>
</tr>
<tr>
<td><strong>Total Prototype Cost</strong></td>
<td><strong>$192</strong></td>
</tr>
</tbody>
</table>

James Clohessy
Combined drought and heat stress trial at CIMMYT-Harare in Zimbabwe

- Hybrids generated from CIMMYT/HarvestPlus breeding material
- Field-based, high-throughput phenotyping of physiological responses
- GWAS and GP for carotenoid, agronomic, and physiological traits

Skywalker UAS

Christine Diepenbrock
Additional Research Projects

Accelerated Development of Commercial Hydrotreated Renewable Jet Fuel from Redesigned Oil Seed Feedstock Supply Chains
Funding Source: USDA-NIFA-DOE BRDI
Investigators: H. Colvin; M. Gore; C. McMahan; M. Jenks; J. Dyer; A. Landis; M. Fraley

Securing the Future of Natural Rubber – An American Tire and Bioenergy Platform from Guayule
Funding Source: USDA-NIFA-DOE BRDI
Investigators: T. Isbell; M. Gore; M. Jenks; J. Dyer; D. Long; D. Archer; S. Frey; D. Galloway; T. Tomlinson

Accelerating Oat Breeding for Nutritional Quality: beta-glucans, lipids, and antioxidants
Funding Source: PepsiCo
Investigators: M. Sorrells; M. Gore; J.-L. Jannink; O. Hoekenga
Additional Research Projects

Elucidating the genetic basis and relationship of root post-harvest physiological deterioration tolerance and carotenoid levels in West African cassava germplasm
Funding Source: BMGF PEARL
Investigators: N. Ndubuisi and M. Gore

Relevance of gender in trait preferences of cassava small-scale farmers in Uganda
Funding Sources: BMGF, NextGen Cassava and CGIAR RTB
Investigators: P. Iragaba; M. Gore; H. Tufan; R. Bezner-Kerr; NaCRRRI

Breeding methods and germplasm for improved nutritional quality of sweet corn
Funding Sources: Hatch NIFA and Cornell Startup Funds
Investigators: M. Gore, M. Smith

Genetic diversity of common bean landraces in US Southwest
Funding Source: Cornell Startup Funds
Investigators: M. Gore and Native Seeds Exchange
Acknowledgements

**Michigan State University**

*Dean DellaPenna (Project PI)*
- Maria Magallanes-Lundback
- Ruthie Angelovici
- Sabrina Gonzalez Jorge
- Alex Mesberg

*Ruthie Angelovici*
- Robin Buell
- Jason Cepela
- Brieanne Vaillancourt
- Elsa Gongora-Castillo

**Purdue University**

*Torbert Rocheford*
- Kristen Chandler
- Brenda Owens
- Tyler Tiede

**Cornell University**

*Edward Buckler*
- Jeff Glaubitz
- Peter Bradbury
- Kelly Swarts
- Cathy Kandianis
- Alex Lipka
- Christine Diepenbrock

This work is supported by NSF Grant 0922493 and the USDA-ARS
Acknowledgements

**Cornell University**
Gore lab members

**University of Arizona**
Pedro Andrade-Sanchez
John Heun

**Kansas State University**
Jesse Poland

**NMSU**
Jinfa Zhang

**U.S. ALARC**
Elizabete Carmo-Silva
Andy French
Doug Hunsaker
Mike Salvucci
Bob Strand
Kelly Thorp
Jeff White

**USDA-ARS**
Richard Percy
David Fang

PGRP Award# 1238187
The 5,000 RILs are genotyped with 14k GBS SNP markers for NAM joint linkage.
Whole-genome resequencing of parents and impute 30M SNPs onto recombination blocks

5,000 RIL Linkage Map

Linkage resolution

NAM resolution

B73 × B73
6,000-9,000 years ago
Y1/PSY1 gain of function mutation is not photoregulated and produces endosperm carotenoids

1 million+ years ago
Tocochromanols in grain Carotenoids in green leaf

“Young” genetic architecture has not had enough time to evolve many endosperm-specific regulatory elements (i.e., less QTL)

White grain - ancestral
Yellow grain – derived

Modified from Yamasaki et al. 2007

Teosinte
Maize inbred/
Maize landrace
Markers at candidate genes associated with a priori QTL predict carotenoid traits as well as genome-wide marker set.

Genomic prediction models: diverse maize panel

Owens et al., 2014, in press
Harvest of Orange Maize for Vitamin A Nutrition Efficacy Trial

Zambia
Africa Acceptance – using orange to overcome preference for white grain and concerns about yellow grain. Orange also associated with more total carotenoids, more flux into pathway. Thus, more that can be modulated to provitamin A.
Thank you to our sponsors: