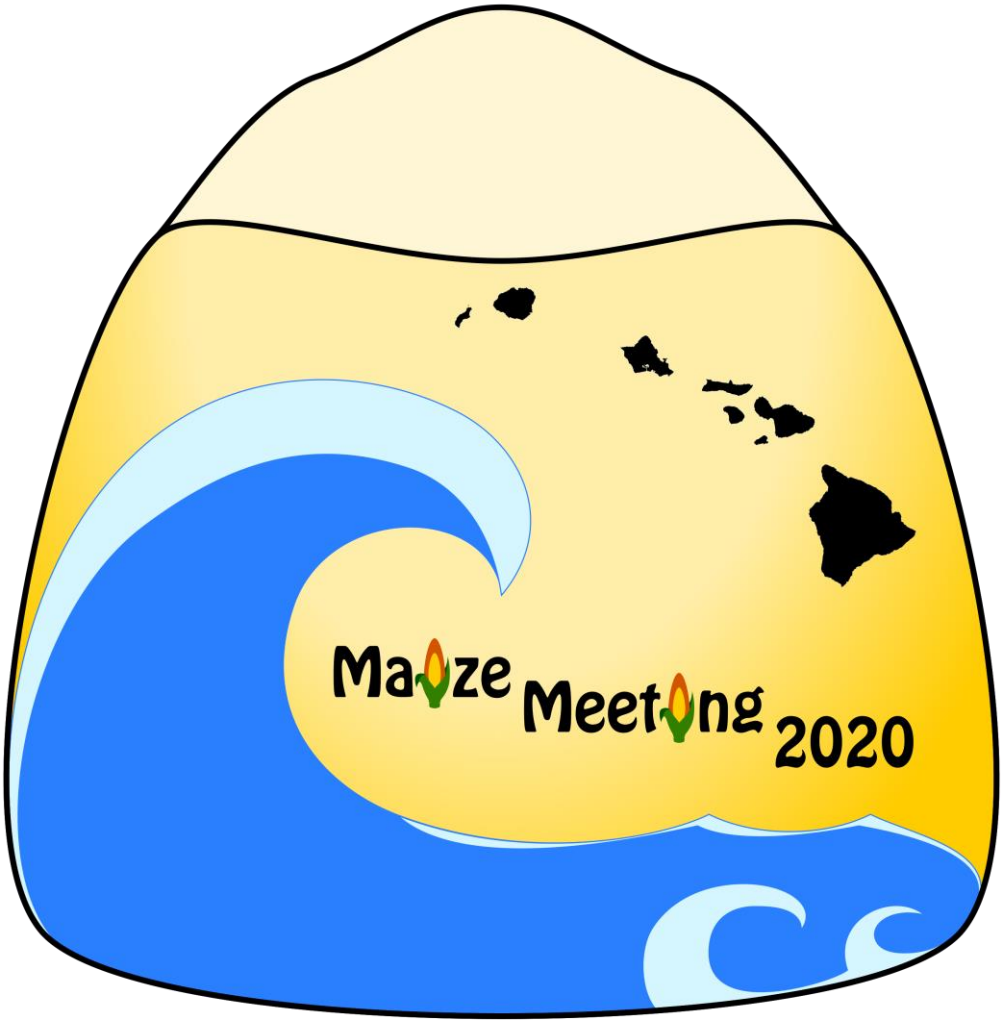
62nd Annual Maize Genetics Conference

Program and Abstracts



March 12 – March 15, 2020

Sheraton Kona

Kailua-Kona, Hawai‘i, USA

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Cover image description

Maize seed with a wave in the foreground and the Hawaiian Islands in the background.

Cover art by

Nicholas Blavet
Institute of Experimental Botany of the AS CR, v.v.i,
Czech Republic

General Information

Meeting Registration

Thursday: 3:00 PM to 9:30 PM: Kaleiopapa Convention Center Foyer
Friday: 7:30AM to 12:30 PM: Kaleiopapa Convention Center Foyer
Saturday: 8:00AM to 12:00 PM: Kaleiopapa Convention Center Foyer

Meals

All meals will be served buffet style outside at the Hawaii Lawn. Please wear your name tag to each meal. Sunday breakfast will be a cash and carry option in the Kaleiopapa Convention Center Foyer. Kona coffee and tea will be available during the morning beverage break and Kona coffee and soft drinks will be available during the 2:30-3:30 PM beverage break at no charge. Water stations will be available all day in the poster rooms.

Talks and Posters

All Talks will be presented in the Keauhou II room at the Kaleiopapa Convention Center.

Posters will be presented in the Keauhou I, III, and IV rooms, adjacent to where the talks will be held. Posters should be hung Thursday starting at 3 PM and stay up until Sunday morning, but must be removed by 9 AM on Sunday. During poster sessions, presenters of odd number posters are asked to stand by their posters 1:30-3:00 PM on Friday and 3:00-4:30 PM on Saturday. Presenters of even numbered posters should stand by their posters 3:00-4:30 PM on Friday and 1:30-3:00 PM on Saturday.

The maize meeting is a forum for presentation and discussion of unpublished material. **Photographing or recording of talks and posters is <u>not</u> allowed.**

Socializing and Networking

After the evening sessions on Thursday and Friday, there will be informal socializing and poster gazing in the Kaleiopapa Convention Center, with refreshments provided until 1 AM. On Saturday evening, there will be informal socializing, poster gazing and refreshments in the Kaleiopapa Convention Center until 11 PM, followed by music, dancing and refreshments until 2 AM in Keauhou II.

Steering Committee

Please share your suggestions and comments about the meeting with the 2020 Steering Committee

Clint Whipple, Chair.....(whipple@byu.edu)
Marna Yandeau-Nelson, co-Chair.....(myn@iastate.edu)
Mike Muszynski, Local Host.....(mgmuszyn@hawaii.edu)
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Erin Sparks.....(esparks@udel.edu)
Maud Tenaillon.....(maud.tenaillon@inra.fr)

<u>Ex officio:</u>
Carson Andorf - MaizeGDB
David Braun - Treasurer
John Portwood - Abstract Coordinator
Marty Sachs - Maize Meeting Guru

<u>Conference Coordinator:</u>
Angela Freemyer

<u>Audio Visual:</u>
Darwin Campbell

Acknowledgements

Many thanks go to John Portwood, Carson Andorf, and the MaizeGDB staff from the USDA-ARS for their tremendous efforts in organizing, assembling, and advertising the conference program. We also greatly thank Angela Freemyer and her team at the University of Missouri Conference Office and Ian Popewitz and Lynne Navis from the Alliance of Crop, Soil, and Environmental Science Societies (ACSESS) for helping to organize the conference, handling registration and dealing with a multitude of other issues. Special thanks are also extended to the Sheraton Kona staff for their help in organizing this conference, and to Darwin Campbell and John Portwood for providing AV and other support. Thanks go to Mei Guo and Todd Jones for their efforts in securing funding to support graduate student attendance at this meeting. Finally, many, many thanks go to Mike Muszynski for his work as local organizer and to Marty Sachs for his wisdom in all things related to the Maize Meeting.

From the Maize Genetics Cooperation Board of Directors:

Maize Genetics Awards (to be announced week of March 2nd):

The 2020 M. Rhoades Early Career Awardee:

Matt Hufford at Iowa State University.

The 2020 L. Stadler Mid-Career Awardee:

Jim Holland at the USDA-ARS and North Carolina State University.

The 2020 R.A. Emerson Awardee:

Jerry Kermicle at the University of Wisconsin – Madison.

The Barbara McClintock Prize for Plant Genetics and Genome Studies

This award has been created to memorialize the unequalled contributions of Dr. McClintock through providing recognition to the most outstanding plant geneticists of the present era. In memory of the many contributions of Dr. McClintock, this Prize will be awarded each year to one or more of the most creative minds and productive scientists in the study of plant genome structure, function and evolution, including the analysis of gene regulation and epigenetics. The 2020 Barbara McClintock Prize for Plant Genetics and Genome Studies goes to James Birchler who will present the McClintock Prize Address.

(See https://maizegdb.org/community/awards for details about each award)

NSF-funded Research Coordination Network for maize genetics:

The National Science Foundation is supporting a 5-year Research Coordination Network project titled "Broadening and Energizing the Maize Research Community". The project began in January, 2018, and is coordinated by the Maize Genetics Executive Committee. The grant funds activities at the Maize Genetics Conference including the MaGNET program and travel awards to increase disciplinary breadth and underrepresented participation. In addition, the funding allows the Maize Genetics Conference to systematically enrich the program during the term of the grant. Mid-year conferences are planned yearly to focus on specific topics that are important to the community. The first mid-year conference was held in Madison, WI in September 2019 and included an overall visioning session as well as focus on Functional Genomics Tools and Resources. The second mid-year conference was held in Madison, WI in September 2019 and focused on Data Collection and Curation, Databases, and Genome Annotation. White papers summarizing conclusions of the mid-year conferences are available on MaizeGDB (https://www.maizegdb.org/mgec). Teams have been assembled within the RCN to focus on: Functional Genomics Tools and Resources; Informatics Tools, Resources, and Services; Training and Student Recruitment; Developing Country Interface and Community Breadth; and Industry Interface. We appreciate the support from the National Science Foundation for this initiative and are excited about the potential for the grant to substantially advance and transform our community.

Data Management Made Simple

Every Maize Researcher should make data Findable, Accessible, Interoperable and Reusable (FAIR; go-fair.org). Here we outline some basic guidelines for FAIR management of your lab's data. It will help everyone in the maize community if you also apply these principles to all papers you review. We are always happy to answer your questions on these issues! https://www.maizegdb.org/contact. For more information, see https://www.maizegdb.org/FAIRpractices

1. **Put your Data in the right Database.**
   - *DNA/RNA/Protein Sequences, genome assemblies should go to NCBI, EBI or DDBJ:* NCBI (US), EBI (Europe), and DDBJ (Asia) provide stable, long-term databases for DNA, RNA and protein sequence data and create stable identifiers (accessions) for datasets. These three share sequence data on a daily basis so data deposited at one is available at all. Each has multiple sub-databases, for example, NCBI has SRA and GEO for un-mapped and mapped sequence reads. Please submit genome assemblies to EBI or NCBI Genomes. We understand this can take some time to complete. We can help, we do not recommend simply submitting contigs to GenBank.
   - *SNPs:* All non-human SNPs should be submitted to EVA at EBI.

See https://www.maizegdb.org/FAIRpractices for more repositories. If your journal article refers to data NOT published with your article, **please make sure to obtain and add a persistent identifier and location of your data in your article.**

2. **Don't rename genes that already have names.**
Renaming genes that already have names is a HUGE problem in maize, especially when an existing name is reused for a different gene. Please look up your gene at MaizeGDB before assigning a name, and follow the maize nomenclature guidelines. (https://www.maizegdb.org/nomenclature).

3. **Attach complete and detailed metadata to your data sets, and use accepted file formats.**
When you deposit data, you are asked for information about your data (metadata). Please give this the same careful attention you give to your bench work and analysis. Datasets that are not adequately described are not reusable or reproducible, and raise questions about the carefulness and accuracy of the research.

4. **Insure your data sets are "machine readable".**
Computers can find data that matches a search query. When describing your data, use permanent identifiers wherever possible use the proper case (LG1 is not the same as lg1), and include GO, PO, PATO terms when possible. Please check and validate that your data is in common, well-used machine readable formats.

5. **Publish your data with your paper.**
Sometimes data are too large to publish as a table or supplementary material with your paper. These data can be deposited in data repositories, which provide accessions or DOIs (stable identifiers). DOIs should be listed in your paper.

6. **Budget time for Data Management.**
Please budget time to do a good job of managing your data as you are with the other aspects of your research.

7. **Familiarize yourself with the FAIR data sharing standards.**
Here are some resources: https://www.go-fair.org, https://doi.org/10.1093/database/bay088.

The MaizeGDB team

The MaGNET Program and 2020 Awards

MaGNET (Maize Genetics Network Enhancement via Travel) is a program that seeks to recruit and retain scientists from diverse backgrounds into the maize research community by encouraging their attendance at the Annual Maize Genetics Conference (MGC). As such, it provides a source of support to help students and early career scientists from under-represented groups learn about maize genetics and connect with scientists already in the community. Awardees are not required to have previous maize genetics research experience, but will hopefully develop an appreciation of the current excitement in the field, and become an integral part of the community in the future. The program also provides an opportunity for awardees to explore potential collaborations and develop career contacts.

Each MaGNET Award helps defray the cost of attending the Maize Genetics Conference, including registration, food, lodging and airfare. In addition, awardees that have never attended the MGC are paired with an experienced 'Maize Mentor', who will help the awardee navigate the conference. Awardees are identifiable by a special notation on their name tags, and many of them are attending the MGC for the first time – please congratulate these scientists and welcome them to our famously hospitable conference!

All applicants must show strong potential for a career in the biological sciences, be either citizens or permanent residents of the USA, and belong to a group traditionally underrepresented in science. To help provide a more integrative and effective experience at the Conference for student awardees, faculty mentors who accompany one or more eligible student applicants are also eligible to apply for a MaGNET award.

2020 MaGNET Awardees

Vanessa Bonebo, Saint Michael's College	Poster #132
Brianna Griffin, Iowa State University	Poster #131
Marlynn Irambona-Serwili, Saint Michael's College	Poster #132
Michelle Lang, Iowa State University	Poster #168

The MaGNET program of the Maize Genetics Conference is supported by grant IOS-1748978 from the National Science Foundation.

Primarily Undergraduate Institutions and Disciplinary Breadth Awards

Primarily Undergraduate Institutions (PUI) and Disciplinary Breadth (DB) are two new financial aid programs that seek to recruit and retain scientists from PUIs and plant-related disciplines into the maize research community by encouraging their attendance at the Annual Maize Genetics Conference (MGC). As such, it provides a source of support to help students and early career scientists from under-represented groups learn about maize genetics and connect with scientists already in the community. The program also provides an opportunity for awardees to explore potential collaborations and develop career contacts.

Each award helps defray the cost of attending the Maize Genetics Conference, including registration, food, lodging and airfare. Awardees are identifiable by a special notation on their name tags, and many of them are attending the MGC for the first time – please congratulate these scientists and welcome them to our famously hospitable conference!

All applicants must show strong potential for a career in the biological sciences, and be either citizens or permanent residents of the USA. To help provide a more integrative and effective experience at the Conference for student awardees, faculty who accompany one or more eligible student applicants are also eligible to apply for a PUI or DB award.

2020 PUI Awardees

Student

Kiana Imani, University of Washington Bothell	Poster #80
Matthew Wendt, Wisconsin Lutheran College	Poster #151
Han Yu, Whitman College	Poster #187

Faculty

Adrienne Kleintop, Delaware Valley University	Poster #11
Brit Moss, Whitman College	Talk #35
Allison Phillips, Wisconsin Lutheran College	----------

2020 DB Awardees

Postdoc

Colleen Drapek, University of Cambridge	Poster #192

Student

Ashley Henderson, West Virginia University	Poster #73

Faculty

Douglas Cook, Brigham Young University	Poster #21
Stephen Piccolo, Brigham Young University	Poster #228

The PUI and DB programs of the Maize Genetics Conference are supported by grant IOS-1748978 from the National Science Foundation.

OPTIONAL PRE-CONFERENCE WORKSHOPS

Tuesday, March 10 - Thursday, March 12

Maize Annotation Jamboree (Hualalai Room)
Organizers: Doreen Ware, Marcela K. Tello-Ruiz, Cristina Fernandez-Marco

Tuesday, 6:00pm - 8:00pm	Welcome Dinner
Wednesday, 9:00am - 8:00pm	Annotation Jamboree: Day #1
Thursday, 9:00am - 4:00pm	Annotation Jamboree: Day #2

Thursday, March 12

9:00am - 4:00pm **Maize Developmental Genetics Meeting**
Organizer: Paula McSteen & Sarah Hake (Mauna Kea Room)

1:00pm - 2:30pm **Maize Genetic Transformation Technology: an Update**
Organizer: Kan Wang (Mauna Loa Room)

Heidi Kaeppler, University of Wisconsin-Madison
Transformation of maize inbred line, LH244, a new public resource for maize functional genomics research

Morgan McCaw, Iowa State University, USA
Genetic transformation of Fast-Flowering Mini Maize

Bill Gordon-Kamm (Corteva AgriScience, USA)
Leaf Transformation in maize: differences and similarities compared to immature embryo transformation

2:30pm - 4:00pm **Maize Community Resources: MaizeGDB & Gramene**
Organizers: Lisa Harper & Marcela Tello-Ruiz (Mauna Loa Room)

4:00pm - 5:30pm **Maize Epigenetics and Chromatin (Maize EPIC) Network**
Organizers: Hank Bass & Vincenzo Rossi (Mauna Loa Room)

(4:00pm) Sarah Anderson, Iowa State University
Widespread imprinting of young genes and transposable elements uncovered with whole genome assemblies

(4:20pm) Thelma Madzima, University of Washington Bothell
MOP1-mediated epigenetic regulation of ABA-induced transcriptional responses in maize

(4:40pm) Kelly Dawe, University of Georgia
NAM pan-genome and (soon to arrive) pan-methylome

(4:55pm) Rob Martienssen, Cold Spring Harbor Laboratory
The MaizeCODE pilot Project

(5:10pm) Hank Bass, Florida State University
Maize EPIC Network Discussion

4:30pm - 6:00pm **Speed Networking with Undergraduates: Connecting to Community**
Organizer: Mark Lubkowitz (Mauna Kea Room)

SCHEDULE OF EVENTS

Talks will be held in Keauhou II.
Posters will be displayed in Keauhou I / III / IV.

Thursday, March 12

3:00 PM – 9:30 PM	**REGISTRATION** (Kaleiopapa Convention Center Foyer)
3:00 PM – 6:00 PM	**POSTER HANGING** (Keauhou I/III/IV)
5:00 PM – 5:45 PM	**MaGNET Awardees and Mentors Introductions** (Hualalai Room)
6:00 PM – 7:00 PM	**DINNER** (Hawaii Lawn)

7:00 PM – 9:00 PM	**SESSION 1 – WELCOME / EXPRESSING THE GENOME** Chair: Clint Whipple / Erin Sparks Talks 1-5. Pages 21-25.
7:00 PM	**WELCOME AND ANNOUNCEMENTS** (Keauhou II)
7:15 PM	**James Satterlee, Cornell University** [T1] *Single-cell transcriptomic analysis of maize shoot apical meristem organization and cell differentiation.*
7:35 PM	**Natalie Clark, Iowa State University** [T2] *Generating multi-scale predictive networks of Northern Corn Leaf Blight resistance.*
7:55 PM	**Peter Crisp, University of Queensland** [T3] *DNA methylomes as a tool for functional annotation of genes and their regulatory regions.*
8:15 PM	**Wei Guo, Purdue University** [T4] *Rapid, heat-induced transgenerational reactivation of a silenced transposable element in maize.*
8:35 PM	**Thomas Hartwig, Max Planck Institute** [T5] *FIND-CIS: an antibody-free, genome-wide method for high-resolution, in vivo mapping of functional cis-elements.*
9:00 PM – 1:00 AM	**INFORMAL POSTER VIEWING, SOCIALIZING AND NETWORKING** (Keauhou I/III/IV and Kaleiopapa Convention Center Foyer)

Friday, March 13

7:00 AM – 8:00 AM	**BREAKFAST** (Hawaii Lawn)
7:30 AM – 12:30 PM	**REGISTRATION** (Kaleiopapa Convention Center Foyer)

8:00 AM – 10:10 AM — SESSION 2 – EMERGING TOOLS AND CHALLENGES
Chair: Todd Jones — Talks 6-10. Pages 26-30.

8:00 AM — **ANNOUNCEMENTS** (Keauhou II)

8:15 AM — **Na Wang, University of Georgia** [T6]
Haploid induction by a maize cenh3 null mutant

8:35 AM — **Lei Liu, Cold Spring Harbor Laboratory** [T7]
Enhancing maize grain yield by CRISPR/Cas9 genomic editing of CLE (CLAVATA3/Embryo-surrounding region) genes for maize breeding

8:55 AM — **Bliss Beernink, Iowa State University** [T8]
Protein expression and gene editing in maize using foxtail mosaic virus vectors

9:15 AM — **Ian Braun, Iowa State University** [T9]
Natural language processing of phenotype descriptions enables automated inference of biological relationships

9:35 AM — **Matheus Baseggio, Cornell University** [T10]
Key steps towards the biofortification of sweet corn: Identifying genes important for improving vitamin and mineral levels in fresh kernels

10:10 AM – 10:30 AM — **BREAK**

10:30 AM – 11:25 AM — SESSION 3 – INTERACTIONS WITH THE ENVIRONMENT I
Chair: Marna Yandeau-Nelson — Talks 11-12. Pages 31 - 32.

10:30 AM — **Bethan Manley, University of Cambridge** [T11]
Independent of Arbuscular Mycorrhizal Symbiosis: Positional cloning and characterisation of a novel arbuscular mycorrhizal mutant in Zea mays.

10:50 AM — **Yi-Hsuan Chu, Michigan State University** [T12]
Elucidating the genome-wide gene regulatory features of transcription factors (TFs) involved in the control of maize phenylpropanoid biosynthesis

11:10 AM — **Natalia de Leon, Maize Genetics Cooperation**
An update on the incorporation of the Maize Genetics Cooperation as a not-for-profit organization.

11:25 AM – 12:25 PM — SESSION 4 – INVITED SPEAKER
Chair: Marna Yandeau-Nelson — Page 18.

11:25 AM — **Introduction**

11:35 AM — **Sarah Hake, USDA-ARS** [IS1]
Organogenesis in maize – lessons from mutants.

Friday, March 13 (continued)

12:30 PM – 1:30 PM **LUNCH** (Hawaii Lawn)

1:30 PM – 4:30 PM **POSTER SESSION 1** (Keauhou I/III/IV)

1:30 PM – 3:00 PM *Presenters should be at odd numbered posters.*

3:00 PM – 4:30 PM *Presenters should be at even numbered posters.*

Beverages will be available from 2:30 to 3:30 PM in the Kaleiopapa Convention Center Foyer

4:40 PM – 6:00PM **SESSION 5 – THE GENES THAT MAKE MAIZE I**
Chair: Michael Muszynski Talks 13-16. Pages 33-36.

4:40 PM **Adam Bray, Donald Danforth Plant Science Center** [T13]
The classic maize mutant Rootless1 is a bHLH transcription factor that modulates crown root number in the field

5:00 PM **Zongliang Chen, Rutgers University** [T14]
A tandem duplication of the maize wushel1 gene promotes major architectural rearrangements in inflorescense meristem

5:20 PM **Yanfang Du, Huazhong Agricultural University** [T15]
Gene duplications at the Fascicled ear1 (Fas1) locus affect adaxial–abaxial cell fate in maize inflorescence meristems

5:40 PM **Harry Klein, University of Massachusetts** [T16]
A dormancy regulatory module is recruited to suppress maize carpels.

6:00 PM – 7:00 PM **DINNER** (Hawaii Lawn)

7:00 PM –9:00 PM **SESSION 6 – AWARDS & MCCLINTOCK PRIZE PRESENTATION**
Chair: Natalia de Leon Page 20.

7:00 PM **Ruth Wagner, MGC Incoming Chair**
M. Rhoades Early-Career and L. Stadler Mid-Career Awards

7:25 PM **David Jackson, MGC Communication Coordinator**
R. Emerson Lifetime Award and 2021 McClintock Prize

7:55 PM **David Braun, University of Missouri**
McClintock Prize Presentation

8:10 PM **Jim Birchler, University of Missouri**
The Gene Balance Hypothesis: How regulatory gene stoichiometries affect expression, the phenotype and evolutionary processes

9:00 PM – 1:00 AM **INFORMAL POSTER VIEWING, SOCIALIZING AND NETWORKING**
(Keauhou I/III/IV and Kaleiopapa Convention Center Foyer)

Saturday, March 14

7:00 AM – 8:00 AM **BREAKFAST** (Hawaii Lawn)
8:00 AM – 12:00 PM **REGISTRATION** (Kaleiopapa Convention Center Foyer)

8:00 AM – 10:00 AM **SESSION 7 – GENOME BIOLOGY AND EVOLUTION**
Chair: Hilde Nelissen Talks 17-22. Pages 37-42.

8:00 AM **Jaclyn Noshay, University of Minnesota** [T17]
The genetic and epigenetic contribution of TEs in shaping the maize genome.

8:20 AM **Candice Hirsch, University of Minnesota** [T18]
Characterizing the maize pan-genome and effects on phenotypic variation.

8:40 AM **Minghui Wang, Cornell University** [T19]
Dissecting recombination landscape in maize using machine learning.

9:00 AM **Devon Birdseye, UC San Diego** [T20]
Maize hybrids show increased expression of plastid protein complexes and decreased expression of ethylene biosynthesis

9:20 AM **Jinliang Yang, University of Nebraska-Lincoln** [T21]
Adaptive evolution of DNA methylation reshaped gene regulation in maize

9:40 AM **Stephanie Klein, Pennsylvania State University** [T22]
Fitness and environmental patterns in maize landraces identify beneficial alleles at single gene resolution.

10:00 AM – 10:30 AM **BREAK**

10:30 AM – 11:30 AM **SESSION 8 – FROM GENOTYPE TO PHENOTYPE**
Chair: Clinton Whipple Talks 23-25. Pages 43-45.

10:30 AM **Norman Best, University of Missouri** [T23]
The lateral suppressor1 gene encodes a GRAS transcription factor required for axillary meristem development in maize.

10:50 AM **Vivek Shrestha, University of Missouri** [T24]
Uncovering the genetic architecture of protein bound amino acids in maize kernels using GWAS combined with gene co-expression network analysis.

11:10 AM **Sarah Anderson, Iowa State University** [T25]
Uncovering imprinting of PAV genes and transposable elements using whole genome assemblies.

11:30 AM – 12:30 PM **SESSION 9 – INVITED SPEAKERS**
Chair: Clinton Whipple Page 19.

11:30 AM Introduction

11:40 AM **Kirsten Bomblies, ETH Zurich** [IS2]
How to tango with four - the evolution of meiotic stability in autotetraploid Arabidopsis arenosa.

Saturday, March 14 (continued)

12:30 PM – 1:30 PM **LUNCH** (Hawaii Lawn)

1:30 PM – 5:00 PM **POSTER SESSION 2** (Keauhou I/III/IV)

1:30 PM – 3:00 PM *Presenters should be at even numbered posters.*

3:00 PM – 4:30 PM *Presenters should be at odd numbered posters.*

Beverages will be available from 2:30 to 3:30 PM in Kaleiopapa Convention Center Foyer

5:00 PM – 6:00 PM **COMMUNITY SESSION - Maize Genetics Cooperation**
MGC Chair: Natalia de Leon (Keauhou II)

6:00 PM – 7:00 PM **DINNER** (Hawaii Lawn)

7:00 PM – 9:00 PM **SESSION 10 – THE GENES THAT MAKE MAIZE II**
Chair: Andrea Gallavotti Talks 26-31. Pages 46-51.

7:00 PM **Thomas Hughes, University of Oxford** [T26]
SCR and NKD genes regulate leaf patterning during maize development

7:20 PM **Nick Lauter, USDA-ARS** [T27]
Cloning and transcriptomic characterization of macrohairless1, a regulator of specialized cell fate commitment in the maize epidermis

7:40 PM **George Chuck, UC Berkeley** [T28]
Necrotic upper tips1 is a florally induced NAC transcription factor that promotes water movement by fortifying protoxylem cell walls

8:00 PM **Jiani Yang, Donald Danforth Plant Science Center** [T29]
Growth hormones BR and GA modulate spikelet meristem identity in Setaria viridis through interface with the ortholog of maize determinacy factor, RAMOSA 1

8:20 PM **Xiaosa Xu, Cold Spring Harbor Laboratory** [T30]
New insights into maize development using single-cell RNA sequencing (scRNA-seq)

8:40 PM **Chong Teng, Donald Danforth Plant Science Center** [T31]
Dicer-like 5 deficiency confers temperature-sensitive male sterility in maize

9:15 PM – 11:00 PM **INFORMAL POSTER VIEWING & HOSPITALITY** (Keauhou I/III/IV and Kaleiopapa Convention Center Foyer)

11:00 PM – 2:00 AM **SOCIALIZING, NETWORKING & DANCE** (Keauhou II)

Sunday, March 15

7:00 AM – 8:20 AM **CASH & CARRY BREAKFAST** (Kaleiopapa Convention Center Foyer)

Posters should be taken down by 9 AM!

8:20 AM – 10:00 AM **SESSION 11 – INTERACTIONS WITH THE ENVIRONMENT II**
Chair: Jeff Ross-Ibarra Talks 32-36. Pages 52-56.

8:20 AM **Shawn Christensen, USDA-ARS** [T32]
Combinatorial stress causes extensive metabolic remodeling and divergent responses in maize defense chemistry

8:40 AM **Katherine Murphy, UC Davis** [T33]
Bioactive diterpenoids impact the composition of the root-associated microbiome in maize

9:00 AM **Jeff Bennetzen, University of Georgia** [T34]
Teaching plant genetics with discovery microbiomics

9:20 AM **Monika Frey, Technical University of Munich** [T35]
More than just the genes: Limitations for biosynthesis of the maize defense compound DIBOA in Arabidopsis

9:40 AM **Yezhang Ding, UC San Diego** [T36]
Convergent evolution on terpenoid metabolic pathways contributes to the protection of diverse crop genera

10:00 AM – 10:30 AM **BREAK**

10:30 AM – 11:40 AM **SESSION 12 – COMMUNICATING WITHIN AND BETWEEN CELLS AND PLANTS**
Chair: Erin Sparks Talks 37-39. Pages 57-59.

10:30 AM **Thomas Dresselhaus, University of Regensburg** [T37]
Signaling along the pollen tube journey.

10:50 AM **Britney Moss, Whitman College** [T38]
Maize auxin response circuits recapitulated in yeast

11:10 AM **Michaela Matthes, University of Missouri** [T39]
Imaging Boron: Illuminating hidden aspects of root architecture in maize

11:30 AM **CLOSING REMARKS**

11:40 AM **ADJOURNMENT**

Posters

Cytogenetics

P1	**Nicolas Blavet** <blavet@ueb.cas.cz>	*Accessory chromosomes in genus sorghum*
P2	**Hua Yang** <yanghu@missouri.edu>	*Comprehensive analysis of gene expression from genomic imbalance*
P3	**Changbin Chen** <chenx481@umn.edu>	*Exploring higher-order chromosome organization patterns by detecting meiotic axis protein attachment sites using ChIP-seq*
P4	**Meghan Brady** <meghan.brady@uga.edu>	*Gene structure and origin of maize abnormal chromosome 10*
P5	**Fangpu Han** <fphan@genetics.ac.cn>	*Identification and characterization of ZWINT-1 as an important protein in the maize cell division process*
P6	**Fangpu Han** <fphan@genetics.ac.cn>	*Maize centromere epigenetics in oat-maize addition lines*
P7	**Fangpu Han** <fphan@genetics.ac.cn>	*R-loops are enriched in maize centromeric region*
P8	**Mateusz Zelkowski** <mz548@cornell.edu>	*Shaping meiotic recombination landscape trough DNA methylation changes in maize.*

Education & Outreach

P9	**Irina Makarevitch** <irinamakarevitch@gmail.com>	*Confronting plant blindness in undergraduate students through authentic research experiences*
P10	**Marcela K. Tello-Ruiz** <telloruiz@cshl.edu>	*Finding and fixing annotation errors through community curation*
P11	**Adrienne Kleintop** <Adrienne.Kleintop@delval.edu>	*Incorporating maize landraces into undergraduate research and education*
P12	**Vivian Bernau** <vivian.bernau@usda.gov>	*Managing and distributing maize diversity: The NPGS maize collection in Ames, IA*
P13	**Vivian Bernau** <vivian.bernau@usda.gov>	*Producing tropical teosinte seed in a temperate environment*

Quantitative Genetics & Breeding

P14	**Daniel Robertson** <danieljr@uidaho.edu>	*A High-throughput non-destructive phenotyping device for detecting disease and pest damage in maize*
P15	**Heather Manching** <hcorn@udel.edu>	*A framework for evaluating performance of ancestral haplotype reconstruction*
P16	**Merritt Burch** <mbb262@cornell.edu>	*A meta-GWAS reanalysis of twenty years of quantitative traits in maize*
P17	**jingyun luo** <jingyunluo@foxmail.com>	*A metabolic genetic roadmap to flavor of waxy maize*
P18	**Jialu Wei** <jlwei@iastate.edu>	*Advancing understanding of maize heat stress response mechanisms by integrated molecular, biochemical, and whole-plant analysis*
P19	**Jianming Yu** <jmyu@iastate.edu>	*Aerial based high-throughput phenotyping for genetic dissection of NDVI in maize*

P20	**Ruijuan Li** <rli@inari.com>	*Allelic expression bias of deleterious protein-coding variants in Maize hybrids*
P21	**Douglas Cook** <maizegenetics@fishface.33mail.com>	*An integrated modeling/breeding experiment to determine the structural factors that influence stalk strength*
P22	**Daniel Robertson** <danieljr@uidaho.edu>	*Are maize stems structurally optimized to resist stalk lodging?*
P23	**Bridget McFarland** <bamcfarland@wisc.edu>	*Assessing the effect of increased planting density on yield component traits in maize*
P24	**Paul Armstrong** <paul.armstrong@usda.gov>	*Automated single kernel measurement of maize composition with NIR spectroscopy*
P25	**Linda Dao** <ldao@ufl.edu>	*Cold tolerance QTL analysis and validation using VIGOR: a machine vision assay for seedling emergence*
P26	**Andrei Kouranov** <andrei.kouranov@bayer.com>	*Demonstration of targeted crossover in hybrid corn using CRISPR technology*
P27	**Eli Hugghis** <hugghise@msu.edu>	*Detection of fusarium-infected wheat seed utilizing image-based analysis*
P28	**Sarah Odell** <sgodell@ucdavis.edu>	*Dissecting quantitative trait variation in a multi-parent maize population*
P29	**Juan Gonzalez** <juangonzalez@ufl.edu>	*Dissection of Quantitative Leaf Blight Resistance in sweet corn using genomics and phenomics*
P30	**Haixiao Hu** <hxhu@ucdavis.edu>	*Dissection of the genetic architecture of adaptation to high elevations in maize with allele-specific expression*
P31	**Samantha Snodgrass** <snodgras@iastate.edu>	*Diverse hybrids demonstrate heterosis beyond heterotic groups*
P32	**Joseph Gage** <jlg374@cornell.edu>	*Diverse maize inbreds show varying patterns of diel gene expression*
P33	**Meng Lin** <ml2498@cornell.edu>	*Elucidating the gene regulatory landscape of natural variation for leaf cuticular conductance in maize*
P34	**Ramandeep Kaur** <ramandeepk6789@gmail.com>	*Fine mapping of qtl on chromosome 9 for drought tolerance in maize*
P35	**Jose Varela** <jvarela@wisc.edu>	*Genetic analysis of endosperm vitreousness and compositional traits in maize*
P36	**Xiaowei Li** <xl743@cornell.edu>	*Genetic analysis of natural variation for levels of Vitamins B_2 and B_6 in maize grain*
P37	**Huyu Liu** <huyu@iastate.edu>	*Genetic analysis of nitrate accumulation in maize stalks*
P38	**Di Wu** <dw524@cornell.edu>	*Genetic and functional genomic analysis of tocochromanol levels in maize grain*
P39	**Brandi Sigmon** <bsigmon2@unl.edu>	*Genetic architecture of maize inflorescence plasticity in response to nitrogen stress*
P40	**jyoti kaul** <kauljyoti1@yahoo.co.in>	*Genetic diversity in kernel compositional traits and resistance to five foliar diseases in tropically adapted inbred lines of Quality Protein Maize*
P41	**Blake Trygestad** <trygesta@msu.edu>	*Genetic resistance to tar spot (Phyllachora maydis) in maize*
P42	**Laura Tibbs** <ltibbs@iastate.edu>	*Genome-wide dissection of B vitamin levels in maize grain in the Ames Diversity Panel*
P43	**Fernando Garcia Espolador** <fernando.garciaespolador@wisc.edu>	*Impact of different reference genomes for SNP calling in a genotyping-by-sequencing pipeline for a tropical maize diversity panel*

P44	**Robert Twohey III** <twohey2@illinois.edu>	*Increasing water use efficiency in Zea mays; An integrative approach to elucidating a complex trait*
P45	**Julia Silva Morosini** <julia.silvamorosini@wisc.edu>	*Inferring panel clustering and dominance effect on genomic prediction assessments involving tropical maize*
P46	**Robert Shrote** <shrotero@msu.edu>	*Integer/categorical particle swarm optimization improves the performance of optimal population value selection*
P47	**Melaku Gedil** <m.gedil@cgiar.org>	*Integrated approaches for molecular marker discovery and deployment for accelerating Striga resistance breeding in maize*
P48	**Jenna Hershberger** <jmh579@cornell.edu>	*Integrating transcriptomics for the improvement of genetic dissection and prediction of provitamin A and vitamin E in fresh sweet corn kernels*
P49	**Jennifer Hawkins** <jennifer.hawkins@mail.wvu.edu>	*Integration of high density genetic mapping with transcriptome analysis reveals candidate genes for tillering in sorghum*
P50	**Abi Gyawali** <agr75@mail.missouri.edu>	*Investigating the effect of pleiotropy due to flowering time in maize kernel composition traits using near-isogenic lines capturing an allelic series*
P51	**Jose Valdes Franco** <jav246@cornell.edu>	*Leveraging genome assemblies and landrace diversity: Evaluating a practical haplotype graph approach in maize*
P52	**Jeffery Gustin** <jgustin@ufl.edu>	*Love me tender: Predicting sweet corn pericarp thickness using single-kernel near infrared spectroscopy.*
P53	**Jacob Washburn** <jacob.washburn@usda.gov>	*Machine learning assisted multi-environment phenotype prediction in maize*
P54	**Aimee Schulz** <ajs692@cornell.edu>	*Maize intraspecific competition dynamics: the genomic architecture behind competitive ability*
P55	**Rafael Della Coletta** <della028@umn.edu>	*Modeling structural variation in genomic prediction models in maize*
P56	**Kyle Swentowsky** <kws67291@uga.edu>	*Perennial regrowth in maize/Zea diploperennis hybrids is controlled in part by a locus on chromosome 2*
P57	**Xianran Li** <lixr@iastate.edu>	*Phenotypic plasticity analyses reveal gene-environment interplay and enable genome-wide cross-environment prediction*
P58	**Brian Rice** <brice6@illinois.edu>	*Pleiotropy in maize inflorescence and leaf traits*
P59	**Vincent Colantonio** <vcolantonio@ufl.edu>	*Population genomics of sweet corn*
P60	**Leandra Parsons** <lmarshall@huskers.unl.edu>	*Production and selection of quality protein popcorn hybrids using a novel ranking system and combining ability estimates*
P61	**Qin Yang** <qyang@nwafu.edu.cn>	*QTL mapping for Gibberella stalk rot resistance in maize*
P62	**Namrata Maharjan** <namrata.maharjan@sdstate.edu>	*Quantitative Trait Loci (QTL) that influence efficacy of Teosinte crossing barrier1 (Tcb1) in maize*
P63	**Yuting Qiu** <yutingq2@gmail.com>	*Resistance to four foliar diseases of maize in four DRIL populations tods four foliar diseases of maize in four DRIL populatioreur foliar diseases of maize in four DRIL populations*
P64	**Daniel Robertson** <danieljr@uidaho.edu>	*Rethinking rind penetration experiments*
P65	**John McKay** <jkmckay@colostate.edu>	*Sensing underground biological traits and the ecology of roots, rhizospheres and aridity: Phenotyping roots at the field plot scale across levels of the factor irrigation*
P66	**Rajandeep Sekhon** <sekhon@clemson.edu>	*Stalk bending strength is a reliable predictor of stalk lodging incidence in maize*

P67 **Lillian Hislop** <lmhislop@wisc.edu> — *Sugarcane mosaic virus resistance in the Wisconsin Sweet Maize Diversity Panel*

P68 **Qiuyue Chen** <qchen295@wisc.edu> — *The genetic architecture of the maize progenitor, teosinte, and how it was altered during maize domestication*

P69 **Raksha Singh** <Raksha.Singh@usda.gov> — *Transcriptional reprogramming of defense related genes and pathways associated with Tar Spot disease in corn*

P70 **Vivek Shrestha** <vs6d9@mail.missouri.edu> — *Uncovering the genetic architecture of protein bound amino acids in maize kernels using GWAS combined with gene co-expression network analysis*

P71 **Zhongjie Ji** <jizhongj@msu.edu> — *Unmanned aerial system image analysis for key physiological traits in maize*

P72 **Diego Jarquin** <jhernandezjarquin2@unl.edu> — *Untangling environmental determinants of crop performance combining genomic selection methods and weather data.*

P73 **Ashley Henderson** <ahende11@mix.wvu.edu> — *Use it or Lose it: Diversity in salt response following the domestication of sorghum landraces*

P74 **Ryokei Tanaka** <rt475@cornell.edu> — *Whole-genome prediction for tocochromanol levels in maize grain: accuracy comparison among different models, traits, and populations*

Transposons & Epigenetics

P75 **Yonghao Sun** <839826968@qq.com> — *3D genome architecture coordinates trans and cis regulation of differentially expressed ear and tassel genes in maize*

P76 **Kevin Ahern** <ka38@cornell.edu> — *Ds mutagenesis of the benzoxazinoid gene cluster on Chromosome 4S*

P77 **Chunguang Du** <duc@montclair.edu> — *A set of maize mutant lines with single Ds-GFP insertions spread throughout the genome*

P78 **Yibing Zeng** <yz77862@uga.edu> — *DNA methylomes of the NAM founders*

P79 **Natalie Deans** <deans.11@osu.edu> — *Distal tandem repeats of the Pl1-Rhoades allele share paramutation and enhancer-like properties*

P80 **Kiana Imani** <kianai7@uw.edu> — *Epigenetic regulation of the BTF3-like transcription factor in drought stressed maize seedlings*

P81 **Thelma Madzima** <madzima@uw.edu> — *MOP1-mediated epigenetic regulation of ABA-induced transcriptional responses in maize.*

P82 **William F. Sheridan** <william.sheridan@und.edu> — *Non-Mendelian segregation of maize embryo-lethal mutants: Genetic or epigenetic?*

P83 **Gina Watanabe** <ginaw@hawaii.edu> — *Packaging centromere-specific integrase into proteinaceous nanoparticles*

P84 **Jay Hollick** <hollick.3@osu.edu> — *Parental RNA polymerase IV affects hybrid performance*

P85 **Jing Xu** <jingxu@webmail.hzau.edu.cn> — *Population-level analysis reveals the widespread occurrence and phenotypic consequence of DNA methylation variation not tagged by genetic variation in maize*

P86 **Jason Lynn** <jlynn@bio.fsu.edu> — *Single-locus immunoprecipitation proteomics (SLIP) analysis of proteins interacting with the b1 tandem repeat paramutation sequence*

P87 **Benjamin Berube** <bberube@cshl.edu> — *Teosinte pollen drive: A novel meiotic drive system in Zea mays*

P88 **Shujun Ou** <oushujun@iastate.edu> — *The divergence of maize NAM founders revealed by syntenic LTR retrotransposons*

P89 **Weijia Su** <weijia@iastate.edu> — *The impact of Terminal Inverted Repeat (TIR) elements in maize genomes*

P90 **Benjamin Oakes** <oakes.105@osu.edu> — *The role of RNA polymerase IV in effecting heritable regulatory changes*

P91 **Michelle Stitzer** <mcs368@cornell.edu> — *Transposable element accumulation reduces fitness in maize*

P92 **Sharu Paul Sharma** <sharu@iastate.edu> — *Transposon-induced inversions activate tissue-specific gene expression in maize*

P93 **Sarah Anderson** <sna@iastate.edu> — *Uncovering imprinting of PAV genes and transposable elements using whole genome assemblies*

P94 **Daniel Laspisa** <dlaspisa@hawaii.edu> — *Why CR2s are more prominent in domesticated maize than teosinte*

P95 **Lyudmila Sidorenko** <lyudmila.sidorenko@corteva.com> — *Zygosity-dependent transgene silencing in maize*

Biochemical and Molecular Genetics

P96 **Usha Bhatta** <ukb45206@uga.edu> — *Comparative genome sequence analysis of teosinte and maize to characterize resistance to the pathogen Ustilago maydis*

P97 **Debamalya Chatterjee** <djc5852@psu.edu> — *Increased expression of antifungal and insecticidal flavonoid phytoalexins in specialty maize lines*

P98 **Prameela Awale** <Prameela.Awale@jacks.sdstate.edu> — *Using antimorph Ae1-5180 as an alternative to ae1 to increase amylose content in maize endosperm*

P99 **Nicholas Morffy** <nmorffy@wustl.edu> — *A dominant mutation in the DNA-binding domain of ZmARF28 alters subcellular location and dimerization*

P100 **Sonja Blankenagel** <sonja.blankenagel@tum.de> — *ABA-mediated differences in drought-related traits in maize: Is ABA 8'-hydroxylase 4 the causal gene?*

P101 **Samuel Armstrong** <armstrsc@whitman.edu> — *Assessing the impact of mutated AUX/IAA proteins in maize inflorescence development*

P102 **Guo Chen** <252931257@qq.com> — *A mitochondrial PPR protein affects the editing of cox2 and nad2 and kernel development in maize*

P103 **Xiaojiao Hu** <huxiaojiao@caas.cn> — *Characterization and mapping of a cytochrome P450 mutant affecting plant architecture in maize*

P104 **Singha Dhungana** <srdm93@mail.missouri.edu> — *Characterization and mapping of the carbohydrate partitioning defective60 mutant in maize*

P105 **Ana Paula Alonso** <Anapaula.Alonso@unt.edu> — *Comparative metabolomics and fluxomics to identify steps limiting oil synthesis*

P106 **Eric Schmelz** <eschmelz@ucsd.edu> — *Connecting genotypes to molecular phenotypes: Metabolite-based genome-wide association studies and multi-omic co-regulation analyses drive gene annotation*

P107 **Erica Bien** <embien16@ole.augie.edu> — *Cross-talk between biochemical defense pathways in Arabidopsis thaliana*

P108 **Lauren Kelly** <lkkelly19@ole.augie.edu> — *Cross-talk between biochemical defense pathways in Arabidopsis thaliana*

P109 **Anna Block** <anna.block@usda.gov> — *Dedicated farnesyl diphosphate synthases in maize produce isoprenoids for growth or defense.*

P110 **Nathan Swyers** <ncs89f@mail.missouri.edu> — *Development of an amenable system for site-specific addition to a maize chromosome*

P111 **Ankita Abnave** <Ankita.Abnave@rockets.utoledo.edu>	*Discovering the gene regulatory networks that govern the phenylpropanoid pathway in maize*
P112 **Steve Moose** <smoose@illinois.edu>	*Discovery and genetic validation of regulatory variants for nitrogen-response traits in maize*
P113 **Michael Paulsmeyer** <paulsme2@illinois.edu>	*Discovery of Anthocyanin3: A negative regulator of the maize flavonoid pathway*
P114 **John Gray** <JOHN.GRAY5@UTOLEDO.EDU>	*Discovery of modules involved in the biosynthesis and regulation of maize phenolic compounds*
P115 **Binning Wu** <wu1515@purdue.edu>	*Enhanced flavan-4-ols corn diet protects intestinal barrier function in mice*
P116 **Gillian Wilkins** <gillian.wilkins@comcast.net>	*Evolution of the cardenolide pathway in Asclepias syriaca*
P117 **Max Blank** <blank@mpipz.mpg.de>	*Functional characterization of cis-regulatory variation underlying drought tolerance in maize*
P118 **Nathaniel Korth** <nate.korth@gmail.com>	*Genetic analysis of fiber biosynthesis in maize seed and impact on the human gut microbiome*
P119 **Alisa Huffaker** <ahuffaker@ucsd.edu>	*Genetic and biochemical deconvolution of pathways controlling maize immunity*
P120 **Xiaowen Shi** <shix@missouri.edu>	*Global impacts of genome imbalance on gene expression in maize aneuploids and polyploids*
P121 **Christina Finegan** <cfinegan@ufl.edu>	*Insight into sweet corn starch biosynthesis using gene expression analysis*
P122 **Amruta Bapat** <amruta03@iastate.edu>	*Insights into the genetic architecture of the ga1 locus in maize genotypes*
P123 **Manwinder Singh Brar** <mbrar@g.clemson.edu>	*Investigating the role of a Beta-glucosidase in the regulation of senescence in maize*
P124 **Jagveer Singh** <jagveer-coafs@pau.edu>	*Marker-assisted introgression of hydroxylase, lycopene-ε-cyclase and opaque2 favourable alleles for reconstitution of nutritionally enriched PMH1 and Buland maize hybrids*
P125 **Rachel Mertz** <mertzr@missouri.edu>	*Metabolomic analysis of maize nodal root growth under precisely controlled water deficit*
P126 **Keting Chen** <kchen@iastate.edu>	*Multi-omics dissection of cuticular lipid production on maize silks*
P127 **Zongkai Liu** <liuzk@cau.edu.cn>	*Mutation of ZmDMP enhances haploid induction in maize*
P128 **Hope Hersh** <hopehersh@ufl.edu>	*Predicting sugar and starch in mature sweet corn kernels*
P129 **Ling Zhou** <zlingxiaoyao@163.com>	*QTL mapping for maize starch content and candidate gene prediction combined with mutation analysis*
P130 **Jae-Hyung Lee** <jalee@cshl.edu>	*RAMOSA3 determines inflorescence branching and interacts with nuclear RNA binding proteins in maize*
P131 **Brianna Griffin** <bdg@iastate.edu>	*REL2 acetylation in plant pathogen interactions*
P132 **Mark Lubkowitz** <mlubkowitz@smcvt.edu>	*SWEETs and SUTs are regulated by distinct and overlapping transcription factors suggesting functional redundancy as well as gene specific functions in carbohydrate partitioning*
P133 **Olga Zimina** <oz32@cornell.edu>	*Single cell methylome analysis of maize meiocytes*
P134 **Norbert Bokros** <norbert.bokros@uky.edu>	*Sweet sorghum stalk strength oscillates in a diurnal pattern*

P135 **Michael Kolomiets** <kolomiets@tamu.edu>
Symbiont-induced systemic resistance to pathogens relies on oxylipins other than jasmonic acid for long distance signaling.

P136 **Rohit Kumar** <mohank@clemson.edu>
Systems genetic analysis of source-sink regulated senescence reveals novel candidate genes and pathways

P137 **Min Ge** <gemin8614@163.com>
The NIN-like protein 5 (ZmNLP5) transcription factor is involved in modulating the nitrogen response in maize

P138 **Charles Hunter** <charles.hunter@usda.gov>
The carotenoid-deficient albescent1 mutant results from lesions in tandemly-duplicated putative plastid terminal oxidase-coding genes

P139 **Rachel Mertz** <mertzr@missouri.edu>
The crossbanded maize mutant Carbohydrate partitioning defective4 exhibits attenuated sugar transport and partial phloem occlusion

P140 **Shaliesh Lal** <lal@oakland.edu>
The essential role of an RNA Binding Motif Protein 48 (RBM48) in U12 splicing is conserved between maize and humans

P141 **Carrie Olson-Manning** <colsonmanning@augie.edu>
The genomic and metabolic consequences of hybridization between two milkweed species (Asclepias)

P142 **Sarah Stein** <sjstein16@ole.augie.edu>
The genomic consequences of hybridization between two milkweed species (Asclepias)

P143 **Travis Hattery** <thattery@iastate.edu>
The silk cuticular lipid landscape of the Wisconsin Diversity Panel: Impacts of genotype, environment, and GxE interactions

P144 **Brian Dilkes** <bdilkes@purdue.edu>
Transcriptome remodeling by the hypersensitive response and associated regulatory variation in maize

P145 **Bharath Kunduru** <bkundur@clemson.edu>
Understanding the biomechanical and metabolic basis of stalk strength in maize using novel phenotyping and statistical modeling

P146 **Jennifer Arp** <jarp@danforthcenter.org>
Variation in C_4 photosynthetic pathways over the maize life cycle

P147 **Amanpreet Kaur** <kaur60@purdue.edu>
What's glutamate signaling got to do with maize plant architecture?

P148 **Xueli An** <xuelian@ustb.edu.cn>
"Idling effect": a working model for anther elongation arrest in ZmMs33 mutant

Cell and Developmental Biology

P149 **Xue Liu** <xueliu@waksman.rutgers.edu>
Adherent1 is a 3-Ketoacyl CoA synthase required for maize cuticle development and organ separation

P150 **Hailong Yang** <yangha16@students.ecu.edu>
Analysis of differential gene expression in the upper and lower meristems of maize

P151 **Matthew Wendt** <matthew.wendt@mail.wlc.edu>
Analysis of stunter2 and stunter3, maize maternal effect mutants with reduced kernel size

P152 **Daniel Blaine Marchant** <dbmarchant@gmail.com>
Beyond the protoplast: Isolating and analyzing fixed cells for single-cell research

P153 **Minjeong Kang** <mjkang@iastate.edu>
Building an academic/industry collaboration to improve transformation of recalcitrant maize inbreds

P154 **Brian Zebosi** <bzebosi@iastate.edu>
Characterization of brassinosteroid deficient semi-dwarf mutant1, modulating shoot architecture in maize

P155 **Aimee Uyehara** <auyeh002@ucr.edu>
Characterization of cytokinesis3 using bulked segregant analysis and live cell imaging

P156 **Thu Tran** <tran@cshl.edu>
Characterization of maize trehalose-6-phosphate synthases (ZmTPSs) and their functions in controlling inflorescence architecture

P157 **Yinan Jian** <jian@cshl.edu> — *Cloning an ear length QTL reveals ethylene as a developmental signal controlling kernel number in maize*

P158 **Katsutoshi Tsuda** <katsuda@nig.ac.jp> — *Control of subcellular localization of a KNOX transcription factor in rice.*

P159 **Masaharu Suzuki** <masaharu@ufl.edu> — *Dissecting genetic networks regulating embryo-endosperm relative size in maize*

P160 **Yutaka Sato** <yusato@nig.ac.jp> — *Dissecting maternal and zygotic control of biotin-dependent signaling required for epidermal cell fate specification in embryogenesis*

P161 **Joseph Gallagher** <jpg@umass.edu> — *Duplicate transcription factor genes GT1 and VRS1 repeatedly evolve roles in growth repression*

P162 **Xingli Li** <xingli.li@ur.de> — *Effects of heat stress during pollen development*

P163 **Edoardo Bertolini** <ebertolini@danforthcenter.org> — *Elucidating the regulatory networks underlying architectural pleiotropy between tassel branching and leaf angle*

P164 **Brad Nelms** <bnelms.research@gmail.com> — *Establishing the timing of haploid genome activation in pollen precursors using allele-specific single-cell RNA-seq*

P165 **Fang Bai** <fbai001@ufl.edu> — *Exploring genetic mechanisms repressing endosperm proliferation in maize*

P166 **Byoung Il Je** <bije@pusan.ac.kr> — *FCP1 peptide signaling is involved in leaf development in maize and SAM bifurcation in arabidopsis*

P167 **Janlo Robil** <jmrobil@mail.mizzou.edu> — *Genetic interactions of the maize large scutellar node1 (lsn1) mutant with auxin and other plant growth hormones*

P168 **Michelle Lang** <mlang1@iastate.edu> — *Identification of novel auxin pathway proteins regulating maize root development*

P169 **Nagesh Sardesai** <nagesh.sardesai@corteva.com> — *Improved maize leaf transformation using Babyboom (BBM) and Wuschel2 (WUS2)*

P170 **Armando McDonald** <armandm@uidaho.edu> — *Investigation the axial cell wall chemistry of two sorghum stalk varieties with high and low lignin contents*

P171 **Annis Richardson** <annis.richardson@ed.ac.uk> — *LIGULELESS2 and friends*

P172 **Michael Petersen** <mwpetersen@wisc.edu> — *Maize transformation and editing resources at the Wisconsin Crop Innovation Center – highlight on LH244*

P173 **Mika Nevo** <nevom@whitman.edu> — *Mapping and characterization of the tls4 mutant in maize reveals potential role for endocytosis in auxin-regulated tassel development*

P174 **Xinxin Ding** <xding4@wisc.edu> — *Microautophagy of storage proteins in maize aleurone cells*

P175 **Carolyn Rasmussen** <crasmu@ucr.edu> — *Microtubules at the cell cortex contribute to division plane positioning in plant cells.*

P176 **Brianne Conlon** <brc82@cornell.edu> — *NARROWSHEATH controls mediolateral outgrowth of lateral organs at the margins*

P177 **Xiaoguo Zhang** <xzhang653@wisc.edu> — *Reticulon 2 regulates endoplasmic reticulum (ER) turnover and represses ER stress in maize aleurone cells*

P178 **Johannes Scharwies** <joscha@stanford.edu> — *Root branching response to moisture gradients*

P179 **Katy Guthrie** <klgdn2@mail.missouri.edu> — *Sequencing and genetic characterization of the Suppressor of sessile spikelets semi-dominant mutants in maize*

P180 **Silas Miller** <millerst@whitman.edu> — *ShadowAuxin: Optimization of quenching FRET pairs for a fluorescent auxin biosensor*

P181 **Todd Jones** <todd.j.jones@corteva.com> — *Society for in vitro biology 2020 world congress on in vitro biology*

P182 **Susanne Matschi** <smatschi@ucsd.edu> — *Structure-function relationship of the bulliform cell cuticle in the leaf rolling response of maize*

P183 **Bibechana Adhikari** <bbchna@iastate.edu> — *Study of gene co-expression networks (GCNs) in drought stress response during early endosperm development in maize*

P184 **Norman Best** <bestn@missouri.edu> — *The lateral suppressor1 gene encodes a GRAS transcription factor required for axillary meristem development in maize*

P185 **Zhaobin Dong** <dongz@berkeley.edu> — *The tasselsheath4 gene establishes developmental fields within floral phytomers via microRNA mediated mutual repression*

P186 **Sarah Hake** <hake@berkeley.edu> — *The Liguleless Narrow1 RLK and Narrow Odd Dwarf, a plasma-membrane localized Ca++ signaling protein, act in overlapping pathways to regulate immunity and maize development*

P187 **Han Yu** <yuh@whitman.edu> — *The REL2 co-repressor enables recapitulation of the maize auxin response circuit in yeast*

P188 **Zhaoxia Li** <zhaoxial@iastate.edu> — *The alternative splicing in response to the increased daily temperature in maize*

P189 **Debamalya Chatterjee** <djc5852@psu.edu> — *The maize unstable factor for orange1 plays an important role in endosperm development*

P190 **Erin Sparks** <esparks@udel.edu> — *The molecular regulation of brace root development*

P191 **Tyler McCubbin** <tm284@mail.missouri.edu> — *Transcriptomic response of maize nodal root growth under precisely controlled water deficit*

P192 **Colleen Drapek** <colleen.drapek@slcu.cam.ac.uk> — *Understanding the role of gibberellic acid accumulation in endosymbiosis*

P193 **Brandon Beall** <bdbeall@iastate.edu> — *Verifying the molecular identity of a cell fate gene in maize endosperm*

Computational and Large-Scale Biology

P194 **Elly Poretsky** <eporetsky@ucsd.edu> — *mutRank: An R Shiny web-application for mutual rank-based coexpression analysis combined with tools for gene candidate prioritization*

P195 **Sidharth Sen** <ssz74@mail.missouri.edu> — *A multi-omics discriminatory analysis approach to identify drought related signatures in maize nodal roots*

P196 **Chunguang Du** <duc@montclair.edu> — *A webserver for helitron prediction and visualization*

P197 **Marcela Tello-Ruiz** <telloruiz@cshl.edu> — *Accessing the MaizeCODE data from Gramene and SciApps*

P198 **Dominic Mier** <ddmier@oakland.edu> — *Assessing the biological ramification of alternative splicing of U12-type introns on eukaryotic gene expression*

P199 **Samuel Leiboff** <sleiboff@berkeley.edu> — *Branchpoints in sex determination: Expression dynamics for making maize ears, tassels, and bisexual relatives*

P200 **Yinjie Qiu** <qiuxx221@umn.edu> — *Building a reference transcriptome for the hexaploid hard fescue turfgrass (Festuca Brevipila) using a combination of iso-seq and illumina sequencing*

P201 **Gun Ho Jung** <ideaway@korea.kr> — *Changes in growth, flavonoids and polyphenol contents according to sowing time of corn in Korea*

P202 **Rebecca Piri** <rdp22327@uga.edu> — *Characterizing knob structure and position in the maize pan genome*

P203 **Zhikai Liang** <liang795@umn.edu>
Cold-responsive gene prediction across Panicoid grasses

P204 **Arun Seetharam** <arnstrm@iastate.edu>
Consolidated approach for characterizing genomic structural variants across the maize NAM founders

P205 **Balreet Pawar** <pawarbk@whitman.edu>
Context-specific network analysis in maize using tissue-specific transcriptomics data

P206 **Jia Qian** <qianjiapingxu@126.com>
Dynamic patterns of protein-coding and noncoding elements across maize development in a maize F1 hybrid and its inbred parents

P207 **Alex Ferris** <acferris@stanford.edu>
Establishment of Ustilago maydis infection in maize anthers

P208 **Jianing Liu** <jl03308@uga.edu>
Gapless assembly of maize chromosomes using long read technologies

P209 **Dale Brunelle** <dale.brunelle@und.edu>
Genetic and bioinformatic analysis for gene identification of emb mutations

P210 **Guangchao Sun** <s.guangchao@gmail.com>
Genomic, metabolic, and transcriptomic responses of the extremophile grass Paspalum vaginatum to nutrient deficit stress

P211 **Marcin Grzybowski** <mgrzybowski2@unl.edu>
High-throughput hyperspectral imaging as a tool to explore natural diversity in biochemical traits over time in maize association panel

P212 **Yan Zhou** <yzhou86@iastate.edu>
Identification of genetic determinants of phenotyping errors in image-based high-throughput phenotyping

P213 **Katerina Holan** <holan2@iastate.edu>
Image-based quantification of Puccinia sorghi pustules in maize

P214 **Hao Wu** <haowu@iastate.edu>
Investigation of NKD1, NKD2 and OPAQUE2 interaction on gene network associated with maize endosperm development

P215 **Changsheng Li** <csli@sippe.ac.cn>
Long-read sequencing reveals genomic structural variations that underlie creation of Quality Protein Maize

P216 **Jack Gardiner** <jack.m.gardiner@gmail.com>
MaizeGDB's MaizeMine facilitates genomic data mining

P217 **Lisa Harper** <lisa.harper@usda.gov>
MaizeGDB: A history of our maize genetics research community

P218 **Ethalinda Cannon** <Ethy.Cannon@USDA.GOV>
MaizeGDB: Hosting a plethora of maize genomes at MaizeGDB including B73 RefGen_v5 and the NAM founder genomes

P219 **Andrea Gallavotti** <agallavotti@waksman.rutgers.edu>
Mapping and functional characterization of cis-regulatory variation in plants

P220 **Marcela Karey Tello-Ruiz** <telloruiz@cshl.edu>
Mining maize with Gramene

P221 **Donald McCarty** <drm@ufl.edu>
New from the UniformMu National Public Resource for maize genetics

P222 **Marilyn Warburton** <marilyn.warburton@ars.usda.gov>
PAST: The Pathway Association Study Tool for interpreting GWAS results in light of metabolic pathway data

P223 **Kelly Dawe** <kdawe@uga.edu>
Pangenome of the maize NAM founder inbreds

P224 **Alyssa Phillips** <arphillips@ucdavis.edu>
Persistence and evolution of polyploid complexes within a species

P225 **Zihao Zheng** <zhzheng@iastate.edu>
Prediction of phenotypic plasticity using root system architecture

P226 **Chenyong Miao** <cmiao@huskers.unl.edu>	*Semantic segmentation of sorghum and maize using hyperspectral data identifies genetic associations with variation in plant size*
P227 **Nancy Manchanda** <nancym@iastate.edu>	*Sequence, assembly and annotation of maize inbred B104: A maize transformation resource*
P228 **Stephen Piccolo** <stephen_piccolo@byu.edu>	*ShinyLearner: Enabling biologists to perform benchmark machine-learning classification algorithms*
P229 **Peter Bradbury** <pjb39@cornell.edu>	*Strategies for imputing genotypes from low coverage DNA sequence using the practical haplotype graph*
P230 **Zachary Turpin** <zmt11@my.fsu.edu>	*The NUPRIME project: Innovations in identifying candidate cis-regulatory elements from small particle MNase footprints*
P231 **Mackenzie Zwiener** <mzwiener3@unl.edu>	*The effects of nitrogen stress on leaf angle in sorghum and maize*
P232 **Miroslava Karafiátová** <karafiatova@ueb.cas.cz>	*The maize B-chromosome content*
P233 **Jan Bartoš** <bartos@ueb.cas.cz>	*The sequence of the mysterious B chromosome reveals some of its secrets*
P234 **Lauren Schulte** <lschulte@bio.fsu.edu>	*Tissue-specific transcriptional activity determined by iRNA-seq analysis*
P235 **Anju Giri** <ag2484@cornell.edu>	*Utilizing adaptive diversity in Tripsacum for improving freezing tolerance in maize*

Invited Speaker Abstracts

Invited Speaker 1 **Friday, March 13 11:35 AM**

Organogenesis in maize - lessons from mutants

(presented by Sarah Hake <hake@berkeley.edu>)
Full Author List: Richardson, Annis[1,2,3]; Lunde, China[2]; Johnson, Kjel; Abraham-Juarez, Jazmin[4]; Hake, Sarah[2,3].
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Positional information is a recurring theme in plant development, whether it is leaf initiation or ligule formation. Leaves initiate repetitively from the shoot apical meristem as divisions push cells into the morphogenetic zone. From the surface of the meristem, it is difficult to tell a leaf initial cell from cells that remain meristematic. Using molecular markers, we can see the down regulation of the transcription factor KNOTTED1 (KN1) in leaf initial cells and a concomitant increase in auxin activity. As the leaf grows away from the meristem, its base is marked by KN1 and tip reveals auxin activity. Each leaf initiation event includes the leaf, the subtending internode and an axillary bud. The leaf contains two distinct domains, the blade and sheath, separated by the ligule. Many mutants identified by Gerry Neuffer have been key in understanding these processes. Two dominant mutants have told us about leaf initiation. One, a mutant in an Aux/IAA gene, fails to make the leaf but still forms the internode. Other defects include lack of midrib and failure to form margins. The other mutant, in an ARF gene, makes extra leaves, also with midrib and margin defects. Proximal distal patterning is fine in both of these mutants. In contrast, another set of mutants affects proximal distal patterning but not leaf initiation. One of the original Kn1 alleles, with an rDt element in the 4th intron, was identified by Gerry Neuffer. It revealed that misexpressed of the meristem gene kn1 led to sheath and ligule tissue in the blade, supporting the idea that KN1 defines the basal region of the leaf in addition to a role in the meristem. Trying to learn more about positional information in the leaf, we have analyzed known mutants such as liguleless1 or lg2 and identified new mutants such as feminized upward narrow, narrow odd dwarf (nod), and the dominant Liguleless narrow (Lgn) mutant, first identified by Neuffer as a half-plant chimera. Lgn and nod reveal a link between ligule development and plant immunity.

Invited Speaker 2 **Saturday, March 15 11:30 AM**

How to tango with four: Meiotic adaptation to whole genome duplication

(presented by Kirsten Bomblies <kirsten.bomblies@biol.ethz.ch>)
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[1] ETH Zurich

Whole genome duplication (WGD) doubles the chromosome content and leads to the formation of polyploid organisms. Polyploids occur throughout the eukaryotic tree of life, but are especially common in plants. However, when the genome first duplicates, neopolyploids face serious challenges to reliable chromosome segregation in meiosis. Newly formed polyploids commonly show defects in chromosome segregation. To understand how regular chromosome segregation can evolve from such inauspicious beginnings, we use as our model system Arabidopsis arenosa, an outcrossing relative of A. thaliana with extant diploid and autotetraploid populations. Autotetraploid A. arenosa has cytologically diploidized meiosis, while newly generated tetraploids have problems in meiosis. We have shown from whole genome resequencing that genes encoding meiosis proteins show strong evidence of selection in the tetraploid lineage. We have begun functional follow-up on several genes. We show that the derived (tetraploid) alleles of several genes we discovered do affect polyploid meiosis in ways that promote stable chromosome segregation in the polyploid context. We hypothesize that these genes together represent a co-evolved polygenic solution to WGD-associated chromosome segregation challenges.

McClintock Prize Abstract

McClintock Prize **Friday, March 13 8:10 PM**

The Gene Balance Hypothesis: How regulatory gene stoichiometries affect expression, the phenotype and evolutionary processes

(presented by: James A. Birchler <BirchlerJ@missouri.edu>)
Author list: Birchler, James A.[1] and multiple collaborators
[1] University of Missouri, Columbia

Changes in chromosomal dosage were found to modulate gene expression in maize years ago. A dosage series of the long arm of chromosome 1 produced primarily an inverse correlation of selected gene expression across the genome. Experiments in Drosophila showed similar results. Single genes, primarily transcription factors, signal transduction components, and chromatin proteins, were identified that produced dosage effects on target genes. RNA-seq experiments in Drosophila and Arabidopsis indicated that global trans effects of trisomy can be varied but the most common deviation from normal is a reduced expression more or less within the inverse range (down to 0.67), although outlying peaks were also found that extend positively and negatively. Recent global studies of gene expression in a large set of aneuploids including monosomies, trisomies, tetrasomies and disomic haploids as well as multiple ploidy levels in maize revealed the spectrum of extensive modulations. Whole genome dosage changes (polyploidy) showed many fewer relative modulations suggesting a stoichiometric or balance component to gene regulation. Re-analysis of studies of gene expression in disomic haploids of yeast and trisomics of mice revealed the topology of gene expression modulations with the most common effect being a reduced expression in trans by hyperploidy. The studies in maize, Drosophila, and Arabidopsis indicate a connection between dosage compensation of the varied genes on aneuploid chromosomes and the trans-acting inverse effect, which has implications for the evolution of sex chromosomes. Duplicate genes have different evolutionary fates depending on the mode of duplication, i.e. the whole genome or segmental regions. The stoichiometric effects are postulated to explain this trend. The recognition of similar aneuploidy effects in monocots, dicots, fungi, insects, and mammals and their connection with dosage compensation suggests that these effects are a general reflection of an imbalance of regulatory gene products in the processes of gene expression.

Short Talk Abstracts

SESSION 1 – EXPRESSING THE GENOME
Chair: Hilde Nelissen Thursday, March 12. 7:00 PM – 9:00 PM

T1

Single-cell transcriptomic analysis of maize shoot apical meristem organization and cell differentiation

(submitted by James Satterlee <jws429@cornell.edu>)
Full Author List: Satterlee, James W.[1]; Strable, Joshua[1]; Scanlon, Michael J.[1]
[1] School of Integrative Plant Science, Cornell University; Ithaca, NY, United States 14853

The maize shoot apical meristem (SAM) gives rise to all of the above ground organs of the plant. To better understand the genetic and hormonal basis of SAM organization and cell differentiation, we took a single-cell transcriptomic approach to characterize in an unbiased fashion the gene expression profiles of cells from the maize SAM, as well as later stages of seedling shoot development. We first improved conditions to enhance the survival of protoplasts isolated from undifferentiated shoot apex tissue, through the addition of L-arginine and modification of the cell suspension pH. We profiled the expression of over 10,000 cells from the maize shoot apex, consisting of the meristem and the six most recently initiated leaf primordia (SAM + P6). To detect rare cells from the meristem we also targeted several hundred cells from SAM + P2 tissue. Using known markers, we identify additional stem cell markers with potential signaling, small RNA biogenesis, and DNA maintenance roles. Furthermore, we find co-expression of *FCP1* and *WOX9B*, two putative meristem regulators, in the same cells. We also propose a peripheral growth regulatory domain characterized by expression of the growth repression-associated gene DRM1 and the putative meristem regulators *GRAS32* and *GRAS33*, two *HAM3* paralogs. Indeed, *GRAS32* and *GRAS33* mutants confer a decrease in SAM height but not width in maize and *DRM1* expression is expanded in quiescent axillary meristems. Finally, we use trajectory inference to identify continuous changes in gene expression that accompany cell differentiation, progressing from the stem cell niche, through organ boundary domains, and into differentiating epidermal, ground, and vasculature tissue. The thousands of shared differentially expressed genes expressed along this trajectory in the SAM + P2 and SAM + P6 datasets suggests the iterative deployment of similar differentiation programs throughout maize seedling shoot development.

Gene / Gene Models described: *FCP1, WOX9B, DRM1, GRAS32, GRAS33*; GRMZM2G165836, GRMZM2G031882, GRMZM2G123896, GRMZM2G051785, GRMZM2G079470
Funding acknowledgement: National Science Foundation (NSF), Schmittau-Novak Small Grant Program, Cornell University

T2 @nmclark91

Generating multi-scale predictive networks of Northern Corn Leaf Blight resistance

(submitted by Natalie Clark <nmclark2@iastate.edu>)
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Northern Corn Leaf Blight (NLB) is one of the most significant corn diseases, causing the largest crop loss of any disease in the Northern United States from 2012-2015. Understanding how gene expression correlates with specific disease phenotypes is critical for developing disease-resistant crops that enhance global food security and limit economic losses. However, the underlying regulatory mechanisms mediating resistance to NLB are not well understood. Thus, the goal of this study is to integrate transcriptome and proteome measurements with disease phenotyping and computational modeling to build predictive regulatory networks of NLB resistance. To address this, 134 Intermated B73xMo17 Doubled Haploid Lines (IBMDHLs) were planted in a field plot using a randomized complete block design. Plants were treated with NLB inoculum at the sixth and seventh vegetative stages. Seven days after inoculation, leaf material was collected from 3 infected plants for each of the lines. Transcriptomic profiling on the infected plant samples was performed using 3' QuantSeq, and mass spectrometry was performed using 11-plex Tandem Mass Tag (TMT) peptide labeling. Using these methods, we were able to detect over 44,000 transcripts, 11,000 proteins, and 42,000 phosphosites. We then performed Quantitative Trait Loci (QTL) mapping on each of these molecular datasets as well as disease QTL mapping. We identified over 25,000 transcript, 5,000 protein, and 4,500 phosphosite QTL (eQTL, pQTL, and phosQTL, respectively), of which approximately 20% co-localize with disease QTL. In addition, only approximately 20% of pQTL and 10% of phosQTL overlap with eQTL, illustrating that the incorporation of proteomic data identifies complimentary regulatory events. We then inferred a network of the genes involved in these different QTLs using Spatiotemporal Clustering for Integrative Omics Networks (SCION: https://github.com/nmclark2/SCION). Together these data provide a comprehensive view of the gene regulatory landscape of corn leaves in response to NLB.

Funding acknowledgement: United States Department of Agriculture (USDA), Iowa State University Plant Sciences Institute

T3 @pete_crisp

DNA methylomes as a tool for functional annotation of genes and their regulatory regions

(submitted by Peter Crisp <p.crisp@uq.edu.au>)
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For all crops with large genomes it is challenging to distill the vast genomic space down to the genetic elements that are important for trait variation. The genes and their cis-regulatory elements (CREs) that determine phenotype comprise only a few percent of the total genome. We have become adept at sequencing and assembling genomes; however, significant challenges remain to refine annotations, including the identification of novel functional elements. Here we provide evidence that focusing on unmethylated regions, particularly in species with large genomes, can provide powerful information for identification of functional genes and CREs. Analysis of DNA methylation and accessible chromatin in six grass species identifies the subset of the genome that is unmethylated and/or accessible. Comparison of chromatin accessibility and DNA methylation reveals that virtually all accessible regions are unmethylated. However, there are many regions that are unmethylated but not accessible, at least in the leaf tissue used for profiling. Focusing on maize, through analysis of other tissues we find that regions with tissue-specific accessibility are unmethylated in all tissues studied. This suggests that the unmethylated intergenic regions that are identified in a single tissue (such as leaf) represent a near complete set of potential regulatory regions. Identifying these regions would normally require chromatin profiling of many cell types or tissues. These unmethylated regions that lack chromatin accessibility in leaf tissue are enriched for patterns of histone modifications, ChIP-seq binding sites, chromatin interactions and also show enhancer activity in reporter assays, suggesting potential functionality. In summary, we demonstrate the ability to predict thousands of novel candidate regulatory elements and functional genes, which are a powerful resource for understanding gene regulation and engineering new traits in the future.

Funding acknowledgement: National Science Foundation (NSF), Australian Research Council

T4

Rapid, Heat-Induced Transgenerational Reactivation of a Silenced Transposable Element in Maize

(submitted by Wei Guo <guo342@purdue.edu>)
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Transposons make up a substantial portion of most plant genomes. Due to their mutagenic potential, most of them are silenced. There is evidence that environmental stress can reverse this epigenetic silencing, but little is known about the mechanism for this reversal. Here, we use a minimal *Mutator* line that includes a naturally occurring variant of the *MuDR* transposon that can heritably trigger epigenetic silencing of that transposon. *MuDR* carries two genes, *mudrA* and *mudrB*, silencing of which is associated with distinct epigenetic pathways. We previously found that *Mediator of Paramutation1* (*Mop1*), a putative RNA-dependent RNA polymerase, is required for the maintenance of *mudrA* silencing. However, silenced *mudrA* is only progressively reactivated after multiple generations in a *mop1* mutant background. In contrast, *mudrB* never becomes reactivated in this background. We find that a complete loss of DNA methylation at *mudrA* in *mop1* does not result in an immediate reactivation of these genes, and that additional repressive histone marks appear to compensate for the loss of DNA methylation. Remarkably, we find that reactivation of *mudrA* can be dramatically accelerated upon heat stress, specifically during the early seedling stage, a process that involves an immediate reduction in the repressive histone marks. In contrast to previous observations, both *mudrA* and *mudrB* are reactivated in *mop1*. This active state is maintained throughout the life of the plant after the initial trigger has disappeared and is stably transmitted to subsequent generations, even when DNA methylation is restored in wild type progeny. Our results suggest that silencing of *mudrA* involves a balance between DNA methylation and histone modification, and that heat stress de-links DNA methylation from epigenetic silencing and can heritably reverse the effects of two independent silencing pathways.

Gene / Gene Models described: *mop1*; Zm00001d003378
Funding acknowledgement: National Science Foundation (NSF)

T5

FIND-CIS: an antibody-free, genome-wide method for high-resolution, *in vivo* mapping of functional *cis*-elements.

(submitted by Thomas Hartwig <thartwig@mpipz.mpg.de>)
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Genetic variants in *cis*-regulatory elements are major contributors to phenotype variation. Yet pinpointing causative regulatory variants remains challenging as classical methods often lack resolution. Here we present F1-mediated identification of non-coding *cis*-element-impacting SNPs (FIND-CIS) to identify, quantify, and genetically verify allele-specific (AS) functional variants. FIND-CIS combines a novel antibody-free, high-resolution transcription factor (TF) footprinting assay, the use of nested F1 hybrids, and a local association pipeline. We applied FIND-CIS and AS RNA-Seq to NAM hybrids to identify functional *cis*-elements responsive under drought conditions. FIND-CIS mapped 70-190k, 20-60 bp interaction footprints (FPs) per hybrid. FPs correlated with putative enhancer sites, TF targets identified via ChIP-Seq, and were often centered on a known TF motif. Within these FPs, we identified 14-25k SNPs per F1 which showed a significant allelic bias (AFPs). AFPs and AS expression of nearby genes were correlated, including known drought-responsive genes such as DREB TFs. Importantly, SNPs shared between hybrids also often shared their allelic bias, providing independent validation of the functional significance of *cis*-elements. Our study provides a robust method for genome-wide analysis of variants affecting *cis*-elements and identifies candidates for genome editing to potentially improve drought tolerance.

Funding acknowledgement: Alexander von Humboldt Stiftung

SESSION 2 – EMERGING TOOLS AND CHALLENGES
Chair: Thomas Slewinski Friday, March 13. 8:00 AM – 10:10 AM

T6
Abstract removed at the request of the author.

T7

Enhancing maize grain yield by CRISPR/Cas9 genomic editing of *CLE* (*CLAVATA3/Embryo-surrounding region*) genes for maize breeding

(submitted by Lei Liu <lliu@cshl.edu>)
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One of the most remarkable achievements of domestication and improvement during the past ~10,000 years was producing maize with enlarged ears to dramatically enhance grain productivity. Ear size is largely determined during early development when the inflorescence meristem (IM) proliferates and produces spikelets. Therefore, increase in IM activity is likely to generate larger ears with higher yield. Meristems are maintained by the well known CLAVATA–WUSCHEL feedback signaling pathways, where secreted CLE (CLAVATA3/EMBRYO SURROUNDING REGION) peptides function as signals in cell-cell communication, proliferation and differentiation. CRISPR null mutants of *Zmfcp1* and *Zmcle7*, *CLV3* homologs in maize, result in over-proliferation of the IM and fasciated ears with more kernel rows, but ears are shorter with less grain yield. However, weak alleles of fasciated-ear genes, *FEA2* and *FEA3*, can increase grain yield.

We therefore created weak alleles of *ZmCLE7* and *ZmFCP1* by promoter bashing using CRISPR/Cas9 with nine sgRNAs targeting each promoter. We found multiple alleles with promoter deletions and inversions, and some of them showed significantly enlarged but normal ears. The most striking allele, which had a 300bp deletion in the *ZmCLE7* promoter, led to a slight decrease of *ZmCLE7* expression, and increase of IM activity to generate a ~25% increase in grain yield in our lab strains of maize, by producing deeper kernels on a bigger ear, an effect that is higher than any other reported natural allele.

We also identified another maize *CLE*, *ZmCLE1E5*, with a similar expression pattern to *ZmCLE7* that is upregulated in *Zmcle7* mutants, suggesting a potentially redundant role in stem cell homeostasis. *ZmCLE1E5* CRISPR null alleles had significantly longer ears with more kernel rows. Our studies suggest that genomic editing can modulate fundamental stem cell pathways by targeting cis-regulatory or coding regions to significantly enhance complex yield traits. These favorable alleles could be immediately used not in only maize breeding but also *de novo* domestication of the wild ancestor teosinte to re-shape modern maize.

Gene / Gene Models described: *ZmCLE7, ZmFCP1, FEA2, FEA3*; GRMZM2G372364, Zm00001d003320, Zm00001d051012, Zm00001d040130

Funding acknowledgement: National Science Foundation (NSF)

T8

Protein expression and gene editing in maize using foxtail mosaic virus vectors

(submitted by Bliss Beernink <kernodle@iastate.edu>)
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Plant viruses are used to carry sequences that mediate rapid and transient induction of gene silencing or expression of heterologous proteins *in planta*. We engineered foxtail mosaic virus (FoMV) so it can be used for virus induced gene silencing (VIGS), virus-mediated protein overexpression (VOX), and most recently, to express a single guide RNA for gene editing in maize. FoMV is a single-stranded RNA virus that expresses its movement and capsid proteins from subgenomic messenger RNAs. To facilitate protein and single guide RNA expression, we constructed a recombinant FoMV that carries a duplicated coat protein promoter just upstream of the native coat protein promoter. The duplicated promoter is followed immediately by a multiple cloning site that enables the insertion of heterologous sequences, such as protein coding sequences and single guide RNAs. Protein expression using this recombinant FoMV was demonstrated using marker proteins such as green fluorescent protein and bialaphos resistance protein. Transient gene editing was demonstrated by expressing single guide RNAs from the virus in transgenic *Nicotiana benthamiana* and maize plants that constitutively express the Cas9 protein. Single guide RNAs expressed from the duplicated promoter mediated edits in the *N. benthamiana Phytoene desaturase (PDS)* gene and the maize *HKT1* gene encoding a potassium transporter. The efficiency of editing was enhanced in the presence of synergistic potyviruses, sugarcane mosaic virus in maize and turnip mosaic virus in *N. benthamiana*, and a viral silencing suppressor, HC-Pro. This work demonstrates the utility of FoMV for VIGS, VOX, and virus-enabled gene editing (VEdGE) in maize.

Funding acknowledgement: DARPA Insect Allies Program, Iowa State University Plant Sciences Institute

T9

Natural language processing of phenotype descriptions enables automated inference of biological relationships

(submitted by Ian Braun <irbraun@iastate.edu>)
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Natural language descriptions of plant phenotypes present in databases such as MaizeGDB and in the scientific literature are a rich source of information for biological research that seeks to untangle relationships between genes and observable phenotypes, such as plant health or size. The volume and unstructured nature of these text descriptions however necessitates a computational approach for leveraging them to predict gene-to-phenotype associations. We computationally translated descriptions of plant phenotypes into structured representations that can be processed to identify biologically meaningful associations. These representations include numerical vectors generated using neural networks and a variety of additional techniques applied from the domain of natural language processing (NLP), as well as representations constructed by automatically mapping text to terms from biological ontologies. We compared phenotype similarities derived from these automated techniques to those derived from manually curated data, and evaluated each approach on predictive tasks such as categorizing genes by functional group or biochemical pathway and predicting protein-protein interactions. Computationally derived representations were comparably successful in recapitulating biological truth to representations created through manual curation, indicating that it is now possible to computationally and automatically produce and populate large-scale information resources that enable researchers to query phenotypic descriptions directly. We present a dataset of computationally-inferred phenotype similarity networks, and discuss tools aimed at the research community for querying and visualizing this information.

Funding acknowledgement: National Science Foundation (NSF)

T10

Key steps towards the biofortification of sweet corn: Identifying genes important for improving vitamin and mineral levels in fresh kernels

(submitted by Matheus Baseggio <mb2446@cornell.edu>)
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Sweet corn is a highly consumed vegetable in the US but makes only a limited contribution to the daily intake of vitamins (A and E), antioxidants (tocochromanols and carotenoids), and minerals that would help in the prevention of health complications. Genome-wide association studies of six tocochromanols, seven carotenoids, and 15 minerals were conducted in a sweet corn inbred line association panel to elucidate the genetic basis of their levels in fresh kernels. Significant associations with likely causal genes were detected for α-tocopherol content (vte4), tocotrienol variation (hggt1 and vte1), β-carotene concentration (crtRB1), and the ratio of flux between the α- and β-carotene branches in the carotenoid biosynthetic pathway (lcyE). Two kernel starch synthesis genes, shrunken2 (sh2) and sugary1 (su1), were associated with tocotrienols and total carotenoids (endosperm traits), suggesting a link between increased sugar content in the endosperm and synthesis of isoprenoids. While the hggt1 and vte1 alleles for increased levels of tocotrienols were almost fixed in the sh2 lines, the crtRB1 haplotype and lycE allele for higher β-branch carotenoids were found at low frequencies among sh2 lines. For minerals, we identified plausible candidate genes associated with accumulation of iron and zinc (nas5), cadmium (hma3 and nramp), nickel (ptr2), and calcium (ras2). These and additional associations at novel loci are currently being investigated with gene expression profiling in fresh kernels. Through this quantitative genetic analysis and the moderate whole-genome predictive abilities for the majority of analyzed traits, our results represent a key step in the optimization of vitamins and minerals in fresh kernels of sweet corn for human health and nutrition.

Gene / Gene Models described: *vte1, vte4, lcyE, crtRB1, hggt1, hma3, nas5, nramp, ptr2, ras2*; GRMZM2G009785, GRMZM2G035213, GRMZM2G012966, GRMZM2G152135, GRMZM2G173358, GRMZM2G175576, GRMZM2G050108, GRMZM2G366919, GRMZM2G057611, GRMZM2G173878
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

SESSION 3 – INTERACTIONS WITH THE ENVIRONMENT 1
Chair: Yongrui Wu Friday, March 13. 10:30 AM – 11:25 AM

T11 @BethanFManley

Independent of Arbuscular Mycorrhizal Symbiosis: Positional cloning and characterisation of a novel arbuscular mycorrhizal mutant in *Zea mays*.

(submitted by Bethan Manley <bm502@cam.ac.uk>)
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Arbuscular mycorrhizal fungi from the *Glomeromycota* phylum form a beneficial symbiotic relationship with the root systems of a majority of plant species, including maize, enabling plants to profit from increased phosphate uptake efficiency. Further understanding of this interaction may prove vital in meeting the challenge of an increasing demand for phosphate supply in agriculture. Establishment of this symbiotic interaction relies on molecular crosstalk between the fungus and the plant roots in the rhizosphere, even before physical contact has occurred. A forward genetic screen has identified *independent of arbuscular mycorrhizal symbiosis (ina*), a maize mutant phenotype that displays a complete block in colonisation when inoculated with *Rhizophagus irregularis*. Positional cloning methods have revealed three candidate genes behind this phenotype, and extensive phenotyping has established that this maize mutant lacks the ability to signal to its symbiotic partner, abolishing the ability to promote their beneficial relationship. To identify the impact of the removal of this signal, a transcriptomics analysis of *Rhizophagus irregularis* spores during their early interaction with this *Zea mays* mutant has been carried out to further examine the extended phenotype of the loss of this plant gene.

Funding acknowledgement: BBSRC, Corteva

T12

Elucidating the genome-wide gene regulatory features of transcription factors (TFs) involved in the control of maize phenylpropanoid biosynthesis

(submitted by Yi-Hsuan Chu <chuyihsu@msu.edu>)
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Phenolic compounds, produced from the phenylpropanoid pathway, are important structural components of the cell wall and participate in the defense of maize to various abiotic stress conditions, as well as to protect against pathogens and herbivores. The knowledge on how the phenylpropanoid pathway is regulated is accumulating in maize and other plants, but genome-wide analyses of the interactions between key pathway regulators and their candidate target genes have yet to be determined. We implemented the recently-developed high-throughput in vitro technique, DNA affinity purification sequencing (DAP-seq), to establish the genome-wide occupancies of 45 TFs involved in the control of the phenylpropanoid pathway. Information on potential TF target genes, preferred TF binding motifs and the effect of DNA methylation on TF binding will be discussed in the context of promoter and distal regulatory elements associated with genomically accessible regions. These results provide new insights on the coordination between TFs and regulatory elements in controlling the transcriptional programs responsible for the accumulation of important plant compounds.

Gene / Gene Models described: *P1, KN1, RA2*; GRMZM2G084799, Zm00001d033859, Zm00001d039694
Funding acknowledgement: National Science Foundation (NSF)

SESSION 5 – THE GENES THAT MAKE MAIZE I
Chair: Andrea Eveland Friday, March 13. 4:40 PM – 6:00 PM

T13

The classic maize mutant *Rootless1* is a bHLH transcription factor that modulates crown root number in the field

(submitted by Adam Bray <abray@danforthcenter.org>)
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Crown roots emerge in whorls at the base of each leaf, starting belowground and continuing aboveground as characteristic "brace" roots that support the shoot. Crown roots comprise the bulk of the maize root system, and have been linked to numerous agronomically important consequences, including water and nitrogen uptake. Here we report the identification and characterization of the gene underlying the classic maize mutant *rootless1* (*rt1*), which is nearly devoid of crown roots. Nanopore sequencing and complementation tests with Ds mutant alleles allowed us to identify the Rt1 gene as a bHLH110 transcription factor. Rt1 overlaps crown root number (CRN) QTL from two studies, allowing the identification of alleles that increase and decrease CRN. The phenotype of the Ds mutants exhibit a reduction of only brace roots. The multiple alleles that modulate CRN demonstrate the quantitative variation in root system architecture controlled by Rt1. Histology of the coleoptilar node showed *rt1* functions in crown root initiation, with the mutant initiating fewer crown roots than wild-type. In-situ hybridizations of wildtype plants show diffuse expression in initiating crown root primordia, but a highly specific expression in the endodermal cell file in emerging primordia, suggesting roles in both initiation and development of crown roots. This expression pattern overlaps with Knotted1, but is distinct from Wox5. Analysis of staged developing primordium showed Rt1 expression is highest in bulging crown root primordia, suggesting it may also be involved with emergence via penetration of the stem. Ongoing experiments seek to identify regulatory targets of Rt1, as well as co-expression patterns with other key developmental regulators. The mounting body of evidence suggests that Rt1 is a major genetic regulator of crown roots in maize with a high level of allelic variation that could be selected for in a breeding program.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

T14

A tandem duplication of the maize *wushel1* gene promotes major architectural rearrangements in inflorescense meristem

(submitted by Zongliang Chen <zlchen@waksman.rutgers.edu>)
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The long-term maintenance of plant apical meristems relies on the accurate control of populations of stem cells which are ultimately responsible for the post-embryonic development and productivity of crop species. The molecular mechanism underlying this process is the well-known CLAVATA-WUSCHEL (CLV-WUS) pathway, a negative feedback-loop that controls the spatial expression of the homeobox *WUS* gene, an essential regulator of meristem size and a key morphogenetic factor. Here we report the characterization and cloning of the dominant *Barren inflorescence3* (*Bif3*) mutant, which shows an extreme proliferation of stem cells in inflorescence meristems. This stem cell over-proliferation eventually leads to the formation of ball-shaped ears and barren tassels. Using a combination of intragenic suppressor screens, CRISPR-Cas9 targeted editing and chromosome walking, we show that the *Bif3* phenotype is caused by a tandem duplication of a 16Kb DNA fragment, containing an extra copy of the *ZmWUS1* gene, which we refer to as *ZmWUS1-B*. This duplication event creates a novel chimeric promoter containing multimerized binding sites for type-B RESPONSE REGULATORs, key transcription factors functioning in cytokinin signaling. This novel promoter enhances *ZmWUS1-B* transcription, which is observed in a unique ring-like pattern in inflorescence meristems. The ectopic expression of *ZmWUS1-B* results in a major rearrangement of inflorescence meristem architecture and altered patterning, accompanied by mis-regulation of key components of the CLV-WUS as well as of various hormonal pathways. Furthermore, genetic and molecular interactions of *Bif3* with known regulators of meristem size show that the core CLV-WUS pathway is conserved in maize. Altogether, these findings answer long-standing questions regarding WUS function in maize and monocot species, and highlight a conserved mechanism for the regulation of *WUS* expression.

Funding acknowledgement: National Science Foundation (NSF)

T15

Gene duplications at the *Fascicled ear1* (*Fas1*) locus affect adaxial–abaxial cell fate in maize inflorescence meristems

(submitted by Yanfang Du <yanfangdu@webmail.hzau.edu.cn>)
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Plant meristems are self-renewing groups of pluripotent stem cells that produce lateral organs in a stereotypical pattern. Organ architecture is patterned by the meristem in various radial dimensions including central-peripheral and adaxial–abaxial axis. Adaxial–abaxial axis of lateral organs is thought to be equivalent to the central-peripheral axis of symmetric organs. However, the genetic basis that determines the differentiation of cells from the same meristem into adaxal or abaxial cells remains unknown. Both the male and female inflorescence meristems of the dominant *fascicled ear* (*Fas1*) mutant fail to grow as a single point and instead show deep branching or finger like structures. Positional cloning of two independent *Fas1* alleles identified a ~160 kb region containing the MADS-box gene *Zmm8* and the YABBY gene *DROOPING LEAF2* (*ZmDL2*). Both genes are duplicated and spatially misexpressed in the mutant inflorescence meristems. We hypothesize that this misexpression leads to suppression of the meristematic activity of the central cells and promotion of the meristematic activity of the peripheral cells, resulting in bifurcated inflorescences. Consistent with this hypothesis, RNA seq analysis reveals decreased expression levels of adaxially expressed *PHAB* and *WUSCHEL* homologs and increased expression levels of abaxially expressed *ASYMMETRIC LEAVES2* (*AS2*), *KANADI* and *YABBY* homologs. Our findings highlight the importance of strict spatiotemporal patterns of expression for both *Zmm8* and *ZmDL2* expression in inflorescence meristems and provide an example of the importance of polarity in organ formation. Moreover, we describe a pathway necessary for proper patterning of ears leading to internal cobs and external kernels.

Gene / Gene Models described: *Zmm8, ZmDL2*; Zm00001d048082, Zm00001d048083
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

T16

A dormancy regulatory module is recruited to suppress maize carpels

(submitted by Harry Klein <hrklein@umass.edu>)
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Growth suppression is essential in plant development. In maize, growth suppression is essential for regulating sexual differentiation, where carpels are suppressed in the male flowers of the tassel. Several mutants have been identified that have carpel suppression defects such as the *tasselseed* and *nana plant* mutants, but these mutants have other floral developmental defects, including reduced stamens and feminized glumes. The molecular mechanisms specifically controlling carpel suppression are largely unknown. Here, we describe a genetic interaction between two genes that specifically regulate carpel suppression in flowers. We discovered that *grassy tillers1* (*gt1*), which encodes a class 1 HD-ZIP transcription factor, and *ramosa3* (*ra3*), which encodes a trehalose-6-phosphate phosphatase, act together to suppress carpels in maize flowers. *gt1* mutants have partially derepressed carpels, derepressed axillary bud growth, and other vegetative growth defects. To identify genes acting with *gt1* to suppress carpels, we conducted an EMS enhancer screen on *gt1* mutants, and found *ra3* enhances the *gt1* phenotype. RNA-seq profiling of *gt1 ra3* double mutant tassels revealed misexpression of sugar signaling and hormone biosynthesis genes. Many differentially expressed genes overlapped with differentially expressed genes in *gt1* mutant tiller buds, suggesting that a bud dormancy program is acting to suppress maize carpels. Indeed, other experiments we have performed support a model for activation of a bud dormancy program. We propose that *gt1* and *ra3* act together to activate a bud dormancy program to suppress carpels.

Gene / Gene Models described: *ts1, ts2, na1, na2, gt1, ra3*; Zm00001d003533, Zm00001d028806, Zm00001d042843, Zm00001d014887, Zm00001d028129, Zm00001d022193
Funding acknowledgement: United States Department of Agriculture (USDA)

T17

The genetic and epigenetic contribution of TEs in shaping the maize genome

(submitted by Jaclyn Noshay <nosha003@umn.edu>)
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A majority (>64%) of the maize genome is comprised of transposable elements (TEs). While many TEs have no functional impact, the insertion of a subset of TEs may reshape the epigenome or gene expression patterns. A set of >60,000 polymorphic TE insertions among B73, Mo17, W22, and PH207 were utilized to study the effects of TEs on nearby chromatin and regulatory elements. Analysis of DNA methylation patterns revealed substantial variability flanking different TE families. This variability was found to be the result of differences in both insertion site preferences as well as post-insertion spreading of DNA methylation from TEs to flanking sequences. Recent studies have characterized thousands of accessible chromatin regions (ACRs) that represent putative DNA regulatory regions. The characterization of these regions relative to TEs revealed hundreds of examples of polymorphic TEs that disrupt ACRs as well as thousands of instances in which an ACR is located entirely within a TE. These findings highlight the potential for TEs to influence gene expression through both disruption and creation of regulatory elements for nearby genes. The comparison of gene expression levels for haplotypes with and without a TE insertion in several larger panels of genotypes revealed TE polymorphisms associated with variable gene expression. TEs that carry an ACR were found to be associated with higher expression of nearby genes demonstrating the potential for TEs to create novel regulatory elements. These analyses highlight the potential for TEs to rewire transcriptional responses and the complex interplay between TE presence and chromatin structure.

Funding acknowledgement: National Science Foundation (NSF)

T18

Characterizing the maize pan-genome and effects on phenotypic variation

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Structural variation has long been known to be an extensive component of the maize pan-genome. Recent advances in sequencing technologies and assembly algorithms have allowed us, for the first time, to characterize this variation across the entirety of the genome. Pacbio-based assemblies of the 26 NAM founder lines are allowing comparative genomic analyses typically conducted across species to be applied to characterize the extensive variation within maize. Across the NAM founder lines, we observe over a gigabase of sequence not contained within the B73 reference genome. These novel sequences, along with other structural variants including deletions, inversions, and translocations, explain phenotypic variation in the maize NAM population that is not captured by SNP variation alone. Across a broader set of 500 diverse inbred lines, we indeed observe a large number of structural variants that were in linkage disequilibrium with SNPs, but also many that are not "tagged" by such variation. We have also shown these structural variants drive dynamic gene expression patterns in inbreds and expression complementation in hybrids. Our current state of knowledge on the content of the maize pan-genome, impacts on expression dynamics, and ultimately phenotypic variation will be presented.

Funding acknowledgement: National Science Foundation (NSF) IOS-1744001 & IOS-1546727, United States Department of Agriculture (USDA) AFRI 2018-67013-27571

T19

Dissecting recombination landscape in maize using machine learning

(submitted by Minghui Wang <mw729@cornell.edu>)
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Meiotic recombination is essential for generating genetic diversity, which is the source of genome evolution, facilitates environmental adaption, and enables breeding. Despite their importance, recombination events are not evenly distributed along chromosomes. Numerous factors, including specific DNA sequence motifs, chromatin characteristics, and distance to chromosome landmarks, have been associated with locations of recombination hotspots. However, the actual impact of these features on recombination landscape is poorly elucidated. To investigate it, we examined 43 genomic and epigenomic features of recombination sites in maize using machine learning. We found that these features allowed identification of recombination sites with over 90% accuracy, with the Random Forest and Deep Learning algorithms performing better than other methods. Nearly 90% prediction accuracy was achieved using a combination of as few as three features: DNA methylation at CG and CHG sites (where H is any nucleotide other than G) and either H4K5 acetylation or nucleosome occupancy. This result suggests that most characteristics associated with recombination sites are redundant. Overall, we found that recombination takes place in a small fraction of the genome that exhibits features distinct from those of the genome at large. Our data point to a predominant role of epigenetic factors in determining recombination landscape in plants. The CO region characteristics exhibited conservation among diverse plant species. In contrast, CO site locations in the genome were mostly not conserved between maize and its close relatives. These data indicate preference of CO sites for faster evolving genome regions. We have also developed a deep learning-based algorithm to identify recombination hotspots semantically from genome sequence context alone. Our results indicate that accuracy of this approach is close to this including chromatin and genome features. These data imply that patterns of chromatin modification underlying recombination sites are closely aligned with the larger DNA sequence context of the location.

Funding acknowledgement: National Science Foundation (NSF)

T20

Maize hybrids show increased expression of plastid protein complexes and decreased expression of ethylene biosynthesis

(submitted by Devon Birdseye <dbirdsey@ucsd.edu>)
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Despite the importance of heterosis in agriculture, its molecular underpinnings have persisted as an unsolved classical problem in biology since its initial report by Charles Darwin. There are few instances of gene expression outside mid-parental range in hybrids, suggesting a post-transcriptional mechanism for hybrid vigor. We employed multiplexed tandem mass tag labeling to analyze the proteomes of green leaves of two maize inbred lines, B73 and Mo17, as well as their F_1 hybrid. The abundance of plastid protein complexes, consisting of subunits encoded by both the nucleus and the plastid, was elevated in the hybrid relative to mid-parent levels, which may account for the greater photosynthetic capacity of hybrids. This pattern was not reflected in RNA-seq data. Furthermore, Mo17 alleles were expressed above mid-parent, while B73 alleles were expressed below mid-parent, suggesting a trans-acting regulator of the proteome. Additionally, ethylene biosynthesis and ethylene-responsive proteins were expressed below mid-parent levels in the hybrid. An ethylene biosynthesis knock-down, *acs2/6*, partially phenocopied the proteome of the hybrid, indicating that a reduction in ethylene biosynthesis may be upstream of part of the hybrid molecular phenotype.

Gene / Gene Models described: *acs2, acs6*; Zm00001d002592, Zm00001d033862
Funding acknowledgement: National Science Foundation (NSF)

T21 @JinliangYang

Adaptive evolution of DNA methylation reshaped gene regulation in maize

(submitted by Jinliang Yang <jinliang.yang@unl.edu>)
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DNA methylation is a ubiquitous epigenetic feature. In maize, more than 40% of cytosines in the genome are methylated. Recently, significant progress has been made in describing the molecular mechanisms driving methylation, yet variation and the evolution of the methylation landscape during maize domestication remain mostly unknown. Here we leveraged whole-genome sequencing (WGS) and whole-genome bisulfite sequencing (WGBS) on populations of modern maize, landrace, and teosinte (Zea mays ssp. parviglumis) to investigate the adaptive and phenotypic consequences of methylation variation in maize. Using a novel estimation approach, we inferred the methylome site frequency spectrum (mSFS) to estimate forward and backward epimutation rates as well as the selection coefficient. We find only weak evidence for selection directly on the methylation state, but identified thousands of population-wide differentially methylated regions (DMRs) and that are enriched in regions showing population genetic evidence of recent selection. Further investigation revealed that DMRs are enriched in 5' untranslated regions, and that maize hypomethylated DMRs likely helped rewire distal gene regulations. For two trait-associated DMRs, vgt1-DMR and tb1-DMR, functional analyses suggested that these DMRs likely serve as cis-acting elements, affecting gene regulation after domestication. Our results enabled a better understanding of the evolutionary forces acting on patterns of DNA methylation and suggested the potential functions of methylation variations during maize domestication.

Funding acknowledgement: United States Department of Agriculture (USDA)

T22 @dangates_j

Fitness and environmental patterns in maize landraces identify beneficial alleles at single gene resolution

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A fundamental goal of evolutionary biology is to understand the genetic basis of adaptation. The loci that confer adaptation to various environments also hold great potential to improve and adapt crop breeding populations to changing climates. In our research we identify loci with patterns of local adaptation using multiple methods and demonstrate how this information complements experimental results designed to identify maize lines with superior adaptation to different stresses. We use data from over 2500 traditional maize landraces representing the breadth of genetic diversity of maize in Mexico to evaluate plant fitness in 13 common gardens across a range of environments to show patterns of local adaptation across. Using genome-wide genotyping-by-sequencing data, we then identify locally adapted loci based upon genotype by environment interactions across experiments conducted in different environments. We further identify genetic associations with environment across all of Mexico, Central, and South America. These environmentally associated loci are highly predictive of adaptive variation in yield and flowering time in our field trials, and we show that alleles associated with low precipitation environments also predict performance in drought trials. Our results indicate that the genetic variation necessary to adapt crops to changing climate exists in open pollinated landraces that have been subject to ongoing environmental adaptation and can be identified by both environmental associations and environmental stress trials. Furthermore, the high diversity and rate of linkage decay of landraces combined with the high marker density of modern genotyping means that GWAS approaches can deliver exceptional precision allowing identification of individual adaptive genes in landrace germplasm that can be used for targeted transfer into modern breeding populations.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

SESSION 8 – FROM GENOTYPE TO PHENOTYPE
Chair: Clint Whipple Saturday, March 14. 10:30 AM – 11:30 AM

T23

The *lateral suppressor1* gene encodes a GRAS transcription factor required for axillary meristem development in maize

(submitted by Norman Best <bestn@missouri.edu>)
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Axillary meristems in maize produce the tillers, ears, tassel branches, and spikelets necessary for plant development. A new mutant, *lateral suppressor1* (*las1*), was identified in an EMS population as a novel locus controlling axillary meristem development. The *las1* mutant fails to initiate ear branches, and minimally affects tassel development. To identify the molecular mutation responsible for the *las1* phenotype a next-generation sequencing and BSA approach was utilized to map the *las1* locus to bin 9.04. A single SNP was identified within the map window that induced a premature stop codon in a GRAS transcription factor. This SNP completely co-segregates with the *las1* phenotype across multiple generations and populations in over 2,000 individuals tested, providing evidence that this mutation is the cause of the *las1* phenotype. To further dissect the genetic pathway that the *las1* mutation affects, double mutants were analyzed between *las1* and other known axillary meristem mutants. The *teosinte branched1* (*tb1*) and *las1* double mutants had no ear branches and a reduced number of tillers indicating that *las1* functions in vegetative axillary meristem initiation or maintenance. To test if *las1* functions in reproductive axillary meristem development, double mutants between *las1* and *barren stalk* (*ba*) mutants were created. The *ba1* and *ba2* mutants fail to produce ear branches and decrease tassel branch and spikelet numbers. The *las1*/*ba2* double mutants exhibited a synergistic tassel phenotype indicating the *las1* functions in a separate but parallel pathway to *ba2* in tassel development. The *las1*/*ba1* double mutants were not significantly different from the weak single *ba1* mutant which suggests that *las1* functions in the *ba1* pathway. Taken together these results indicate that *las1* regulates both vegetative and reproductive axillary meristem development and adds a novel regulator to this developmental process.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

T24 @KviveManiStha

Uncovering the genetic architecture of protein bound amino acids in maize kernels using GWAS combined with gene co-expression network analysis

(submitted by Vivek Shrestha <vs6d9@mail.missouri.edu>)

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Seeds are a major source of protein in both human and livestock, however, seeds of major staple crops are deficient in several essential amino acids (EAA) which can lead to severe malnutrition, even if one's calories requirements are met. Many attempts to increase the EAA has demonstrated only limited success since seed can rebalance their amino acids composition even when major changes are introduced in their proteome. However despite the tight regulation within any given genotype seed amino acid composition display extensive natural variation. The latter can be utilized to uncover the genetic basis of the seed amino acids regulation and may identify new targets for seed amino acids biofortification. Hence we performed genome wide association study (GWAS) on 81 protein bound amino acids (PBAAs) – absolute levels, relative composition and derivative traits based on the amino acids metabolic pathways. Our analysis yielded 364 unique significant SNPs resulting in 1978 candidate genes from a 200kb window spanning the SNPs. To whittle down the candidate genes we have used an orthogonal dataset of a publicly available seed developmental gene coexpression dataset. Our underlying assumption was that genes that are part of the genetic architecture of the seed PBAA will likely to co-express in modules enriched in metabolic processes or protein related metabolism. We performed WGCNA with 1978 genes and did modules enrichment. The most significant enriched biological processes found were translation, cellular amino acid biosynthetic process and cellular respiration. Our results strongly suggests that the genetic variation in translational machinery itself can be involved in the natural variation of protein bound amino acid levels and composition. To date most seed amino acids biofortification relied on targeting metabolic pathway, TF or specific storage proteins. However our data suggests that new venue should be explored towards the manipulation of the translational machinery itself.

Funding acknowledgement: Missouri EPSCoR

T25 @sna8

Uncovering imprinting of PAV genes and transposable elements using whole genome assemblies

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Genomic imprinting, or parent-of-origin biased gene expression, has been reported in the endosperm of many plant species. Imprinted genes exhibit preferential expression of the maternal or paternal allele and are often associated with allele-specific variation for DNA methylation or histone modifications. While some genes have conserved imprinted expression patterns across plant species, there are also examples of genes with imprinting status that varies across different haplotypes within the same species, leading to the question of how genetic variation and epigenetic variation interact to influence gene expression patterning. Assessing how genomic imprinting arises has been limited by the technical difficulties associated with calling allele-specific expression using a single reference genome where only loci that are shared between genotypes and contain SNPs that distinguish alleles can be assayed. To overcome this limitation, we performed RNA-seq on reciprocal crossed hybrid endosperm between maize genotypes with complete, de-novo genome assemblies. By mapping to whole genome assemblies of both parents and comparing expression across reciprocal crosses, we were able to identify imprinted expression of both shared and non-shared portions of the genome. This allowed the identification of imprinting for several genes with present-absent variation (PAV) among genotypes. This approach also enabled the analysis of imprinting at transposable elements. We find > 100 imprinted transposable elements, over half of which show presence/absence variation in genome content between genomes. Interestingly, while genes show both maternally-biased and paternally-biased patterns of imprinting, the vast majority of imprinted transposable elements exhibit maternally-biased expression. By analyzing examples variable imprinting across genotypes, we are beginning to define the mechanisms that underlie parent-specific expression patterns in the endosperm.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Iowa State University

T26 @TomHughesPlants

SCR and *NKD* genes regulate leaf patterning during maize development

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In most cases, the C_4 photosynthetic pathway is underpinned by characteristic Kranz anatomy, with concentric wreaths of bundle sheath (BS) and mesophyll (M) cells surrounding closely spaced veins (V), resulting in a recurring V-BS-M-M-BS-V pattern. We have shown that in maize, this pattern is in part redundantly regulated by the duplicated orthologs of the Arabidopsis *SCARECROW (SCR)* gene. Both *ZmSCR1* and *ZmSCR1h* transcripts accumulate in ground meristem cells during leaf development. Consistent with this, *Zmscr1;Zmscr1h* mutants exhibit a reduction in ground meristem cell-divisions, resulting in a significant increase in vascular bundles with only one or sometimes no separating M cells. Furthermore, ectopic sclerenchyma develop both ad and abaxially relative to intermediate veins, in positions where M cells would normally form. During Arabidopsis root development, the SCR pathway is associated with the activity of a number of INDETERMINATE-DOMAIN (IDD) transcription factors. During maize leaf development, we have recently found that a transcriptional negative feedback loop occurs between the *SCR* genes and the *NAKED-ENDOSPERM (NKD)* IDD genes. *ZmNKD1* and *ZmNKD2* have previously been shown to redundantly regulate aleurone development, but *Zmnkd1;Zmnkd2* mutants exhibit normal leaf patterning. Quadruple *Zmscr1;Zmscr1h;Zmnkd1;Zmnkd2* mutants, however, exhibit more severe perturbations to leaf patterning than *Zmscr1;Zmscr1h* double mutants. In particular, there is a striking increase in the number of veins with no separating M cells, resulting in multiple 'fused' vascular bundles. Given the known interaction of IDD genes with SCR during Arabidopsis root development, we propose that a similar network involving SCR and NKD regulates maize leaf patterning.

Gene / Gene Models described: *ZmSCR1, ZmSCR1h, ZmNKD1, ZmNKD2*; GRMZM2G131516, GRMZM2G015080, GRMZM2G129261, GRMZM5G884137
Funding acknowledgement: Bill & Melinda Gates Foundation, Biotechnology and Biological Sciences Research Council

T27

Cloning and transcriptomic characterization of *macrohairless1*, a regulator of specialized cell fate commitment in the maize epidermis

(submitted by Nick Lauter <nick.lauter@usda.gov>)

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The maize leaf epidermis is a marvelous developmental genetic system because it is intricate, orderly, and dynamic. Macrohairs are particularly useful within this model because they are responsive to signals at the organism, organ, sub-organ, tissue and cellular levels. Paradoxically, they are the first of 14 epidermal cell types to exhibit fate commitment, but the last to complete morphogenesis. This occurs as the leaf descends from the whorl, and is marked by induration with silica and erection by non-cell autonomously recruited columnar cells. As glass spikes towering above the epidermis, macrohairs mitigate biotic and abiotic stresses by defending against herbivory, refracting light and retaining heat. While scores of genes affecting trichome initiation and morphogenesis have been cloned in Arabidopsis, the only cloned genes affecting maize trichomes act at higher levels, such as central-peripheral patterning and acquisition of adult identity. To gain a molecular entry point at the cell fate-commitment level in maize, we leveraged one weak and three null-mutant alleles to clone *macrohairless1*. Two null alleles abolish transcription, while the third causes key amino acid changes. The weak allele is expressed at WT levels, but contains a large in-frame deletion, suggesting partial function that is consistent with its' known ability to be suppressed by an additional genetic factor. Results from four transcriptomic experiments contrasting *macrohairless1* mutant versus WT plants show that components of GTPase signaling and cell-cycle regulation are strongly affected by loss of MHL1 protein. However, very little overlap with trichome initiation genes from Arabidopsis is observed, suggesting either a notable difference between monocot and dicot networks, or an unveiling of an intermediate layer of regulation. Finally, the complex relationship between macrohairs and bulliform cells will be discussed in the contexts of lateral confinement and neighbor inhibition patterning mechanisms.

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T28

Necrotic upper tips1 is a florally induced NAC transcription factor that promotes water movement by fortifying protoxylem cell walls

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When water deficits coincide with flowering in maize they result in severe developmental consequences such as leaf wilting, tassel browning and sterility, a condition known as "tassel blasting." Thus, during the floral transition special mechanisms must exist to promote sufficient water transport to the growing floral apex. In order to understand the genes underlying this process, we identified the *necrotic upper tips1* (*nut1*) mutant that mimics tassel blasting and shows signs of both heat and drought stress, despite being fully watered. Early vegetative development is normal, and the *nut1* phenotype is evident only after the floral transition. *nut1* stems have difficulty moving water as shown by dye uptake and movement assays, while the leaves show decreased relative water content. Plastic sections, cryofracture SEM and TEM of *nut1* vasculature revealed defects in protoxylem thickness and integrity. Later, these defects indirectly affect the metaxylem, leading to collapse of the vascular strand. The *nut1* mutant is caused by an *Activator* transposon insertion into a unique single copy NAC transcription factor gene. *nut1* is expressed only after the floral transition in the root, stem and leaf sheath, but not in meristematic tissue or leaf blade. Immunolocalization using a NUT1 specific antibody showed that the protein is specific to initiating protoxylem before it disappears upon maturation. Using DAP-seq, ChIP-qPCR and RNA-seq, NUT1 downstream targets were identified that function in secondary cell wall biosynthesis, apoptosis, and maintenance of cell wall thickness and strength. These results show that unique transcription factors function within the vasculature to maintain cell wall integrity during periods of high water movement. The existence of the *nut1* pathway demonstrates that heat and drought stress may share a common physiological mechanism, and thus this gene may be a useful breeding target to address such stresses in the future.

Funding acknowledgement: National Science Foundation (NSF)

T29

Growth hormones BR and GA modulate spikelet meristem identity in *Setaria viridis* through interface with the ortholog of maize determinacy factor, *RAMOSA 1*

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A major determinant of inflorescence architecture in cereals is timing of spikelet meristem (SM) identity, where an indeterminant axillary branch meristem acquires a determinate fate and terminates in a spikelet bearing pollen and/or grain. The degree of branching and position of spikelets are determined by this timing and impact grain yield and harvestability. In maize, classic mutants with defects in axillary meristem determinacy or identity, e.g., the *ramosa* (*ra*) mutants, show increased branching in tassels and ears, however little is known about their molecular mechanisms, which can be harnessed to fine-tune optimal inflorescence architectures. Here we use model grass *Setaria viridis* to dissect the mechanisms underlying SM identity and determinacy in maize. *Setaria* offers a unique system for studying this question since axillary branches terminate in either a spikelet or sterile bristle; the latter due to an imposed fate overriding SM identity. A mutagenesis screen isolated a collection of meristem identity mutants, including those producing only spikelets or bristles on the inflorescence. Mapping of causal loci indicated that brassinosteroids (BRs) and gibberellic acid (GA) were intimately involved in the spikelet/bristle fate decision, and treatment with exogenous GA or BR inhibitor produced inflorescences with all bristles or spikelets, respectively. Two mutants displayed homeotic conversions between organs: *bristleless 1* (*bsl1*) is defective in the ortholog of rice *D11*, a rate-limiting enzyme in BR biosynthesis, and *spikeletless* (*spkl*) was validated as the ortholog of maize *ra1*. Genetic analyses indicated *bsl1* was epistatic to *spkl*. We propose a model where SPKL/RA1 regulates SM identity genes through interface with BR and GA pathways. A maize mutant in *Zmd11/bsl1* showed comparable phenotypes and is being used to test genetic interactions in maize. Co-expression networks across developmental transitions and mutants in both species are leveraged to dissect conserved and divergent mechanisms modulating SM fate.

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T30

New insights into maize development using single-cell RNA sequencing (scRNA-seq)

(submitted by Xiaosa Xu <xxu@cshl.edu>)

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Different meristem types within the ear or root determine their architecture and maize productivity. An understanding of these meristems requires insights into developmental domains, and the gene networks required to specify them. However, these domains are classified only by morphology, or by insights from classical genetics, but this knowledge is limited by genetic redundancy and pleiotropy. Here, we investigated the transcriptional profiles of 15,000 single cells from B73 ear primordia across six replicates, and a similar number from roots. In ears, we detected expression from 28,000 genes, with an average of 5,600 transcripts detected per cell, and clustering identified 16 reproducible cell groups via Meta-analysis. The resulting ear development scRNA-seq atlas provides a detailed map of cellular identities from a maize CLAVATA3 (*ZmCLE7*) marked stem-cell cluster, a *KNOTTED1* marked L2 meristem cluster and a *YABBY* gene marked organ primordia cluster, among others. Importantly, we could map 76 of the 77 maize inflorescence development genes defined by mutant phenotype to specific cell clusters. Each cluster also contained an additional ~20-200 new candidate cell type or domain specific markers, and some were validated using *in situ* hybridization. We verified our findings using Fluorescence Activated Cell Sorting (FACS) of reporter lines, such as *pYABBY14-TagRFPt*, and found a highly significant overlap between genes enriched in FACS and in YABBY scRNA-seq clusters. Our resource can inform genetic analysis by accurately predicting genetic redundancy, such as finding a functional paralog of *RAMOSA3* (*RA3*), and aid in building co-expression networks at a single cell level, e.g. to identify transcriptional regulators of an ear length QTL. In summary, we developed a community resource identifying hundreds of novel candidate regulators of cell fate or development, in the maize inflorescence and root, to inform maize genetics at a fundamentally new level.

Gene / Gene Models described: *ZmCLE7, KNOTTED1, YABBY14, RAMOSA3*; GRMZM2G372364, GRMZM2G017087, GRMZM2G005353, GRMZM2G014729

Funding acknowledgement: National Science Foundation (NSF)

T31 @tjcteng

Dicer-like 5 deficiency confers temperature-sensitive male sterility in maize

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Small RNAs play important roles during plant development by regulating transcript levels of target mRNAs, maintaining genome integrity, and reinforcing DNA methylation. *Dicer-like 5* (*Dcl5*) is proposed to be responsible for precise slicing in many monocots to generate diverse 24-nt phased, secondary small interfering RNAs (phasiRNAs), which are exceptionally abundant in meiotic anthers of diverse flowering plants. The importance and functions of these phasiRNAs remain unclear. Here, we characterized several mutants of *dcl5*, including alleles generated by the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) *Cas9* system and a transposon-disrupted allele. We report that *dcl5* mutants have few or no 24-nt phasiRNAs, develop short anthers and defective tapetal cells, and exhibit temperature-sensitive male fertility. We propose that DCL5 and 24-nt phasiRNAs are critical for fertility under growth regimes for optimal yield.

Gene / Gene Models described: *Dcl5*; Zm00001d032655
Funding acknowledgement: National Science Foundation (NSF)

T32

Combinatorial stress causes extensive metabolic remodeling and divergent responses in maize defense chemistry

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The characterization and improved integration of stress-induced defense is important for maize production, as billions of bushels are lost each year due to nature-imposed damage. Important components of maize defense are small molecule natural products that regulate stress responses and directly counter imposing threats. While the identification and characterization of specific specialized metabolites has helped to elucidate important classes of defense molecules, there remains a great need for the global characterization of defense chemistry against abiotic and biotic stress, including variations of combinatorial stress that routinely challenge crop systems. In this study, we performed targeted and non-targeted metabolomics on maize exposed to individual and combinatorial stresses caused by heat, drought, and *Cochliobolus heterostrophus* infection and observed significant reorganizations of the maize metabolome. Comparative analyses of individual stresses demonstrated analytical distinction between *C. heterostrophus* infected tissues and the studied abiotic stresses, indicating minimal overlap between the differentially elicited metabolomes. Combinations of heat+*C. heterostrophus* inoculation displayed a ~2-fold increase in disease progression compared to inoculated controls. As a possible factor in the heat-induced susceptibility, the antimicrobial p-coumaric acid was significantly reduced in the heat+infection plants. In contrast, drought stress amplified maize defense responses to *C. heterostrophus*, demonstrating a positive relationship between the number of days drought stress prior to inoculation and the concentrations of defensive phytohormones, death acids, antimicrobials, amino acids, and other primary and secondary metabolites. Transcript accumulation of common maize defense genes was also positively and significantly affected by drought stress prior to inoculation. Collectively, these results reveal the complex nature of maize defense chemistry in plants under environmental stress and show how different abiotic stresses can have diverse effects on maize responses against microbial pathogens.

Funding acknowledgement: United States Department of Agriculture (USDA)

T33

Bioactive diterpenoids impact the composition of the root-associated microbiome in maize

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Maize, and other plants, deploy both primary and species-specific, secondary metabolites to communicate with other organisms and cope with environmental challenges. This includes interactions with soil-dwelling microbial communities, where plants may exchange sugars for important nutrients and protection against environmental perturbations, directly benefitting plant health. However, the molecular mechanisms that coordinate these plant-microbe interactions remain elusive. We report that maize specialized diterpenoid metabolites with known antifungal bioactivities also influence rhizosphere bacterial communities. We show that antibiotic dolabralexin diterpenoids highly abundant in roots of some maize varieties can be exuded from the roots. Comparative 16S rRNA gene sequencing determined the bacterial community composition of the maize mutant *Zman2* (*anther ear 2*), which is deficient in dolabralexins and closely related bioactive kauralexin diterpenoids. Under well-watered conditions, the *Zman2* rhizosphere microbiome differed significantly from the isogenic wild-type sibling with the most significant changes observed for Alphaproteobacteria of the order Sphingomonadales. By contrast, there was no difference in the microbiome composition between the mutant and wild-type was observed under drought stress. Metabolomics analyses support that these differences are attributed to the diterpenoid deficiency of the *Zman2* mutant, rather than other metabolome alterations. These findings suggest physiological functions of maize diterpenoids beyond known chemical defenses, including the assembly of the rhizosphere microbiome community.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Department of Energy (DOE)

T34

Teaching plant genetics with discovery microbiomics

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Lab courses routinely instruct students in a set of standardized and fairly robust techniques that will provide some knowledge as to how scientific research is performed, but does not yield any scientific discoveries during the course itself. In contrast, the fairly new and expanding field investigating plant::microbiome interactions provides the opportunity for students to learn techniques in both wet bench and computational science while concurrently making scientific discoveries that advance the field and are eminently publishable. We have chosen the interaction between soil microbes and maize or other grass roots as our study/learning system, and emphasize both microbial and plant genetics as the areas of focus. Despite challenges in the initial implementation of this strategy, the undergraduate students were already discovering and characterizing new microbial ecotypes and species by the end of the first semester that the class was taught. They went on to see how these communities interact with each other in community reconstruction experiments with the novel microbes they had cultured. In the second iteration of this course, we have expanded the research to include phage investigations, complex multipartite communities (e.g., maize, microbes, phage), and the specific study of biofilms on the maize root. Many of the maize root::microbe characteristics are both durable and different in B73, Mo17 and other inbreds, offering the opportunity to map the maize QTL/genes responsible for the interaction outcomes. The culturing and characterization (by whole genome sequencing, physiological tests and computational analyses performed in class) of a large cohort of microbes will allow future testing of specific plant genotype-by-microbe genotype interactions by these same students (or their peers) in subsequent independent research projects. Both the new biological discoveries in microbiomics and maize root biology made by these students and student opinions on the strategy and outcomes of the course will be presented.

Funding acknowledgement: DOE and Giles Professorship

T35

More than just the genes: Limitations for biosynthesis of the maize defense compound DIBOA in *Arabidopsis*

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Benzoxazinoids (BX, e.g. DIBOA) are defense metabolites mainly present in the grasses that confer broad range resistance against microbial pathogens and herbivores. The importance of BXs for protection was recognized early on and breeders selected lines containing high BX levels. The BX biosynthetic pathway has been elucidated and all genes are available. The pathway shares the intermediate indole with primary metabolism, i.e. tryptophan biosynthesis. Hence a connecting point for BX biosynthesis exists in all plants and an establishment as alien pathway should be feasible, thereby the plant's chemical defense should be strengthened. BX biosynthesis was successfully constituted in *Arabidopsis* by transfer of six maize *Bx* genes, however with low efficiency. Inefficient competition with primary metabolism and presence of toxic intermediates are the major bottlenecks in BX biosynthesis detected in *Arabidopsis*. In maize both obstructions might be overcome by generation of a multi-enzyme complex (metabolon) that positively influences the metabolic flux into and within the pathway. The components required for BX-metabolon formation in addition to the catalytic enzymes are unknown. Such structures are missing in *Arabidopsis* since a substantial leakage of intermediates of BX biosynthesis is observed in transgenics. Prominently the first pathway-specific metabolite indolin-2-one (ION) is detected. ION is a biological active compound and is modified as xenobiotic by plants in general. ION activates salicylic acid connected defense pathways in *Arabidopsis* with a trade-off in growth and fertility. Hence, evolutionary ION production will be subject to counter selection. Avoidance of deleterious amounts of ION seems to be essential in pathway establishment. Maize has a basal tolerance to ION that could have favored recruitment of the ION hydroxylase BX3 in evolution. The "brute force" transgenic expression of defense pathways may be limited in efficiency since evolutionary gained means to reduce toxicity and to preserve the balance of the metabolome are missing.

Gene / Gene Models described: *Bx3*; GRMZM2G167549
Funding acknowledgement: German Research Foundation (DFG) through SFB924, Federal Ministry of Education

T36

Convergent evolution on terpenoid metabolic pathways contributes to the protection of diverse crop genera

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Plants synthesize a diverse suite of specialized metabolites that provide protective and defensive roles against herbivores and pathogens. Many plant lineages produce hundreds of distinct terpenoids yet the knowledge of their existence, structural identities and biosynthetic pathways often remains hidden. To uncover additional maize defense pathways against fungal pathogens, metabolomic profiling was conducted on the fungal-elicited stems. Co-occurring within elicited maize stems are a diverse array of oxygenated selinene derivatives, zealexins, kuaralexins and the previously unreported metabolite α-santalen-12-oic acid. As key blends protecting Indian sandalwood trees (*Santalum album*), wild tomato (*Solanum habrochaites*) and many other plants, α-santalene derivatives have long been sought for potent activity against pathogens and herbivores. How α-santalene is made in maize and more generally α-santalen-12-oic acid is synthesized in plants remains unclear. To define the gene(s) responsible for maize α-santalen-12-oic acid biosynthesis, we utilized combined genetic mapping approaches including two NAM RIL populations and the Goodman diversity panel for GWAS to collectively identify terpene synthase 9 (*ZmTPS9*) as the gene responsible for the biosynthesis of α-santalene. Biochemical characterization in *E. coli* confirmed that ZmTPS9 produces α-santalene and β-bisabolene as the two major products. Further gene expression analysis and enzymatic assays revealed that three maize cytochrome (CYP) P450s (CYP71Z16/18/19) oxidize α-santalene to α-santalen-12-oic acid. In addition, a CYP71Z family P450 from wild tomato has the catalytic activity to produce α-santalen-12-oic acid. In vitro antimicrobial assays demonstrated that low yet physiologically relevant concentrations of α-santalen-12-oic acid strongly inhibited the growth of maize fungal pathogens. Our results support convergent evolution leading to α-santalen-12-oic acid biosynthesis contributes to plant defense in diverse crop genera.

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T37

Signaling along the pollen tube journey

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In the majority of plants including all grasses, pollen tubes carry two sperm cells as a passive cargo for fertilization. During their journey, pollen tubes have to overcome a number of prezygotic hybridization/speciation barriers including penetration of papilla hair cells/stigmata, growth towards and inside the transmitting tract, guidance inside the egg apparatus ultimately leading to pollen tube burst, gamete activation and double fertilization. Intensive cellular communication takes place along the pollen tube pathway to distinguish self from alien pollen tubes and to promote own and reject foreign tubes. Here, we will report unpublished data related to pollen tube invasion in papilla hair cell structures of maize and further signaling occurring during pollen tube growth. We discovered that ROS production, Ca^{2+} spiking and programmed cell death (PCD) is associated with pollen tube invasion in compatible interactions. Peptides of the Rapid Alkalinization Family (RALF) are secreted by pollen tubes and are perceived by receptors of the CrRLK1L-family to regulate tube growth and growth direction changes. Similarities and differences between pollen tube invasion in the maternal tissues of maize and Arabidopsis will be compared.

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T38

Maize auxin response circuits recapitulated in yeast

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The hormone auxin regulates myriad processes during the life of a plant - from root and shoot development to environmental responses. Although auxin signaling function has diverged and expanded, differences in the molecular functions of signaling components have largely been characterized in *Arabidopsis thaliana*. Recapitulation of minimal Arabidopsis nuclear auxin response circuits (ARCs) in yeast cells has enabled extensive characterization of the range of tunable auxin-induced signaling dynamics, revealing a central role for Auxin/Indole-3-Acetic Acid (Aux/IAA) repressor proteins in controlling the rate of developmental events *in planta*. We have now recapitulated the maize ARC in yeast in order to functionally annotate maize auxin signaling components, focusing on genes expressed during inflorescence development. We identified a subset of maize Aux/IAAs expressed in developing inflorescences and utilized the yeast system to reveal that these repressors: (1) degrade in response to auxin, (2) can repress AUXIN RESPONSE FACTOR (ARF) transcriptional activity with the assistance of the maize co-repressor protein RAMOSA1 ENHANCER LOCUS2 (REL2), and (3) when co-expressed with a maize auxin receptor exhibited a highly-sensitive auxin response. We have also begun testing how sensitive the ARC is to alterations in expression level of signaling components, with preliminary work showing nearly-identical responses across a range of Aux/IAA expression levels. Much of this work was carried out by more than 50 undergraduate students at Whitman college who participated in 4 different course-based undergraduate research experiences (CUREs) and summer internships. This work is providing new insights that inform our understanding of how auxin signaling modules are tuned in an important crop species, as well as providing substantial authentic research training in plant synthetic biology for a new generation of biologists.

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T39

Imaging Boron: Illuminating hidden aspects of root architecture in maize

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Boron (B) deficiency is one of the most widespread micronutrient deficiencies worldwide, leading to severe yield losses in maize. B is taken up by the plant as boric acid through the roots. The earliest phenotypic defects observed under B deficiency are in meristems, resulting in shorter primary roots and stunted vegetative growth. The mechanisms underlying these developmental defects are poorly understood in part because of technical challenges in visualizing B. Boric acid analogs, like phenylboronic acid (PBA), have previously been used to mimic B deficiency symptoms by interfering with B's function in crosslinking pectin subunits of the cell wall, however their effects on maize development have not been explored. We showed that germination assays using PBA and its derivative 4-Fluoro-PBA (FPBA) led to seedlings with shorter primary roots and fewer lateral roots, which are typical root phenotypes observed under B deficiency. We showed that the induced primary root length defects could be rescued by boric acid, providing evidence that this phenotype is the result of B deficiency. We hypothesized that FPBA could be used as a radiotracer for B binding sites in plants by incorporating the radioactive [^{18}F] fluoride. Treatment of maize seedlings with the synthesized [^{18}F] FPBA showed localization to the tip of the primary root and lateral root initiation sites. This study reports the first time that a B radiotracer has been developed in plants, based on a chemical that mimics B deficiency, opening up the possibility for visualizing other elements for which imaging has been challenging. The visualization of B binding sites in the maize root with a radiotracer is unprecedented, allowing for future mechanistic studies of the effect of B deficiency on meristems.

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Posters

P1

Accessory chromosomes in genus sorghum

(submitted by Nicolas Blavet <blavet@ueb.cas.cz>)
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Accessory chromosomes are non-essential elements presented in the genomes in addition to standard A-chromosome complement. Commonly, they are named "B-chromosomes" and have been described in many eukaryotes. They escaped from Mendelian inheritance, do not bring any advantage to individual, but in contrast can be harmful at some dosage level. Genus Sorghum belongs to the tribe Andropogoneae as maize, which is one of the model plants for B-chromosome studies. In Sorghum, presence of B-chromosome has been reported previously in five species. Here, we present the first study of B-chromosome in this genera utilizing molecular approaches and NGS. We found B positive individuals in anonymous population of Sorghum purpureosericeum (2n=2x=10). As there is a strong tissue specific elimination of Bs from roots, leaves and stems in this species, the anthers were always used as a material for screening. Pollen nuclei from flowering plants were analysed using the flow cytometry and in selected individuals B presence was confirmed at meiotic level. Population of both B+ and B- nuclei from the same accession were flow sorted and sequenced using Illumina. The comparative repeat analysis (using RepeatExplorer) revealed a few candidate clusters, from whose B specific markers were derived. Further, based on repeats we developed a robust cytogenetic marker, which allow us to visualize B-chromosome in situ.

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P2

Comprehensive analysis of gene expression from genomic imbalance

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The phenotypic consequence of addition or subtraction of part of a chromosome is more severe than changing the whole genome. This relationship is referred to as genomic balance. Previous genomic balance research in maize focused only on protein and mRNA expression for a small number of genes, but the gene dosage effects at the global level are still unknown. Our laboratory has collected a nearly complete set of translocations between various chromosome arms and the supernumerary B chromosome of maize. To investigate how an extra chromosomal region affects gene expression at the haploid level, we first identified haploids by crossing female hyperploid heterozygotes, which contain 19 A, 1 AB and 2 BA chromosomes, to a haploid inducer line. Karyotyping by fluorescence in situ hybridization (FISH) was used to distinguish haploids with extra chromosome arms (disomy) from euploid haploids (euploid). Consistent with previous results, disomic plants grew much slower and were shorter than the euploids. Next, comparison of DNA sequences obtained from disomy and W22 haploid leaf tissue allowed the identification of breakpoints on the A chromosomes in the different translocations. Based on RNA sequencing of disomies and euploids, the expression levels of most genes located on the varied chromosome arms (cis) in disomies were proportional to chromosome dosage, while a notable subset of cis genes showed dosage compensation with no expression changes regardless of chromosome dosage. For genes not located on the varied chromosome arm (trans), ratio distribution and scatter plot analysis showed that most disomies had a generalized down regulation but TB-4Sa, TB-7Lb and TB-9Sd had a generalized upregulation with TB-9Lc and TB-10L19 showing a mixture of up and down regulation. Future work aims to determine which genes in cis are responsible for expression changes in trans genes, and how genomic balance affects small RNA expression.

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P3

Exploring higher-order chromosome organization patterns by detecting meiotic axis protein attachment sites using ChIP-seq

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Maize, like other organisms relies on genetic variation to adapt to changing environment. A major source of genetic variation in eukaryotes is homologous recombination in meiosis, a process in which parental chromosomes exchange parts with each other. During this process, a protein structure forms the axis between homologous chromosomes (two pairs of sister chromatids) and mediate synapsis and recombination. This DNA-protein structure, called the synaptonemal complex (SC), starts by the installation of chromosome axis proteins whose key members include ASY1 and ZYP1. Mutation in axis proteins in other organisms result in meiotic defects including reduction in crossover formation and illegitimate recombination. However, the mechanism of how SC axis proteins affect recombination is not well understood. We therefore conducted a ChIP-seq of maize anthers at Zygotene of meiosis prophase I with antibodies against ASY1 and ZYP1 to map the installation of these proteins in the genome and gain insights as to how their distribution might affect recombination. Antibodies against both proteins were produced and validated in immunolocalization experiments. Here, we report the results of ASY1 and ZYP1 ChIP-seq experiments and present data on their distribution along the maize genome. The implication of their distribution on the DSB and recombination landscape is also discussed. The results allow us to examine how DSB and recombination hotspots, as well as crossover landscape might be influenced by higher order chromosome organization found in synaptonemal complexes.

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P4

Gene structure and origin of maize abnormal chromosome 10

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The maize abnormal chromosome 10 (Ab10) haplotype demonstrates meiotic drive, and as a result has played a significant role in maize evolution. The haplotype is characterized by a long arm that is ~1.3x the length of the normal chromosome 10 (N10) long arm, but the origin of this additional sequence is unknown. We are identifying the gene structure of the Ab10 unique regions through the study of a series of mutant Ab10 haplotype bearing lines. We are using a comparative genomics approach to identify homologous genes and/or syntenic regions in an effort to make inferences about the origin of the Ab10 haplotype. Understanding the evolutionary history of the Ab10 haplotype will provide a fuller understanding of how modern maize came to be and the way that meiotic drive systems develop and are maintained.

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P5

Identification and characterization of ZWINT-1 as an important protein in the maize cell division process

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The kinetochore complex plays a crucial roles in centromere assembly, chromosome orientation and segregation to ensure proper cell division, which is required for the genomic stability in all eukaryotes. ZWINT-1, kinetochore protein that connects the outer KMN network and the RZZ complex, has been identified to function during the cell cycle in human cells and yeast. However, the function and relationship between the ZWINT-1 and other kinetochore proteins remain unclear in flowering plants.Here, we identified a maize ZWINT-1 homolog, which displays a high expression level in growing tissue.Yeast two-hybrid analysis shows that the N-terminal domain of maize ZWINT-1 interacts with the C-terminal domain of KNL1 protein, which isthe sameas in human cells. Cytogenetic observation in maize meiosis with a specific anti-ZWINT-1 antibody reveals that it localizes to centromeric regions in metaphase. Functional study with CRISPR-Cas9 technology produced maize lines with mutations in ZWINT-1. They exhibited a slow growth phenotype compared to wild type at the callus stage. Further study of chromosome behavior during cell division with these lines will shed light on the role of ZWINT-1 in maize.

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P6

Maize centromere epigenetics in oat-maize addition lines

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Stably inherited alien maize chromosomes in oat-maize addition (OmA) lines can be produced by hybridizing maize and oat. Immunofluorescence shows that oat CENH3 can load on maize centromere regions in OmA. The mechanism how oat CENH3 can load on maize centromeres and the changes at maize centromeres in OmA is of interest. We found that there are similar sequences in oat to centromeric retrotransposon of maize (CRM), although the transcription of CRM in OmA is different between maize and oat. By analyzing anti-CENH3 ChIP-seq data in OmA, we found that oat CENH3 could bind on different portions of CRM 1 similar sequences. In addition, we identified a long terminal repeat (LTR) retrotransposon of oat centromere and two C genome (of hexaploidy oat) specific satellites by fluorescence in situ hybridization. Moreover, abnormal meiotic behaviors of oat chromosomes in OmA were observed. In maize, circular RNAs produced by CRM 1 can bind on the chromatin DNA, which is required for CENH3 localization. Similar-sized circular RNA of OmA was also observed by atomic force microscopy, suggesting potential mechanisms of adaptation of maize chromosomes in OmA.

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P7

R-loops are enriched in maize centromeric region

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R-loops are stable chromatin structures comprising an RNA:DNA hybrid and a displaced single-stranded DNA. R-loops have been implicated in gene expression and transcriptional termination, as well as in replication blocks and genome instability. Here, we use the ssDRIP-seq technique for genome-wide identification of R-loops in maize. We found R-loops formation strongly correlated with both GC and AT skews. Antisense R-loops sharply peak around transcription start sites (TSSs), while sense R-loops are enriched around transcription end sites (TESs), and these peaks show positive correlation with gene expression. R-loops are prevalent in repetitive genomic regions, such as 45S ribosomal DNA (rDNA), 5S rDNA and the centromere. Centromeric retrotransposons (CRMs) are strongly associated with R-loop formation. However, the R-loops of CRM1 elements are mainly formed in the 3'LTR while R-loops of CRM2 are formed in 5'LTR. Furthermore, we identified the nuclearly localized R-loop removing enzyme ZmRNaseH. Over expression of ZmRNaseH leads to a global reduction of R-loops and strongly affects maize development. Our date indicate that R-loops are common features in the maize genome and centromeric R-loops may play important roles in centromere function.

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P8

Shaping meiotic recombination landscape trough DNA methylation changes in maize.

(submitted by Mateusz Zelkowski <mz548@cornell.edu>)
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Meiotic crossovers (COs) have a fundamental role in creating new genotypes that may exhibit novel trait combinations. However, large segments of maize chromosomes have limited CO formation, complicating the breeding process. In plants, CO locations exhibit reduced DNA methylation levels. Hence, we are examining the role of DNA methylation in shaping the CO landscape in maize. To do this, we are studying mutants defective in *zmet2*, a gene encoding a chromomethyltransferase, and *ddm1*, which encodes a chromatin remodeling factor. Mutations in *zmet2* result in a reduction of DNA methylation levels at CHG and CHH sites (where H is any nucleotide other G), whereas mutating *ddm1* affects all three types of methylation sites, CG, CHG, and CHH. Maize has two redundant copies of *ddm1* but homozygous double mutants are embryo lethal. This situation forces us to use a homozygous/heterozygous combination *ddm1-1*/+ *ddm1-2*/*ddm1-2* to study the role of the *ddm1* gene in meiosis. In both the zmet2 and *ddm1* mutants, we found increases CO numbers, by 10% in *ddm1* and by 50% in *zmet2*, as well as altered CO localization patterns, compared to wild type. *ddm1* mutants displayed a CO shift towards chromosome arms, whereas mutating *zmet2* resulted in more COs being formed in pericentromeric regions of chromosomes. We also examined formation of double strand breaks (DSBs) in chromosomal DNA, which initiate meiotic recombination. The numbers of meiotic DSBs, as detected by the presence of chromosomal foci of RAD51, a protein involved in DSB repair, were in both mutants similar to those of wild type. Surprisingly, in the *zmet2* mutant, we observed a shift of DSB locations from pericentromeric regions towards chromosome arms and from transposable elements towards genic regions, compared to the patterns in wild type. Overall, our studies show that altering DNA methylation levels in maize results in complex changes in recombination landscape.

Gene / Gene Models described: *Ddm1-1; Ddm1-2; zMet2*; GRMZM2G177165; GRMZM2G071025; GRMZM2G025592
Funding acknowledgement: National Science Foundation (NSF)

P9

Confronting plant blindness in undergraduate students through authentic research experiences

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The term "plant blindness" describes the lack of students' interest in working with plants. Most undergraduate students pursuing Biology degrees in small, predominantly teaching, liberal arts institutions are interested in health-related careers. They want to learn about human-oriented models and investigate problems related to human health. As this tendency is partially due to the inherent properties of the human brain, intentional efforts are needed to bring students to the world of plant research. Here, we present a series of different modules of authentic research experiences focused on investigating plant response to abiotic stress in maize seedlings. These multi-week modules, incorporating investigating gene expression, quantitative genetics, gene mapping, and phenotypic analysis, were implemented in various courses and were demonstrated to increase students' awareness of and interest in plant research. This poster will discuss the approaches to successful implementation of these course-embedded research experiences, as well as barriers and challenges to sustaining them in the settings of primarily undergraduate institutions.

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P10

Finding and fixing annotation errors through community curation

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High-quality gene models with accurate gene structure are essential for scientists to design experiments targeting specific genes. Traditionally, validating those gene models requires manual curation. This is a labor-intensive and time-consuming process in which one or a few individuals evaluate and correct computational predictions using all available experimental evidence.Before manual curation, automated gene finders identify the parts of a sequence that encode genes, regulatory sequences, and repetitive elements. These pipelines are becoming faster as the algorithms improve, and are more accurate as they are able to incorporate more biological data to use in their predictions. But they still make errors (think fused or split genes). In a pilot study (Tello-Ruiz et al, 2019), we tested two distinct approaches to identify mispredictions from automated gene finders using the quality values generated by the MAKER-P gene annotation pipeline, and the alignments between translated protein sequences and its homologs across species using the Gramene gene tree visualizer. During our first maize annotation jamboree (CSHL, Nov. 2017), we selected a subset of B73_RefV4 maize genes for curation. We showed that all the gene models analyzed using both methods had annotation errors, predominantly different exon length, missing or extra exons and UTRs. A group of eight students had the opportunity to correct the errors using the Apollo gene editor, and support the community curation of the maize reference genome. Ideally, this method could be used as a means to enable students and citizen scientists to participate in the annotation of any sequenced eukaryotic genome.To date, we have organized three maize annotation jamborees, and plan to hold one more prior to the 2020 Maize Genetics Meeting. The implementation of these approaches was successful when piloted with eighteen sophomore-level undergraduate students in an Honors Genetics course at Middle Tennessee State University and will be discussed.

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P11

Incorporating maize landraces into undergraduate research and education

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Landraces are regionally adapted varieties with distinctive characteristics that have been maintained for generations. Maize landraces are an important source of genetic diversity. However, many important maize landraces remain rarely studied. The purpose of this project was to incorporate South and Central American maize landraces into an undergraduate Plant Science program. The objectives of this research were to: 1) Utilize maize landraces for individual undergraduate student research projects 2) Incorporate maize landraces into Plant Science courses and 3) Evaluate adaptability of South and Central American maize landraces to the Mid-Atlantic U.S. region. A total of 65 different maize landraces from South and Central America and seven North American inbreds and heirlooms were grown in two replications in a randomized complete block design. Observations on maturity, plant and ear height, and lodging were taken as part of an undergraduate student research project. In addition, the collection was also incorporated into an undergraduate Plant Pathology course for a corn disease diagnosis assignment. Although lodging and effects on maturity were observed, the results indicated that the majority of South and Central American landraces could be grown in the Mid-Atlantic U.S. and used for undergraduate student projects and classes. This research will be repeated next summer with additional landraces.

P12

Managing and distributing maize diversity: The NPGS maize collection in Ames, IA

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In addition to the Maize Genetic Stocks collection held in Urbana, IL, the USDA National Plant Germplasm System (NPGS) includes a collection of more than 20,000 accessions of cultivated temperate- and tropical-adapted maize and wild relative genetic resources from around the world. This collection is held at the North Central Regional Plant Introduction Station (NCRPIS) in Ames, Iowa and is backed up in Fort Collins, Colorado. Approximately 75% of the collection (14,922 accessions) is available for distribution. Seed viability is monitored on a 10-15 year cycle. When viability drops below 50%, or if the kernel inventory falls below 1000 seeds, an accession becomes unavailable for distribution until it can be regenerated. Temperate-adapted material is typically regenerated in Ames, Iowa. Nurseries provided by collaborators and contractors in the continental US, Saint Croix, Puerto Rico, and Mexico are used to regenerate non-temperate material from unique environments. Seed regeneration is costly and is carefully managed to ensure that genetic integrity preserved. It is also an opportunity to gather observations. GRIN-Global, the germplasm database of the NPGS, currently holds 301,785 trait observations on 16,882 maize accessions, and 14,981 ear, kernel, and cob images on 6,307 accessions, in addition to accession passport and provenance data. Germplasm requests can be made through the NPGS GRIN-Global public website: https://npgsweb.ars-grin.gov/gringlobal/search.aspx. In 2019, NCRPIS distributed more than 20,000 packets of maize to requestors around the world.

Funding acknowledgement: United States Department of Agriculture (USDA)

P13

Producing tropical teosinte seed in a temperate environment

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The USDA National Plant Germplasm System's maize collection held in Ames, IA includes 439 wild accessions from the genus *Zea*. Currently, only 17% (74 accessions) are available for distribution. Regenerating maize wild relatives native to Mexico and Central America in Iowa is difficult because 1) short days are necessary to induce flowering, 2) their plant physiology is not amenable to bagging inflorescences so isolation from other teosintes and cultivated maize is required during flowering, and 3) 100+ plants are needed to avoid imposing a genetic bottleneck. By incorporating a growth regulator into our regeneration protocol, we are able to produce plants that can be more easily moved between the field and the greenhouse. For example, in December 2019 73,532 seeds were harvested from 134 plants of *Zea luxurians* (Durieu & Asch.) Bird (PI 441933). For this accession, 250 seeds were germinated in a growth chamber in June 2019, then transplanted to pots in the greenhouse. Following seedling establishment the pots were moved outside to grow vegetatively during the long summer days (up to 15 hours) in Ames, Iowa. Bonzi, a growth regulator, was applied mid-summer to promote tillering and reduce overall plant height. As the fall frost date approached and daylength shortened, pots were moved back to the greenhouse and LED lights were set to promote flowering. After flowering was initiated, fans were used to create air movement and plants were agitated daily to facilitate pollen spread. Seed was harvested daily with a handheld vacuum. Plants were threshed by hand after they had dried to ensure all seed had been collected. Seed was cleaned using a seed blower, fractionator, and optical sorter. With this protocol established, we are able to reliably regenerate at least one accession each year.

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P14

A High-throughput non-destructive phenotyping device for detecting disease and pest damage in maize

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Disease and pest damage in maize stalks are major causes of stalk lodging. This research aims to develop and validate methods to non-destructively determine the transverse stiffness of maize stalks, which is highly related to the presence of disease and pest damage. A handheld device for determining the transverse stiffness of maize stalks in the field has been developed. It is a single-user non-destructive device and has an operational battery life of up to 6 hours. The device enables high-throughput phenotyping of live plants and quick identification of individuals affected by diseases (such as stalk rot) and pest damage. Engineering finite element models were also generated to investigate the effects of pest damage and disease on transverse stiffness. Specimen specific models were generated using geometric information obtained from CT scans of maize stems. The models were loaded in transverse compression and the effects of pith voids and or reduced pith integrity (e.g., degraded material properties) were investigated directly. Experiments were conducted to validate results from the device and the finite element analysis models. The device provides farmers a quick, easy, and reliable method to measure transverse stiffness and detect disease or pest-damaged plants in the field. The finite element models will enable scientists to determine the physiological factors that affect transverse stiffness in maize stalks. This research will ultimately lead to lower stalk lodging and higher yields in the future.

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P15

A framework for evaluating performance of ancestral haplotype reconstruction

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The genome is a mosaic of ancestral haplotypes blocks that, if known, can be useful for quantitative trait mapping, exploring the evolutionary history of an individual or population, and investigating patterns of recombination. Reconstructing ancestral haplotypes is non-trivial and accuracy can be variable depending on the tool used and the population/individual it is performed on. Here we present a framework for evaluating ancestral haplotype reconstruction based on the software, RABBIT. Reconstructed haplotypes are evaluated based on four criteria: 1) accuracy of ancestral assignment (AAA), 2) accuracy of genotype assignment (AAG), 3) phasing accuracy (SER), and 4) correlation of crossover counts (CCO). This is demonstrated using a Tropical Synthetic (TropicS) population of maize that was created from seven tropical inbred lines and subjected to parallel selection for early flowering time across a latitudinal transect for two generations. Evaluation of the population resulted in an AAA of 85%, an AAG of 95%, a SER of less than 0.008%, and a CCO of over 71%. We also compare the performance of two algorithms for haplotype phasing and explore potential sources of inaccuracies across the genome. Furthermore, this software was used to reconstruct ancestral haplotypes for 12,000 heterozygous individuals from the TropicS population. Ultimately, integrating genome-wide imputation, reconstruction of ancestry blocks and tests for selection will provide new insight into the response to selection in the TropicS. These analyses are ongoing and the latest results from this study will be presented

Funding acknowledgement: United States Department of Agriculture (USDA)

P16

A meta-GWAS reanalysis of twenty years of quantitative traits in maize

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Identification of loci that contribute to the complex genetic architecture controlling maize traits has intrigued maize geneticists for many years. In this study, we remap phenotypes from across maize populations to find the features that characterize complex traits. Past mapping experiments have been difficult to compare to each other because they have focused on one population, utilize different genome versions, and have only analyzed a few traits. In this study, we collected thousands of previously published morphological, metabolic, and expression phenotypes across the Nested Association Mapping population (NAM), SeeDs of Discovery, the Goodman Association Panel, and the USDA-ARS NCRPIS collection ('Ames' panel) in multiple environments. We then mapped these datasets in a meta-GWAS using consistent model parameters. Due to the high genetic heterogeneity within and between these populations, we use a combination of global and local principal components to control for linked loci arising from population structure and kinship. We hypothesize that the properties of these mapped variants can predict loci that have a functional impact, uncover regulatory networks of gene activity, reveal genotype by environment effects, and uncover loci showing statistical pleiotropy. We will compile these results in an online, queryable database for maize geneticists and breeders to mine for associations of interest for gene prioritization and gene editing.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P17

A metabolic genetic roadmap to flavor of waxy maize

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Waxy maize (*Zea mays L. sinensis Kulesh*), as a popular fruit and vegetable crop as well as an important source of micronutrients in the human diet, has experienced domestication selection and subsequent genetic improvement during its long cultivation history, exhibiting significant divergence from modern common maize. Through integration omics analysis and comparative genetic analysis of a 318 waxy maize inbred lines population with 517 regular modern maize inbred lines, we found that not only the famous known gene *waxy1* and starch related pathway have been selected during its breeding history, numbers of other genes and pathways, such as benzoxazinoid and brassinosteroid pathway, are also involved in the selection of waxy maize both on genomic and transcriptional level, and shape the dramatic differences between waxy and regular maize. More importantly, we found that the divergence of these metabolic pathways are probably due to artificial selection of grain flavor-associated traits in waxy population. In total, 188 primary metabolites and 1590 secondary metabolites are qualified in the waxy population and some were metabolites were identified as the important contributions to flavor and consumer liking by large scale tasting experiences. The genome-wide association study permitted the identification of genetic loci that affect most of the flavor-associated metabolites, including sugars, amino acids, and phenylpropanoid related metabolites. Our findings not only shed lights on the selection of waxy maize, but also provides useful resources and knowledge for high nutritional maize breeding.

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P18

Advancing understanding of maize heat stress response mechanisms by integrated molecular, biochemical, and whole-plant analysis

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Heat stress is becoming a more serious threat to agricultural production worldwide than before due to global warming and climate change. Development of heat tolerant maize varieties is of crucial importance, which can be facilitated by gaining knowledge of heat stress response mechanism. However, limited progress has been made towards unravelling the molecular and genetic basis of heat response and tolerance in maize. In our study, we selected 27 representative heat tolerant and susceptible inbred lines from a maize diversity panel, and developed three connected populations (B76 × B106, B76 × NC350 and NC350 × B106). Our aims were to: 1) quantify natural variation in heat stress response and identify underlying mechanisms and 2) identify genetic loci that contribute to differential heat stress response through quantitative trait loci (QTL) analysis. Hence, we are combining whole-genome transcriptome profiling, lipidome profiling, and extensive physiological characterization of diverse maize inbred lines, and robust genetic mapping of targeted populations to establish a clearer understanding of heat stress response. A set of 432 leaf samples of 27 inbred lines for RNA-seq were collected at 4 time points from 2 tissues. Preliminary results with pilot samples identified 786 differentially expressed genes (DEGs) under heat stress: 36(4.6%) transcription factors and 22(2.8%) heat shock genes. A co-expression network was constructed, and modules involved in photosynthesis and jasmonic acid signaling pathway played an important role in heat stress response processes. In addition, 22 QTL were identified within two existing populations (B73 × NC350 and B73 × CML103). For the next step, we are going to integrate time-series multi-omics data from different developmental stages, together with QTL mapping through three connected populations, to establish a broad reference framework for heat stress research in maize.

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P19

Aerial based high-throughput phenotyping for genetic dissection of NDVI in maize

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Plant phenotyping under field conditions plays an important role in agricultural research. Efficient and accurate high-throughput phenotyping strategies enable a better connection between genotype and phenotype, which is critical for crop improvement. Unmanned aerial vehicle-based high-throughput phenotyping platforms (UAV-HTPPs) provide novel opportunities for large-scale proximal measurement of plant traits with high efficiency, high resolution, and low cost. In this study, we extracted time-series data for normalized difference vegetation index (NDVI) from multispectral images at 5 time points across the growing season of 1,752 diverse maize accessions with a UAV-HTPP. We identified genotypic differences and analyzed the dynamics and developmental trends of NDVI during different maize growth stages. Clustering analysis with time series NDVI classified 1,752 maize accessions into 2 groups possessing distinct NDVI developmental trends. Then the time series NDVI data were used in penalized-splines (P-splines) model to obtain genotype-specific curve parameters. Genome-wide association study (GWAS) using static NDVI values observed from individual time points and P-splines estimated NDVI curve parameters as phenotypic traits detected signals significantly associated with the traits. Additionally, GWAS for P-splines fitted NDVI values discovered the dynamic change of SNP effect for the trait associated genetic loci, which may suggest the role of gene-environment interplay in controlling NDVI development. Our results suggest the usefulness of UAV-based remote sensing for genetic dissection of NDVI.

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P20

Allelic expression bias of deleterious protein-coding variants in Maize hybrids

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The traditional phenotype-based breeding and the more recent genomic selection techniques have made significantly improved economically valuable and genetically complex traits in agricultural species. However, further progress in genetic improvement of such complex traits requires a better understanding of the underlying genetic variants and functions thereof. Various efforts have been attempted to address this issue. However, current methods and systems are limited in their capacity to assess the fitness impact of individual genetic variants. Accordingly, there is a need for improved methods and systems for assessing genetic variants. The assessed genetic variants can then be prioritized and used as candidates for genetic modification or targets for selection to improve desired traits in agricultural species. Here, we examined the idea of using allele specific expression (ASE) to help identify and prioritize genetic variants which impact fitness in maize. Specifically, we tested gene level ASE in hybrid between B73 and Mo17 and its association with the functional impact of amino acid substitutions which differ between the parents. Our results suggested that ASE can be used as one endophenotype to help prioritize maize deleterious sites for modification by genome engineering.

P21

An integrated modeling/breeding experiment to determine the structural factors that influence stalk strength

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Rather than modifying plants by trial and error, engineering tools could be used to analyze and then design the architecture of plants to meet specific criteria. While the possibilities are tremendous, we are not yet able to design crops in this way because the field of crop biomechanics is quite young and undeveloped. This poster outlines a research approach to advance this type of research. Rather than presenting research, the purpose of this poster is to provide an opportunity for researchers to discuss/critique the proposed research approach.
The objective of this research is to gain new understanding of the genetic and structural factors that influence maize stalk strength using an iterative cycle involving Computational Modeling, Large-Scale Field Experiments, and Structural Assessments. The project will consist of the following components:
Computational Modeling: Models of maize stalk failure will be created, rigorously validated, and then be used to investigate relationships between structural parameters, strength, and structural efficiency.Large-Scale Field **Breeding Experiments**: Genetic and structural diversity is required for rigorous validation of computational models and to assess the heritability of key phenotypic traits. The necessary genetic and structural diversity will be generated by creating new hybrids via test-cross & intra-cross breeding.
Structural Assessment: Specimens from field experiments will be assessed in terms of tissue properties, morphology, and structural performance. These data will be used to rank hybrids, validate computational models, suggest ways in which models must be refined, test hypotheses, and determine the genetic heritability of key phenotypic traits.
This research will result in 4 concrete advances: (1) a validated modeling platform for analyzing maize architecture; (2) a rank-ordering of the structural features that influence stalk strength; (3) over 100 new varieties of maize and their associated structural characteristics; and (4) estimates of genetic heritability of key structural phenotypes.

P22

Are maize stems structurally optimized to resist stalk lodging?

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Stalk lodging results in millions of dollars in lost revenue each year. Despite a growing body of literature on the topic of lodging, the structural efficiency of maize stalks has not been investigated previously. The ability to withstand wind loading is dependent on the applied load from the wind, and the stem's material and morphological characteristics. In this study, we investigate the taper and morphology of mature maize stalks to determine if rind tissue is efficiently allocated to withstand wind induced bending stresses that cause stalk lodging. 945 fully mature, dried commercial hybrid maize stem specimens (48 hybrids, ~2 replicates, ~10 samples per plot) were subjected to: (1) three-point-bending tests to measure their bending strength and (2) rind penetration tests to measure the cross-sectional morphology at each internode. The data were analyzed through a structural optimization algorithm to determine if rind tissues were efficiently organized to resist wind induced bending stresses. Hybrids with higher average bending strengths were found to allocate rind tissue more efficiently than weaker hybrids. However, even strong hybrids were structurally suboptimal. There remains significant room for improvement of the load bearing structure in maize stalks. It was also found that maize stems are morphologically optimized for wind loading near the top of the stem. This seems to be consistent with the authors' observations in field conditions; although border rows (plants at the border of the field) may experience loading along the full length of the stem, the majority of maize plants seem to only be loaded by the wind near the panicle at the top of their stem. It was also found that hybrids that are stronger tend to have more consistent levels of optimization, indicating the possibility that there exists a genetic component to the morphological optimization of maize stems to their wind loading environment.

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P23

Assessing the effect of increased planting density on yield component traits in maize

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Grain yield in maize has been increasing at a steady pace for the last 80 years in North America. Increasing the number of plants per hectare, or planting density, has been the most influential aspect associated with such yield increases. The goal of this work is to understand the variation of phenotypic expression in yield component traits using populations that reflect older, minimally selected and commercially relevant germplasm across a range of planting densities. To assess the effect of different planting densities in plant development and components of yield, an "Ever-Increasing Density" (EID) plot format was used as an alternative to planting adjacent experiments at different densities. The EID scheme used altered plant spacing within each plot to systematically simulate planting densities from 6,970 plants/acre to 104,550 plants/acre. The populations used share a reference parent (PHW65) and represent groups of older, less selection (MoG), minimal selection (Mo44), and similar to commercially relevant germplasm (PHN11). Hybrids of 361 doubled haploid lines from these biparental populations were crossed to PHT69 and evaluated using the EID modified split-plot design with two replications in Madison, WI in 2018. Ears were collected from each planting density in a plot and analyzed for ear, cob and kernel traits using high-throughput ear imaging. The component traits that are calculated are kernel length, depth, width, and area; ear number, length, width and cob color. Data from this evaluation will be compared with yield and agronomic data collected as part of the Genomes to Fields initiative in 2018 that involved the same genotypes. Preliminary results indicate differences in yield component traits, specifically kernel row number and number of ears produced, across treatments and populations. Evaluating plant response at varying densities will reveal which yield component traits are most affected and should be considered when increasing yield.

Funding acknowledgement: United States Department of Agriculture (USDA)

P24

Automated single kernel measurement of maize composition with NIR spectroscopy

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Measurement of single kernel traits may provide a fast and economic method for the sorting of desirable seeds used in maize breeding. In particular, the use of automated methods of detect doubled haploid (DH) seeds would be highly desirable as opposed to hand screening based on coloration. Currently one method of sorting of DH seeds by oil content is being done using an automated nuclear magnetic resonance instrument to measure oil content, however throughput is slow. Presented is the description of a novel near infrared reflectance instrument that can measure oil content and achieve sorting rates of 2 to 3 kernels/s. Prediction model accuracy for oil content developed from chemometric models using partial least squares regression - cross-validation, yielded root mean square errors (RMSE) of less than 1 %. Additional constituent measurements may also be of benefit for general characterization such as protein measurements which has similar measurement accuracy to oil.

Funding acknowledgement: United States Department of Agriculture (USDA)

P25

Cold tolerance QTL analysis and validation using VIGOR: a machine vision assay for seedling emergence

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Temperature is a crucial factor to determine maize seedling emergence, and cold tolerant varieties are needed for early spring plantings. We evaluated cold emergence for 267 recombinant inbred lines (RILs) from the Intermated B73 x Mo17 (IBM) population using VIGOR, a soil-based machine vision platform. Individual cameras monitor 168 kernels in parallel with time-lapse imaging. Images are processed by an application in the public CyVerse cyberinfrastructure. Machine scores are comparable to human scores with a median time difference of 30 minutes. RILs were treated with warm and acute cold. In the cold stress treatment, seeds were sown at 5°C for 5 days followed by transfer to 24°C. Emergence rate, time, and percent were recorded. Quantitative Trait Loci (QTL) analysis on these emergence parameters detected a previously identified QTL as well as a novel QTL on chromosome 10. The novel QTL was found in both warm and cold conditions. To confirm the QTL on chromosome 10, 22 near isogenic lines (NILs) that had either B73 or Mo17 segments within the QTL interval were assayed with the same temperature conditions. There were statistically significant differences between Mo17 and B73 haplotypes within the QTL interval in terms of emergence frequency and time. In warm conditions, the Mo17 allele decreased emergence frequency 6% and delayed emergence by 4.9 hours irrespective of inbred genomic background. Under cold conditions, the Mo17 allele in the B73 genomic background had slower emergence by 4.4 hours and lower emergence frequency. These results demonstrate that high resolution phenotyping improves the detection of natural variants contributing to seed and seedling quality in maize.

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P26

Demonstration of targeted crossover in hybrid corn using CRISPR technology

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Naturally occurring homologous recombination during meiosis drives genetic diversity during sexual reproduction. Targeted recombination at allelic loci in F1 plants would provide a significant advantage to the plant breeding process by accelerating QTL fine mapping, facilitating trait introgression, and increasing genetic gain. We sought to demonstrate the ability to target recombination at specific genomic loci in an F1 plant during mitosis utilizing the CRISPR Cpf1 system and further compare the efficiency to naturally occurring recombination. Our results demonstrate that targeted recombination between parental chromosomes can be introduced at early stages of plant regeneration. Our evidence suggests the targeted recombination was driven by the non-homologous end joining (NHEJ) repair pathway presumably during mitotic cell division. In this experiment the targeted recombination was stable, heritable and significantly higher than the natural recombination rate. These results are a step towards the use of guided nuclease technology to simplify the creation of desired genome combinations in progeny and accelerate breeding.

P27

Detection of fusarium-infected wheat seed utilizing image-based analysis

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Fusarium Head Blight is one of the most devastating plant diseases in the world. The scab disease has caused over $3 billion dollars in losses due to its degenerative effect on the nutritive, physical, and chemical qualities in the grain. Fusarium Head Blight, also referred to as "scab", is caused by the Fusarium spp. with its dominant pathogen being Fusarium graminearum. Scab infection in wheat (Triticum spp.) is shown by the bleaching of the spike head, beginning in one of its spikelets, and spreading to the rest of the spike. This often will result in the shriveling of the wheat kernels and a pinkish, bleached appearance as the disease progresses. The disease also produces a mycotoxin called deoxynivalenol (DON) that can be very harmful to animals and humans as it disrupts normal cellular function and can lead to nausea, fever, headaches, and vomiting. DON contaminated grain causes extreme discounts as the USDA recommends DON levels not to exceed 1 part per million(ppm) and 2ppm is marked as unacceptable for wheat used in human foods. Therefore, it is important to identify Fusarium diseased seed to reduce the possibility for DON infection. The aim of this study is to develop an image-based identification model for the identification and distinguishing of healthy and diseased wheat seeds. Utilizing image-based methods for disease identification would help researchers to improve the efficiency of rating Fusarium diseased kernels (FDK), which is typically done by hand. This would also give us a more subjective method for evaluating disease severity.

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P28 @sg_odell

Dissecting quantitative trait variation in a multi-parent maize population

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The study of evolutionary quantitative genetics requires the ability to link differences in phenotype to genotypic variation. The search for quantitative trait loci (QTL) that explain complex traits such as yield, plant height, and flowering time has been ongoing in maize. Multiparent advanced generation intercrossing (MAGIC) populations contain more genetic diversity than biparental mapping populations and reduce the confounding factor of population structure that is an issue in association mapping populations. Here we present the results of using a MAGIC population of double haploid maize lines created from 16 diverse founders to perform QTL mapping, comparing QTL identified using three different methods of representing genotype data. We validate the ability of each method to find QTL using a previously identified flowering time QTL, vgt1, for which the causal variant is known. High density genotype data and whole-genome sequencing of the founders allows us to resolve haplotypes shared between founders and test the power of these different approaches. A closer look at presence-absence variation for the MITE insertion underlying vgt1 and flowering time phenotypes suggests a potential epistatic interaction in select founder lines.

P29 @juanplants

Dissection of Quantitative Leaf Blight Resistance in sweet corn using genomics and phenomics

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Florida and Georgia make up almost 75% of the total fresh market sweet corn acreage in the Southeastern (SE) United States. Southern and Northern Corn Leaf blight are foliar diseases caused by closely related ascomycete fungi, *Bipolaris maydis* and *Exserohilum turcicum* that threaten yield losses every year. Due to the SE climate, disease pressure is highly adaptable and constant. Such disease pressure necessitates **multi-gene quantitative disease resistance** because single-gene, race-specific resistance is not stable and breaks down as fungal populations evolve. Developing resistant cultivars requires introgression of resistant germplasm—a balancing act between diversity and maintenance of important marketable traits. Preliminary results show that **this trait is heritable** and has breeding potential. Towards this, we are conducting a Genome-wide Association Study (GWAS) to **quantitatively dissect disease resistance**. We present a **systematic screen of diverse sweet corn** germplasm for corn leaf blight resistance using computer vision. Computer vision is applied here to automatically measure diseased leaf area. We demonstrate that **computer vision can provide a reproducible, quantitative, and objective measurement of disease severity**—thereby improving phenotyping over traditional qualitative approaches. We expect this novel phenotyping approach to increase the power to detect genomic regions controlling resistance to Southern and Northern Corn Leaf Blight. Results from the screen will serve as the basis for a GWAS and inform breeding of resistant germplasm specifically adapted to the Southeastern growing region.

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P30

Dissection of the genetic architecture of adaptation to high elevations in maize with allele-specific expression

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Little is known about the genetic architecture and effect sizes of genetic loci of adaptation to high elevations (AHE) in maize. Here we use allele-specific expression (ASE) to scan the genome to pinpoint genes associated with AHE. To obtain more general results rather than specific to any two lines of bi-parental populations, we selected 26 outbred landraces each from high (> 2000 m) and low (less than 1600 m) elevation sites in Mexico and South America, and created F_1 hybrid families of all the 104 accessions by crossing each landrace to B73. The 104 F_1 families were planted at the high and low elevation Mexican field conditions with two replications per location. Two tissues (leaf tip and leaf base) per plant were collected at the v4 leaf stage for making strand-specific RNAseq libraries. To prevent mapping biases that could cause erroneous ASE calls, we will build sample-specific pseudo transcriptome by integrating landrace genomic variants into B73 transcripts. With RNAseq data of two individuals from each family at each of the two field locations, we will be able to test gene expression divergence associated with elevation and identify genes that show cis-regulatory divergence between low and high elevation populations. This enables us to learn both the genomic loci in local adaption and molecular pathways they control. Insights gained regarding loci underlying local adaptation from this study can feed back into modern maize improvement, yielding valuable benefits in the face of climate change.

Funding acknowledgement: National Science Foundation (NSF)

P31 @snodgrasshopper

Diverse hybrids demonstrate heterosis beyond heterotic groups

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The genetic mechanism of heterosis has been under serious study for over 100 years, resulting in several possible theoretical models. The complementation or dominance model has the most empirical support, positing the accumulated masking of recessive, deleterious alleles underlies heterosis. This implies more distantly related populations that have accumulated more genetic variants will produce hybrids with higher heterosis. Most studies have focused on SNP variants between parents to test mechanistic models of heterosis. Recent genomic research has demonstrated the importance and commonality of structural variation. While there is strong theoretical support for incorporating these genetic variants into heterosis models, only a handful of studies have demonstrated the variants' potential impact to these models.

We have developed a full diallel population with reciprocals from thirteen diverse inbred maize lines to address these questions. Phenotypic data of this diallel may define the relationship between heterosis and genetic distance of the parents. Our pilot study phenotyping a subset of this population demonstrates heterosis is common across maize hybrids and is not constrained by historically important heterotic groups. In addition to discussing the pilot results, we outline future work to identify single parent expression (SPE). The SPE pattern is observed when the hybrid and one parent express a gene while the other parent does not. This extreme expression complementation, which has previously been observed in wheat and maize, lets us test the importance of regulatory and structural variation and the support of the complementation model.

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P32

Diverse maize inbreds show varying patterns of diel gene expression

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Little is known about the diel (daily, having a 24-hour cycle) patterns of gene expression in maize, much less about how those patterns vary between diverse inbreds. In this study we use findings from Arabidopsis, regulatory grammar (position and orientation of transcription factor binding sites), and gene expression network analysis to describe differences in diel gene expression patterns among diverse maize inbreds. Daily patterns of gene expression have been extensively studied in Arabidopsis, resulting in the characterization of numerous genes that contribute to control of the circadian clock. Several of these genes are transcription factors that show diel expression patterns. Orthologous genes in maize have also been shown to be involved in circadian rhythm, leading us to hypothesize that these transcription factors are contributing to diel cycling of expression in their downstream target genes. We profiled gene expression in adult leaf tissue for 24 of the NAM parents every 2 hours for 24 hours under field conditions. We found that approximately a third of expressed genes show evidence of diel cycling patterns. A search for binding motifs of transcription factors that regulate circadian rhythm in Arabidopsis revealed that maize genes with those same binding motifs nearby are more likely to show diel patterns of expression. These results demonstrate transferability of findings from Arabidopsis to maize, and lay the groundwork for forthcoming exploration of diel patterns of gene expression in maize.

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P33

Elucidating the gene regulatory landscape of natural variation for leaf cuticular conductance in maize

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The cuticle, a hydrophobic layer of cutin and waxes synthesized by plant epidermal cells, is the major barrier to water loss during stomatal closure and under water-limited conditions. Dissecting the genetic architecture of natural variation for leaf cuticular conductance (gc) is important for identifying genes relevant to improving crop productivity in drought-prone environments. To this end, we conducted a genome-wide association study (GWAS) of gc with ~10M SNPs imputed on a maize inbred association panel, allowing for the identification of nearly 60 distinct genetic loci that included ISTL1 and FAB1 based on the top 0.001% associated SNPs. With the identical association panel, a transcriptome-wide association study (TWAS) using gene expression data collected from developing maize adult leaves also detected ISTL1, and other candidate genes plausibly involved in cuticular wax biosynthesis and transport, among the top 1% of genes (~170 total) associated with gc. To increase statistical power for candidate gene identification, an ensemble approach that unified GWAS and TWAS results revealed additional candidate genes associated with gc that are involved in the biosynthesis (CER3 and CER7) and possibly vesicle trafficking (SEC14) of cuticular waxes. The data generated from this study will provide novel insights into the role of regulatory variants in the development of the maize leaf cuticle, and will ultimately assist breeders to develop drought-tolerant maize for target environments.

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P34

Fine mapping of qtl on chromosome 9 for drought tolerance in maize

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Drought is considered as one of the major limiting factors in sustainable maize production all over the world as it causes yield reduction by an average of 15% to 20%. The objective of the study was to identify and to transfer QTL associated with drought tolerance into spring maize inbreds through marker assisted backcross breeding (MABB). A total of 135 F_8 recombinant inbred lines (RILs) from the cross between CM 123 as the susceptible (female) parent and CM 140 as the tolerant (male) parent along with parents were evaluated under control and drought stress conditions for two consecutive seasons. The QTL on chromosome 1, 3, 4, 6, 7 and 9 were identified for drought tolerance under both stress and control conditions. The present study focused on fine mapping of QTL for number of kernel per ear (*qKPE*) present on chromosome 9 (*bnlg1401-umc1634*) explaining phenotypic variance of 23.14% under stressed environment. This region was narrow down by designing 50 new SSR markers between the bracketed QTL (*qKPE*). Seventeen SSR markers showed the polymorphism between CM 123 and CM 140. These markers along-with previous mapped markers were employed on RIL population. The QTL analysis narrowed down the genetic distance to 3.8 cM from 11.5 cM and physical distance to 691 kb from earlier distance of 15 Mb flanked by two new SSR markers *viz*. PAU_1143 and PAU_1137. The *qKPE* is also introgressed through MABB into two spring maize inbreds LM23 and LM24 of hybrid PMH10 for water use efficiency. The foreground selection has been carried out in two generations i.e., BC_1F_1 and BC_2F_1. Also, Background selection has been done in BC_1F_1 to check the background recovery of recurrent parent. The plants carrying the QTL with highest recurrent parent background recovery were selected and again backcrossed to respective parent for generation of BC_3F_1 population.

P35

Genetic analysis of endosperm vitreousness and compositional traits in maize

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Maize endosperm hardness, often classified as degree of vitreousness, is a relevant nutritional trait due to its connection with lysine and tryptophan concentration as well as its relationship with starch digestibility. There is a strong negative correlation between the percentage of kernel vitreousness and ruminal starch availability, which makes this trait a target in silage breeding. A direct measurement of the trait is accomplished by tedious manual dissection of kernels. The objective of this study is to expand the available tools to accurately predict vitreousness and apply this method to characterize natural variation and ultimately map regions of the genome associated with this trait. For the first objective, we have developed a endosperm vitreousness Near Infrared Spectroscopy (NIRs) calibration curve using more than 1,500 kernels individually characterized to build the training set. A set of 150 genotypes from the Wisconsin diversity (WiDiv) panel were selected to maximize phenotypic variation of the training set and planted in 2018 in Madison, WI. To asses natural variation, 800 inbred lines from the WiDiv panel were planted using a randomized complete block design (RCBD) with two replicates in Arlington, WI in 2018. Also, four multi-parent advanced generation intercross (MAGIC) populations were planted in Madison, WI in 2016 and 2017 arranged as RCBD with two replicates each. Both the WiDiv and the MAGIC populations were harvested at physiological maturity and representative samples were scanned using NIR to measure endosperm vitreousness, total kernel starch, protein and oils. The NIR calibration equation produced a coefficient of determination (R^2) of 0.84 using partial least square regression (PLSR) and validation using the leave-one-out (LOO) method. The WiDiv population showed a wide distribution of vitreousness levels ranging from ~35% to ~93% which makes this population a good candidate to perform association studies of vitreousness. Entry mean heritabilities for vitreousness, kernel protein, starch and oil are 0.82, 0.91, 0.86 and 0.83, respectively. Initial genetic mapping results will be discussed.

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P36

Genetic analysis of natural variation for levels of Vitamins B_2 and B_6 in maize grain

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B vitamins, a class of water-soluble enzyme cofactors and their derivatives essential for human health, are limiting in maize grain, thus contributing to human nutritional deficiencies for those subsisting on maize-based diets. To understand the genetic control of vitamins B_2 and B_6 levels in maize grain, we measured vitamin B_2 (riboflavin) and four forms of vitamin B_6 (pyridoxal, pyridoxamine, pyridoxic acid, pyridoxine) on ~2,000 inbred lines of the Ames panel evaluated across two years. The broad-sense heritabilities of these B vitamin traits ranged from 0.57 (riboflavin) to 0.90 (pyridoxine). Although the four vitamin B_6 traits are synthesized by a shared biosynthetic pathway, only one pair of compounds (pyridoxamine and pyridoxine) had a correlation stronger than 0.30. Genome-wide association studies were performed on these B vitamin traits with ~12M SNPs, resulting in the detection of eight distinct genetic loci significantly associated with one or more of the vitamin B_6 compounds at a genome-wide FDR of 0.05. To improve statistical power for identifying novel and prioritizing existing candidate genes, we performed transcriptome-wide association studies and eQTL analyses with gene expression data collected via 3' Quant RNA-sequencing analysis of developing grain samples harvested at 24 days after pollination from ~1,000 lines of the Ames panel. Selected candidate genes will be investigated through reverse genetics approaches to further explore their role and function in the accumulation of these two B vitamins in maize grain. Findings of this study will provide novel insights into the genetic basis of these limiting vitamins and beneficial alleles identified from this project will be useful for the nutritional enhancement of maize grain.

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P37

Genetic analysis of nitrate accumulation in maize stalks

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Nitrogen (N) is the most commonly applied nutrient and one of the costliest inputs in corn production. Typically, N fertilization increases agricultural productivity but its use can have negative effects on the environment. Understanding the genetic control of nitrate uptake and utilize has the potential to facilitate the development of genotypes that more efficiently utilize applied N, reducing input costs and contributing to a more sustainable agricultural system. Past genetic investigations have largely focused on comparing plants grown with or without added N fertilizer. To understand the genetic control of nitrate accumulation, we measured N accumulation in the basal stalks of the SAM diversity panel, which consists of 357 genotypes, under multiple levels of applied N and then conducted GWAS. Longer term, we seek to understand the relationship between basal stalk nitrate accumulation and plant performance which may enable us to provide evidence-based fertilization recommendations to farmers.

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P38

Genetic and functional genomic analysis of tocochromanol levels in maize grain

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Tocochromanols are a class of antioxidants important in both plant fitness and human health in the form of vitamin E. In this study, we leveraged the allelic diversity captured in the maize Ames panel of ~1,500 inbred lines, which were imputed with ~12 million SNPs, to identify genes contributing to the biosynthesis of tocochromanols in maize grain. When conducting a GWAS of nine tocochromanols traits, associations concordant with prior studies were detected for *vte4* and α-tocopherol, as well as *vte1*, *hggt1* and *hppd1* for tocotrienols. Additionally, two chlorophyll biosynthesis genes, *por1* and *por2*, recently reported to explain the majority of variation for tocopherol grain levels in the U.S. NAM panel, were significantly associated with grain tocopherol levels in the Ames panel. Additionally, 3′ Quant RNA-sequencing was conducted on developing kernels collected from more than 1,000 lines of the panel, allowing us to more fully explore the regulatory control of the tocochromanol pathway. In parallel, we designed a light/dark treatment experiment on seven NAM founders and generated single and double mutations at *por1* and *por2* through CRISPR/Cas9 mediated genome editing to explore the underlying mechanism. The light/dark experiments were performed in 2018 and 2019, with tocochromanol levels measured in mature kernels and RNA-seq analysis performed in 24 DAP developing kernels. The dark-treated ears had significantly lower tocopherol levels relative to light-treated and control ear samples, but these changes could not be definitively attributed to expression-level changes among treatments. *por1por2* double homozygous knockout mutations yielded a stunted, yellow plant phenotype, while a single homozygous knockout mutation at *por1* or *por2* did not result in appreciable plant phenotypic changes. The analysis of results from these ongoing functional genomic studies will be presented and connected to our efforts to genetically improve vitamin E levels in maize grain.

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P39 @SchnableLab

Genetic architecture of maize inflorescence plasticity in response to nitrogen stress

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Nitrogen is an essential constituent for proper growth and development in maize and is also an expensive input for farmers for maximizing yield. Response to nitrogen deficiency is plastic as nitrogen stress can lead to significant decreases in yield due to physiological and morphological effects during development. Part of this variation may be due to differences in root-rhizobiota interactions from differences in root exudates and the recruitment of beneficial microbes in individual maize genotypes. To better assess the phenotypic impact of nitrogen stress on maize inflorescences under field conditions, we grew out the maize 282 diversity panel during two field seasons under normal and nitrogen deficient conditions and phenotyped for flowering time, inflorescence length, branch zone length, spike length, primary branch number in maize tassels, as well as, ear length, ear width, row number, ear fill, kernel abortion, and kernel dimensions in maize ears. A Genome-Wide Association Study (GWAS) was then performed to identify trait associated SNPs for these inflorescence traits. Candidate loci include genes involved in stress response, plant growth and development, and root growth. These data will then be combined with rhizobiome results to determine if the phenotypic plasticity in response to nitrogen stress is correlated to differences in soil and root microbial composition, abundance, and activity, as well as, drone imagery and leaf spectral measurements to assess how effectively maize reproductive stress severity can be estimated from these proxy data types. In future, these data will be used to optimize maize germplasm for improved reproductive resilience under low-nitrogen conditions.

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P40

Genetic diversity in kernel compositional traits and resistance to five foliar diseases in tropically adapted inbred lines of Quality Protein Maize

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An attempt has been made to identify source of variation, analyze genetic diversity, and quantify multivariate relationships among nutritionally important traits and resistance to five key foliar diseases in tropically adapted 152 inbred lines of Quality Protein Maize (QPM). The results displayed significant variability for traits viz. specific gravity, test weight, tryptophan, lysine, protein, starch, oil and sugar contents, and quality index, respectively. The data also highlighted correlative associations among some of these traits. Using Principal factor analysis (FA) procedures based on correlation matrix with eigen value one or more than one criteria, the first four factors pertaining to nine kernel physical and compositional traits contributing most heavily to the variability accounted for 81.52% of total variance. Based on screening under epiphytotic conditions at hot spot locations, the lines displayed a wide range of reactions to respective five foliar diseases, namely Maydis leaf blight (MLB), Turcicum leaf blight (TLB), Polysora rust (PR), Common rust (CR) and curvularia leaf spot (CLS). The results of principal factor analysis indicated that the first two factors accounted for 70.21% of total variance. Further, the proportion of lines with resistance to all the five diseases was worked out as 11.54%. These included one medium DMRQPM 106, and five late maturing lines namely HKI 164-7-4, HKI 163, HKI 5072-2BT, CML 165 and CML 451Q.On the basis of kernel physical and compositional traits and resistance to foliar diseases, promising QPM lines were identified as sources of variability for multiple traits that can be used in developing high-quality disease resistant maize hybrids. The results also presented substantial opportunities to further improve these traits and also suggested exploration of a new source of elite breeding stocks containing a high level of the target traits in QPM cultivars.

P41

Genetic resistance to tar spot (Phyllachora maydis) in maize

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Tar spot is a new and rapidly spreading disease in the United States caused by the fungus Phyllachora maydis. This Ascomycota fungus infects maize leaves to create hardened black lesions on leaf material. It has shown to cause up to 50% yield loss in maize fields. The goal of this study was to map genetic resistance to tar spot in diverse maize and to assess whether aerial images could be used to observe disease progression over time. 543 total maize genotypes were screened and rated from the Wisconsin Diversity panel and Iowa State's Germplasm Enhancement of Maize (GEMs) program. The experiment was planted as a replicated field trial in 2019 near Allegan, MI using natural pressure as inoculum. Disease ratings were taken at six timepoints as a percent disease coverage of the ear leaf. We collected natural color and multispectral aerial images of the field trial at eight timepoints throughout the growing season to estimate disease progression over time. We calculated the area under disease progress curves for each replication. We were able to model environmental variation due to natural disease spread and showed that our ratings were heritable. We found that tar spot disease ratings were not correlated with flowering time, plant height, or ear height. We conducted a genome-wide association study for disease resistance and found significant loci for further study. We will collect a second year of field trial data to confirm our results, while working to introgress resistance to tar spot into more elite temperate varieties.

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P42

Genome-wide dissection of B vitamin levels in maize grain in the Ames Diversity Panel

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Maize is low in B vitamins, leading to deficiencies and associated health problems among those who depend on maize-based diets, especially in vulnerable groups including women and children in developing countries. This collaborative project investigates the genetic basis of natural variation in B vitamin content in maize grain by integrating techniques including genome-wide association studies (GWAS), UniformMu transposon lines, genomic prediction, and 3′ RNA-seq. In 2015 and 2017, approximately 2,000 inbred lines from the Ames Diversity Panel were grown, and B vitamin content in mature grain from this panel was measured by LCMS. B vitamin traits measured included thiamine (13C) and thiamine (d3) (B1 traits); and nicotinic acid, nicotinamide, and trigonelline (B3 traits). The calculated traits thiamine, niacin, and active niacin were determined from these measured traits. Genome-wide scans using three different methods (mixed linear model, multi-locus mixed model, and FarmCPU) were conducted for these phenotypes using a high-density imputed genotypic dataset containing more than 12 million SNPs. Significant associations were found for all traits, and candidate genes underlying these peaks were identified. To validate these associations, UniformMu lines with insertions in or near candidate genes were selected and grown in 2019. B vitamin levels in grain harvested from these will be measured and compared to levels in wild-type grain. Genomic prediction models were also constructed based on the 2015 and 2017 Ames Panel phenotypes. In 2018, immature ears were harvested for 3′ RNA-seq at 24 days after pollination from ~1,000 lines of the Ames Panel. Based on these data, transcriptome-wide association studies (TWAS) and eQTL analyses are ongoing to complement GWAS results. We expect that this project will facilitate the selection of maize lines for improved B vitamin content, resulting in improved nutrition and health in people who grow and eat these varieties.

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P43

Impact of different reference genomes for SNP calling in a genotyping-by-sequencing pipeline for a tropical maize diversity panel

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Genotyping-by-sequencing (GBS) protocol is a useful procedure to obtain larger number of genetic markers in diverse populations. Calling SNPs in GBS pipeline involves aligning the obtained reads to a reference genome. Different reference genomes are expected to affect the quality and number of markers retained depending on the population genotyped background. In this scenario, we aimed to compare the use of distinct tropical and temperate reference genomes for SNP calling in a tropical maize diversity panel. For that, we used 360 Brazilian inbred lines genotyped using Illumina NextSeq. We adopted a Tassel-5-standalone pipeline for GBS protocol and the software Bowtie2 for the alignment. The reference genomes studied were B73 versions 4 and 5, Oh43, CML247, and CML103. We considered two SNP datasets, non-filtered (NF) and filtered (FD), and we assessed SNP calling according to the following parameters: proportion of reads aligned, total number of SNPs before and after filtering the markers, average coverage depth, and overall populational metrics. The number of SNPs in FD ranged from 14,601 (CML247) to 14,818 (Oh43), and the coverage depth was between 3.45 (B73v4) and 5.05 (B73v5). The proportion of reads aligned was negatively correlated with the number of SNPs in both, FD (-0.667) and NF (-0.424) and the length of the reference genome (-0581 and -0.452 for FD and ND, respectively). These observations suggest that duplicated fragments in some reference genomes are likely absent in others regardless of their origin as tropical or temperate. Additionally, the association of the alignment of a smaller number of reads with a higher number of SNPs may occur due to a monomorphic gene stacking in the same positions. Our results indicate the importance of an in-depth investigation on available genomes before selecting one since as reference as they directly impact the accuracy of genetic analyses in breeding programs.

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P44

Increasing water use efficiency in *Zea mays*; An integrative approach to elucidating a complex trait

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Water limitations currently exist for crop production and will become a greater problem over the next several decades due to global climate change. As rainfall events become more sporadic, crops with increased water use efficiency (WUE) will provide growers with greater yield stability under variable conditions. The USDA National Agricultural Statistics Service reported that nearly a quarter of the maize grown in the United States is already marketed as having drought tolerance traits. Breeders generally select for increased drought tolerance due to the difficulty in measuring plant water use. However, although WUE is related to drought tolerance, it is fundamentally different in that the efficient use of available water can reduce the likelihood that the plants will experience drought stress. Whole-plant WUE is a complex trait that is affected by biochemical, physiological and morphological phenotypes. Identifying the genetic factors influencing these traits will better allow us to measure and select for increased WUE. Using stable carbon isotope composition ($\delta^{13}C$) in the leaf, we have been able to identify variation in transpiration efficiency. This variation has led to the identification of lines that can be used for both the genetic dissection of $\delta^{13}C$ and breeding. In addition, we are studying the contribution of nighttime transpiration to total plant transpiration. An understanding of why nighttime transpiration occurs could enable a more efficient regulation of stomata and increase WUE. Finally, leaf morphology (number, length, width, and thickness) can affect whole-plant water use. We present the identification of genetic loci controlling specific leaf area (SLA), a proxy for leaf thickness. Through these interdisciplinary approaches, we have identified phenotypes that contribute to whole-plant WUE and have used genetics and breeding to work toward improving crop WUE.

Funding acknowledgement: United States Department of Agriculture (USDA)

P45 @julia_morosini

Inferring panel clustering and dominance effect on genomic prediction assessments involving tropical maize

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Genomic selection (GS) is effectively used in maize breeding to provide hybrid performance prediction, since producing and testing all possible combinations of available inbreds in the field is typically impractical. Even though hybrids are the most commonly used genotypes in maize, estimated marker effects are usually considered mainly additive and GS is not consistently adjusted for reciprocal recurrent selection (RRS). In this study, we evaluated the prediction of tropical maize hybrids considering distinct models including additive effect, additive+dominance effects and inferred heterotic groups (HG). For that, 906 single crosses obtained from a diallel scheme of 49 inbred maize lines were genotyped in silico using 34,571 SNP and evaluated for grain yield in four environments in Brazil. The models assessed included: additive using a single population (A), additive+dominance using a single population (A+D), and additive+dominance using a North Carolina II (NC) factorial design. The HG used in the NC scenario were assigned based on the specific combining ability (SCA) estimates from the A+D model. Prediction abilities (PA) were obtained using a 5-fold cross-validation approach. The A model provided smaller PA (0.56) compared to A+D (0.62), which corroborates the importance of modeling the contribution of non-additive effects towards heterosis. NC provided a significantly increased SCA of the single crosses (0.173) compared to A+D (0.052). However, there was no substantial increase in PA (6%) from A+D model compared to NC, which indicates that a smaller set of crosses genetically well targeted by HG is as efficient as using all inter- and intra-crosses in terms of PA. Our findings suggest that clustering HG based on SCA from the A+D model is a useful approach for RSS when no previous information is available about population genetic structure. Finally, the differential modeling of marker effects for each HG is crucial to a sustainable RSS breeding program.

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P46

Integer/categorical particle swarm optimization improves the performance of optimal population value selection

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Genomic prediction and selection have been used in the last two decades to accelerate plant breeding efforts in major crop species like maize, soybean, and wheat. Several genomic selection strategies have been proposed with algorithmic difficulties ranging from polynomial-time hardness to non-deterministic polynomial-time (NP) hardness. In this study, I examine the effect of algorithm choice on the performance of Optimal Population Value (OPV) selection, a genomic selection strategy of NP-hard difficulty. I compare the performance of a steepest ascent hillclimber (HC) algorithm and an integer/categorical particle swarm optimization (ICPSO) algorithm in optimizing OPV. I reveal that ICPSO leads to better OPV solutions and can enhance genetic gain in simulated breeding populations. This research highlights the importance of optimization algorithm choice in solving breeding selection problems and offers a new avenue through which plant breeders can improve crops to provide for the needs of a growing world.

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P47

Integrated approaches for molecular marker discovery and deployment for accelerating *Striga* resistance breeding in maize

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Maize yield is dramatically reduced by severe infestation of *Striga hermonthica* where the parasitic weed is endemic, further diminishing the already low productivity. Genetic improvement of maize inbred lines for host plant resistance to *S. hermonthica* can be accelerated by integrating genomic tools in the breeding scheme. Understanding, characterizing, and identifying the mechanisms of resistance of the host is an important initial step towards strategically pyramiding of multiple genetic factors into a single cultivar to boost resistance durability. Various approaches including expression analysis, QTL identification, and investigation of Strigolactone deficient mutant were employed. RNAseq derived transcriptome profile of susceptible and resistant maize genotypes revealed differentially regulated genes involved in various pathways including defense genes, cellular transporters, and genes involved in secondary metabolism, suggesting that the mechanism of resistance involves multiple biochemical and physiological mechanisms. Validation of these genes by quantitative real-time PCR is underway. Genome wide association study, conducted on a panel of 150 maize inbred lines, identified a total of 14 major SNPs that were significantly associated with four traits (Striga damage rating and Striga emergence at 10 weeks after planting, ear per plant and grain yield under Striga infestation). Screen house evaluation of mutant maize lines carrying naturally occurring transposon Ds insertion, which inactivated carotenoid cleavage dioxygenase 8 (ccd8), showed nil to very low germination of Striga plants. In a confirmation study, GR-24 (a synthetic analogue of Strigolactone) reversed the effect of the defective Strigolactone production. Introgression of the mutant gene to elite inbred lines through marker-assisted backcrossing is underway. The findings from these studies will be integrated with other complementary studies such as QTL identification by linkage analysis in multiple F_2 and BC_1 segregating populations as well as comparative genomics results.

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P48 @jennahersh

Integrating transcriptomics for the improvement of genetic dissection and prediction of provitamin A and vitamin E in fresh sweet corn kernels

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Even though sweet corn is highly consumed in the US, it makes only a minor contribution to dietary intake of carotenoids (provitamin A, lutein, zeaxanthin) and tocochromanols (vitamin E, antioxidants). Considerable variation for fresh kernel carotenoid and tocochromanol levels has been found in sweet corn germplasm, but full utilization of these genetic resources will require a better understanding of the genetic architecture of these traits. In our previous work, genome-wide association studies of carotenoid and tocochromanol levels in fresh kernels were conducted in a sweet corn inbred line association panel. Significant associations were detected for carotenoid traits with *crtRB1* and *lcyE*, and for tocochromanol traits with *vte4*, *vte1*, and *hggt1*. Moderate to high prediction abilities were obtained for carotenoid and tocochromanol fresh kernel traits when using genome-wide markers. The power and resolution of these studies were limited by the small population size and long-range patterns of linkage disequilibrium of the sweet corn population. Transcriptome-wide association studies have the potential to help overcome these weaknesses, as gene expression data are both independent of linkage disequilibrium and provide insight into regulatory variation, allowing for a more precise identification of causal genes. To take advantage of these benefits, we will conduct a 3′ RNAseq analysis of fresh kernel samples of the same sweet corn inbred line panel. This will allow for transcriptome-wide association studies and transcriptome-based prediction of carotenoid and tocochromanol fresh kernel traits. Fisher's combined test will be used to integrate results from both genome- and transcriptome-wide association studies, resulting in more deeply resolved genetic architectures. Together, the results of this study will help breeders to make important selection decisions more rapidly and accurately.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P49 @HawkinsLabWVU

Integration of high density genetic mapping with transcriptome analysis reveals candidate genes for tillering in sorghum

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Phenotypes such as branching, photoperiod sensitivity, and height were modified during plant domestication and crop improvement. Such traits are essential for maximizing crop production and meeting global demands. Here, we integrate quantitative trait loci (QTL) mapping of a RIL population derived from an interspecific cross between S. propinquum and S. bicolor (Tx7000) with RNA sequencing to fine map genes responsible for agronomically important traits in sorghum. Our high-density genetic map is composed of 2,397 bin markers and spans an average genetic distance of 578 cM. We identified 34 QTL across 2 field locations and 1 greenhouse experiment for tiller number, height, stem diameter, biomass yield, flowering time, arial branching, and perenniality. In addition, we identified 6,189 genes that were differentially expressed during early tiller bud elongation. When combining the results from our QTL analysis with the RNAseq results, genes such as DRM1, FAR1, and ERF/Apo2 were both differential expressed and lie within QTL, suggesting strong genetic controls involved in tiller elongation. Our study demonstrates the usefulness of our population in detecting QTL associated with agronomically important traits in sorghum and provides a valuable resource for genetic improvement to the sorghum community.

Funding acknowledgement: National Science Foundation (NSF)

P50 @abiskar_gyawali

Investigating the effect of pleiotropy due to flowering time in maize kernel composition traits using near-isogenic lines capturing an allelic series

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Photoperiod response has been a strong barrier to the adaptation of a crop to a new environment. In maize, tropical germplasm is well adapted to short day growing conditions because of its proximity to the center of origin in southern Mexico. However, tropical maize, if grown in long day conditions, will have delayed flowering time and this mal-adaptation is not limited to increased plant height and disease-pest susceptibility. Early-flowering maize in the long day environment can be achieved by selecting maize lines for early flowering. However, the mal-adaptation due to photoperiod can mask the effect of beneficial alleles for some seed quality traits, and selection for early flowering can result in the loss of some beneficial alleles at the cost of adaptation. To address these questions of pleiotropy and linkage drag, we created a set of Near Isogenic Lines capturing an Allelic Series (NILAS). NILAS is a set of inbred lines that contain small introgressions from a chromosomal segment from a donor parent (DP) for a range of functional alleles in an elite recurrent parent (RP) background. Marker assisted selection was used to target the introgression of four photoperiod quantitative trait loci (QTL) that were previously identified as a barrier for temperate adaptation of tropical germplasm. For each of the four QTL, NILAS was developed by crossing seven tropical DP with two temperate RP. Furthermore, each introgression is comprised of 12 overlapping introgressions resulting in total of 672 lines. All the NILAS were grown and evaluated in eight environments, each located at a different latitude extending from Puerto Rico (18^0N) to Wisconsin (40^0N). Here we are using days to anthesis (DTA) and days to silking (DTS) as the photoperiod response to detect, if any, the masking effect of flowering time on four seed quality traits: starch, crude protein, phosphorus and fat.

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P51

Leveraging genome assemblies and landrace diversity: Evaluating a practical haplotype graph approach in maize

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Maize landraces have been bred in a plethora of environmental conditions around the globe for generations, allowing their genetic components to change and adapt over time. The Seeds of Discovery project from CIMMYT generated a GBS panel of over 3,000 landrace accessions annotated with GIS data from multiple locations in 18 countries across Latin America. The maize community has been working to generate whole genome assemblies for an array of maize accessions, including the recent release of the 25 NAM founders, 8 of which correspond to CIMMYT Maize Lines (CML). Recent research has shown that including a diverse set of distinct populations allows for the more accurate identification and evaluation of the genotypic effects in complex traits. Here, we leverage the recently available genome assemblies as a source of haplotypes for imputation, aiming to improve on the standard approaches for the genotyping pipelines through a maize Practical Haplotype Graph (PHG). Finally, we assemble approximately 20 additional CML accessions at the gene space level, resulting in a richer representation of the haplotypes controlling the environmental adaptation of the maize landraces. By implementing this maize PHG, in conjunction with the landrace environmental data, we aim to enable the better mining of beneficial alleles in germplasm banks, to improve on the identification and generation of better climate adapted varieties.

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P52

Love me tender: Predicting sweet corn pericarp thickness using single-kernel near infrared spectroscopy.

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Successful sweet corn hybrids possess numerous characteristics that optimize producer profit and satisfy consumer tastes. Breeders incorporate these traits into their selection criterion when developing new inbred and hybrid lines. Phenotypic characterization can limit the number of evaluated lines if the trait is difficult or time-consuming to measure, particularly if the data are collected at fresh eating stage. Tools that reduce data collection time can increase the number of lines evaluated and lead to faster genetic gain. Near-infrared (NIR) spectroscopy is widely used for estimating the chemical composition of mature seeds and grains. We developed a custom NIR platform that collects reflectance spectra from individual kernels. Single-kernel NIR data can be translated into accurate predictions of chemical composition such as oil and protein content, as well as physical characteristics such as mass and density. We developed a single-kernel NIR model to predict mature kernel pericarp thickness, which is an important trait contributing to consumer likability and pathogen resistance during imbibition and germination. Pericarp thickness in a diverse sweet corn panel ranged from very thin (25 µm) to thick (>200 µm) and the error associated with NIR-based prediction was 18 µm. The model was used to predict pericarp thickness in 478 lines of a sweet corn diversity panel. These phenotypes can be used directly to select for optimal tenderness and pathogen resistance. In addition, we plan a Genome Wide Association analysis to identify genetic loci associated with pericarp thickness variation. Identification of large effect loci would allow marker assisted selection for optimal tenderness in sweet corn and point to genes involved in the relatively unknown genetic program that determines pericarp thickness in maize.

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P53

Machine learning assisted multi-environment phenotype prediction in maize

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Predicting an organism's phenotype from the combination of its genotype and environment has long been a goal of both basic and applied research. However, this goal is complicated by the fact that both the genomes of organisms, and the environmental conditions in which they grow and develop are complex. Drawing upon analytical tools from quantitative genetics, physiological growth modeling, and machine learning, a series of methods were developed for predicting plant phenotypes across more than 70 environments (year and location combinations) and 1000 genotypes from the Genomes to Fields Initiative (G2F). These methods include optimized physiological growth models and convolutional neural networks, and use inputs like weather, soil, and genetic data to predict crop grain yield/plant fitness. The accuracy of these predictions varies substantially (0-90%) by environment, genotype, and modeling method. Further analysis provides insights into genetic, environmental, and sampling considerations that impact the likelihood of predictive success. These methods will afford researchers and practitioners a greater ability to predict and understand an organism's phenotype under various environmental and genetic conditions. Potential applications of the approaches include agriculture, breeding, climate mitigation, ecosystem management, and others.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P54

Maize intraspecific competition dynamics: the genomic architecture behind competitive ability

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The presence of specific neighbors can have a direct phenotypic effect on an individual within a population. These differences in phenotype due to one's neighbors are called associative effects, and can be interpreted as a heritable environmental effect in quantitative genetics. Over time, maize has been selected for cooperative rather than competitive tendencies as a result of increased planting densities with identical genotypes. Uniformity in potentially competitive traits such as plant height has remained an important breeding goal; taller plants are able to shade out shorter plants and acquire a greater degree of resources, ultimately limiting the yield of neighboring plants. However, the question remains as to what extent other traits such as leaf angle and root architecture, and the genes underlying those traits, play a role in determining competitive or cooperative ability. We hypothesize that, depending on the genotype, plants will have varying degrees of competitive ability and impact on their neighbors' resulting level of fitness. In this study, we used phenotypic data from the maize Nested Association Mapping (NAM) population containing 26 families across 11 environments to map how the genetics of a neighbor impact the observed phenotype of an individual. We will further investigate the genetics underlying a plant's competitive ability through QTL mapping and genome wide association studies for SNPs impacting neighboring plants, utilizing additional populations. Mapping competitive ability can potentially benefit breeders in maize and other crops by identifying lines with negative or positive associative effects that could be leveraged to make greater selection gains.

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P55

Modeling structural variation in genomic prediction models in maize

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Genomic prediction is a tool that allows breeders to predict hybrid performance based on genetic markers and decrease the time to develop superior hybrids. In practice, genomic prediction still shows relatively poor accuracy, mainly when predicting performance across multiple or untested environments. Currently, prediction models generally rely on single nucleotide polymorphisms (SNPs) as a source of genetic variation because they are abundant in the genome and ease to evaluate via molecular assays. However, structural variants (SVs), which are associated with environmental adaptations like tolerance to toxic soils or to biotic and abiotic stresses, are also prevalent in maize. We hypothesize that utilizing SVs in genomic prediction models will increase prediction accuracies, as they may explain variation in performance among multiple environments more accurately than SNPs alone. To test this hypothesis, we have simulated quantitative traits using genotypic information from a population of 525 RILs derived from diallel crosses of seven maize inbred parental lines. We simulated traits with additive effects and additive and dominance effects, assuming different heritabilities (0.2, 0.5 or 0.9), number of loci (3, 25 or 75), as well as types of causative variants (SNPs, SVs or both) controlling the trait. Each genetic architecture was simulated for multiple environments using the R package *simplePHENOTYPES* (https://github.com/samuelbfernandes/simplePHENOTYPES). Finally, we tested genomic prediction models using SNPs and/or SVs as markers using RR-BLUP, and compared prediction accuracy for each genetic architecture-marker combination using a T2, T1 and T0 validation scheme or a 5-fold cross validation scheme for traits with or without dominance effects, respectively. These simulations provide a way of assessing the usefulness of SVs in genomic prediction models and will also provide information for interpreting the results of future empirical analysis.

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P56

Perennial regrowth in maize/Zea diploperennis hybrids is controlled in part by a locus on chromosome 2

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Perennial regrowth is a trait with major potential significance for agriculture and developmental biology yet it has not been thoroughly investigated in grasses. Most grass genera contain both annual and perennial species and Zea diploperennis is the closest perennial relative to maize which can cycle between vegetative and reproductive growth repeatedly. Taking advantage of the ability for maize and Z. diploperennis to form viable hybrids, we generated two F2 mapping populations by crossing the tillering maize inbreds P39 and Hp301 with Z. diploperennis. In a controlled greenhouse experiment using the P39/Z. diploperennis population, we discovered 104/489 (21%) of plants retained regrowth. Using a QTL-seq approach, DNA from 98 regrown and 30 non-regrown plants were separately pooled and the regrown pool was shown to be enriched for a region on the short arm of chromosome 2. CAPS markers were designed on this chromosome arm and subsequent genotyping supports the conclusion that this locus acts dominantly to control perennial regrowth. CAPS markers were also designed near a second locus on chromosome 7 previously shown to control this trait but this genotype segregated normally in regrown plants, suggesting this locus does not contribute to perennial regrowth in this mapping population. We have begun additional trials using both mapping populations under greenhouse and field conditions. In our field trial, 38/228 (16.7%) and 276/876 (31.5%) of plants from the P39 and Hp301-derived populations, respectively, have regrown following a hard freeze in early winter 2019. This regrowth does not statistically correlate with a variety of traits measured including flowering time and plant height but does correlate well with small tillers observed prior to freezing. Future work will involve genotyping these F2 individuals to perform QTL. We are also developing fine-mapping and inbred line resources by repeatedly back-crossing regrown individuals to the appropriate maize parent.

Funding acknowledgement: National Science Foundation (NSF)

P57

Phenotypic plasticity analyses reveal gene-environment interplay and enable genome-wide cross-environment prediction

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Identifying mechanisms and pathways involved in phenotypic plasticity and gene-environmental interplay is one of the long-standing challenges in biology. With extensive field-observed complex traits, environmental profiles, and genome-wide SNPs in three major crops (maize, wheat, and oat), our objective is to systematically explain, model, and predict crop performance in natural environments. A key step is the identification of environmental indices, which are biologically relevant and estimable for new environments. With the identified indices, the observed performance dynamics could be modeled by two reaction-norm parameters. These two parameters were segregating among populations. The varied performance among different environments could be attributed to specific genes responding to the environmental indices. Interestingly, different sets of genes were identified for describing the flowering-time plasticity in maize, while overlapping sets of genes were found in wheat and oat. Accurate and on-target performance predictions for diverse germplasm in new environments suggested the promising potential of this framework. This integrated framework enables a biologically informed dissection of the genetic architecture of complex traits and facilitates a genome-wide prediction of performance across varied environments.

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P58

Pleiotropy in maize inflorescence and leaf traits

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Inflorescence in maize (*Zea mays* L.) reflects the interplay between tassel, ear, and vegetative traits. To be successful, modern maize breeding requires optimal inflorescence, specifically the temporal syncing of pollen shedding and receptive silks. It is possible that some genetic components underlying these traits might contribute to two or more of them simultaneously, a phenomenon called pleiotropy. To ensure that the biology underlying inflorescence is understood and utilized as effectively as possible in maize breeding, it is critical that the advantages and disadvantages of these pQTL approaches are rigorously assessed using maize data. Therefore, this study performed univariate, multivariate, and PC-based GWAS using publicly available maize leaf and inflorescence trait data. Additionally, maize genotypic data were used to conduct a simulation study to aid in comparing the true and false positive detection rates of simulated quantitative trait nucleotides (QTNs) using these approaches. We hypothesized that the resulting peak-associated SNPs are linked to loci that simultaneously contribute to the variability of maize leaf and inflorescence traits.

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P59

Population genomics of sweet corn

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Sweet corn is predominantly defined by mutations within genes that affect the sugar content of the endosperm, such as *sugary1* and *shrunken2*. Due to these mutations and the importance of a few founder sweet corn varieties to modern germplasm, sweet corn is known to have undergone a major selection bottleneck. To better understand the genomic differences between modern sweet corn and other types of maize, we performed population genomic analyses using publicly available genotype by sequencing data across ~5,000 maize accessions, 590 of which are sweet corn. We performed population structure and phylogenetic analyses and found sweet corns to be predominately clustered within one main clade. By comparing populations of *sugary1* and *shrunken2* genotypes against populations of other types of maize, we looked for regions of the genome that are being constrained by selection. Calculations of Fst, Tajima's D, and XPCLR were used to define regions of the genome undergoing selection sweeps. As expected, we discovered genomic regions around the *sugary1* and *shrunken2* genes that were under strong selection. Interestingly, we also discovered areas of strong selection around genes which are known to play roles in flowering time, starch biosynthesis, and sugar transport. Overall, by looking at modern sweet corn germplasm through the eyes of population genomics we can further understand what areas of the genome are being selected for, which can help provide targets for the further genetic improvement of sweet corn.

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P60

Production and selection of quality protein popcorn hybrids using a novel ranking system and combining ability estimates

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Popcorn is agronomically inferior and genetically limited compared to other maize subspecies. To increase genetic diversity and improve popcorn agronomics, dent germplasm has been introduced to popcorn with limited success and generally, major loss of popping. Between 2013 and 2018, twelve Quality Protein Popcorn (QPP) inbreds containing Quality Protein Maize (QPM) and popcorn germplasm were produced that maintained popping while carrying the *opaque-2* allele conferring heightened kernel lysine. This is an opportune trait in the growing market for healthier snacks and a model for mining dent corn traits into popcorn. In this study, we crossed independently generated QPP inbreds to explore the effects of heterosis on popcorn protein and popping quality and plant agronomics and selected hybrids for further production. To rank and select hybrids, we utilized a novel hybrid-ranking model while examining the maternal and paternal combining abilities of QPP inbred lines and specific combining abilities of crosses for all traits. We also observed a biological manifestation of heterosis by categorizing hybrids by pedigree that resulted in a stepwise progression of trait improvement. These results corroborated our hybrid selection and offered insight in basic heterosis research. Popcorn quality and agronomic trait covariances also suggest the synergistic introgression of highly vitreous dent maize (QPM) into popcorn, providing a likely explanation for the successfully maintained vitreous endosperm, protein quality and popping traits in the presence of a completely remodeled proteome. This analysis is enabling QPP hybrid selection for large-scale comparison to currently marketed elite popcorn varieties.

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P61

QTL mapping for *Gibberella* stalk rot resistance in maize

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Stalk rot caused by *Fusarium graminearum* is one of the most destructive diseases in maize worldwide. Disease resistance to stalk rot is highly polygenic, and is influenced by genetic background and environmental factors. So far, there are two QTLs, *qRfg1* (*ZmCCT*) and *qRfg2* (*ZmAuxRP1*) associated with *Gibberella* stalk rot resistance, have been cloned. Here, we conducted QTL mapping in two $F_{5:6}$ recombinant inbred line populations and one $F_{2:3}$ population. To investigate if *qRfg1* and *qRfg2* loci segregating between the parental lines, PCR genotyping and sequencing were performed. We found that all the parental lines carry the susceptible *qRfg1* allele and an unknown *qRfg2* allele. The pathogen *F. graminearum* was artificially inoculated in the field and the disease was determined using four parameters, including disease scale, the total number of internodes discolored, the length of discolored stalk, and the number of internodes greater than 75% discolored. The four parameters were significantly positively correlated in different populations ($r=0.82$-0.96, $P \leq 0.001$). We found two significant QTLs were stable among different parameters. We will confirm the effect of each QTL using near-isogenic lines. Our results will help breeders to select resistance alleles in their breeding programs.

Gene / Gene Models described: *ZmCCT,ZmAuxRP1*; GRMZM2G381691,GRMZM2G063298
Funding acknowledgement: Natural Science Foundation of China

P62

Quantitative Trait Loci (QTL) that influence efficacy of *Teosinte crossing barrier1* (*Tcb1*) in maize

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Pollen cross-contamination can be problematic for breeders and producers of specialty varieties. Various physical methods to avoid contamination are difficult or ineffective. Several genetic systems are available to prevent contamination, including *Teosinte crossing barrier 1* (*Tcb1*). Silks of a plant possessing dominant *Tcb1-s* rejects pollen possessing the recessive allele *(tcb1)*. However, successful fertilization occurs when *Tcb1-s* pollen falls upon *tcb1* silks. The efficacy of dominant *Tcb1-s* can be reduced with repeated backcross, which may suggest there are modifiers of *Tcb1*. To find modifiers, we set up a QTL mapping experiment using 202 F1s of intermated B73 x Mo17 (IBM) recombinant inbred lines (RILs) crossed with pure breeding W22 *Tcb1-s* lines. After flowering, five ears from each F1 were pollinated by plants with recessive *tcb1* but having *R1* and *C1* color factor to produce anthocyanin colored kernels (B73, Mo17, and W22 are all recessive *r1 c1*). A second pollination was made the following day either by selfing or sibbing. Because half of the pollen from the F1 plants possessed *Tcb1-s*, the second pollination fills the ear out with colorless kernels. After harvest, ears were scored based on the proportion of colored kernels, indicating *Tcb1-s* blocking efficacy. QTL mapping was conducted using "R/qtl" package in R studio. Mean color contamination was the phenotype and genotypic data were obtained from MaizeGDB. QTL mapping yielded two significant peaks (LOD threshold = 3.62; 10000 permutation; = 0.05). One peak was on the long arm of chromosome 4 (4L; LOD = 4.43) and the other was on the short arm of chromosome 5 (5S; LOD = 4.1). These QTLs explained 16.1% of phenotypic variability and had an additive effect. We believe these results need to be taken with caution because of the peculiar conditions of last summer, which will be explained.

Funding acknowledgement: South Dakota Agricultural Experiment Station

P63

Resistance to four foliar diseases of maize in four DRIL populations tods four foliar diseases of maize in four DRIL populatioreur foliar diseases of maize in four DRIL populations

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Maize is infected by multiple pathogens and foliar diseases can reduce the effective photosynthetic area of the plant and ultimately reduce yield. Host plant resistance is an important solution since it is more economy and environmental-friendly than pesticide application. Developing lines that carry multiple disease resistance (MDR) is thus of great importance. This study focused on four major foliar diseases: Goss's wilt (GW), grey leaf spot (GLS), northern corn leaf blight (NCLB) and southern corn leaf blight (SCLB). In our study, linkage mapping for GW was conducted in four disease resistance introgression line (DRIL) populations that had Oh7B as the common recurrent parent. Mapping results for GW were combined with existing studies for GLS, NCLB and SCLB in the same four populations. We conducted 1) individual linkage mapping analysis to identify QTL specific to each disease and population; 2) joint-linkage mapping analysis to identify QTL that were consistent across the four populations for each disease; and 3) multivariate analysis to identify the potential MDR regions for each population. Using these approaches, we identified chromosomal regions that are statistically significant for each disease in different populations. By identifying QTL from both individual mapping and joint-linkage mapping analyses, we were able to find regions that either conferring resistance or susceptibility to multiple diseases across different populations.

P64

Rethinking rind penetration experiments

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Rind penetrometer experiments have been utilized for more than 100 years to estimate the bending strength and lodging resistance of several crop species. However, despite much research the method has not been adopted by commercial breeding programs and findings from past studies are often not reproducible. This is likely related to the lack of testing standards and operating procedures related to rind penetrometer experiments. Furthermore, selective breeding for rind penetration resistance has been shown to reduce lodging resistance in some cases. We conducted an engineering investigation of the rind penetration test to determine what material properties it measures and to determine the effect of puncture rate, and penetrating probe geometry. In addition, we developed a novel rind puncture methodology that can be used to obtain measurements of rind thickness, diameter, moment of inertia and a novel quantity termed the integrative puncture score. The method relies on a device that penetrates the plant with a small probe and records both the force and displacement of the probe as the stalk is punctured. A customized algorithm then analyzes the force displacement data and determines both the diameter and rind thickness of the plant with a high degree of accuracy. Results from this new methodology have been compared to diameter and rind thickness measurements obtained via high-resolution micro-CT scans from a set of 1000 corn stalks. The data shows good agreement with the more costly and time-consuming CT measurements ($R2 > 0.9$). In addition, the new method demonstrates far greater repeatability than caliper measurements which are highly confounded by the shape of the caliper and the amount of gripping force applied by the user. Furthermore, the novel integrative puncture score quantity was shown to be a far greater predictor of stalk strength that the typical rind penetrometer measurement.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P65

Sensing underground biological traits and the ecology of roots, rhizospheres and aridity: Phenotyping roots at the field plot scale across levels of the factor irrigation

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Maize breeding and the massive yield increase is one of the great technological achievements of the 20th century. Unfortunately the productivity of these elite hybrid genotypes requires unsustainably large Nitrogen and water inputs. Negative consequences of this intensive production system include degradation of aquatic habitats and the release of greenhouse gases. Moving away from this unsustainable local optimum provides an opportunity to consider approaches where large-scale production is managed to act as a carbon sink. I will present results from an ARPA-E funded ROOTS project, where we are examining genetic variation in maize roots, including their plasticity to water availability and maize root traits that will increase soil carbon sequestration. 370 inbred lines from the maize SAM panel (Leiboff et al 2015) were grown in replicates field plots with 2 levels of the factor irrigation, in both Maricopa, AZ and Fort Collins, CO in 2018 and 2019. Intact roots systems were sampled destructively by measuring root pulling force at 2 timepoints, then washed, imaged, and sampled for composition. Additional root traits were generated from feature extraction of the images. These traits, along with leaf water content and standard above ground traits, were analyzed to describe standard quantitative genetic parameters. Next, breeding values of traits were used for GWAS analysis and to identify relevant material for pre-breeding and testing the efficacy of enhanced root varieties for increasing sustainability of maize production in various target environments.

Funding acknowledgement: Department of Energy (DOE)

P66 @sekhonlab

Stalk bending strength is a reliable predictor of stalk lodging incidence in maize

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Stalk lodging in maize results in substantial yield losses that are preventable through genetic improvement. However, the lack of a robust and economical phenotyping method for assessing stalk lodging resistance is a major hindrance to breeding efforts and genetics studies. We have developed a phenotyping platform DARLING (Device for Assessing Resistance to Lodging IN Grains) that induces failure patterns consistent with natural stalk lodging events, and measures stalk bending strength in field-grown plants. Here we examine the association between data gathered from this new phenotyping platform with counts of stalk lodging incidence on 47 maize hybrids representing a subset of genetic diversity. For comparative purposes, we examine four additional predictive phenotypes commonly assumed to be related to stalk lodging resistance, namely, rind penetrometer resistance, cellulose, hemicellulose, and lignin. Lodging incidence data were gathered on 47 hybrids, grown in 98 distinct environments, spanning four years and 41 unique geographical locations in North America. Using Bayesian generalized linear mixed effects models, we show that stalk lodging incidence is associated with each of the five predictive phenotypes. Based on a joint analysis, we demonstrate that, among the phenotypes considered, stalk bending strength measured by the new phenotyping platform is the strongest predictive phenotype of naturally occurring stalk lodging incidence in maize, followed by rind penetrometer resistance and cellulose content. This study demonstrates that field-based measurements of stalk bending strength provide a reliable estimate of stalk lodging incidence. The stalk bending strength data acquired from the new phenotyping platform will be valuable for phenotypic selection in breeding programs and for generating mechanistic insights into the genetic regulation of stalk lodging resistance.

Funding acknowledgement: National Science Foundation (NSF)

P67 @HislopLily

Sugarcane mosaic virus resistance in the Wisconsin Sweet Maize Diversity Panel

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Sugarcane Mosaic Virus (SCMV) is a ssRNA viral disease that detrimentally impact yield in *Zea mays* across the United States. The goal of this experiment was to identify sweet maize lines that demonstrate resistance to SCMV, confirm the presence of genomic regions previously identified in field maize associated with resistance *(*the *Scmv1* and *Scmv2 loci*), or characterize resistant loci unique to sweet maize. This experiment was conducted with the support of NIFA funding. 8 replicates of 563 sweet maize accessions were tested for SCMV resistance. Plants were inoculated 14 days after planting and observed for signs of infection 28 days after planting. Forty six of the lines were found to be completely resistant to SCMV. We used an Illumina NextSeq500 to genotype 92971 marker sites using Genotyping By Sequencing for 417 of the tested accessions. Population structure of the panel was observed and GWAS was conducted to identify loci corresponding to resistance. The *Scm1* loci was confirmed with GWAS through the presence of significant loci at position S6_23727065 (p = 1.02e-12), flanking the *Scm1* gene located at Chr6: 24034207..24035363. Other loci of interest were identified through GWAS which will require further validation. This research will aid sweet corn breeders in selecting for SCMV resistance and increase information on publicly available germplasm that will be useful to future breeding projects.

Funding acknowledgement: United States Department of Agriculture (USDA)

P68

The genetic architecture of the maize progenitor, teosinte, and how it was altered during maize domestication

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The genetics of domestication has been extensively studied ever since the rediscovery of Mendel's law of inheritance and much has been learned about the genetic control of trait differences between crops and their ancestors. Here, we ask how domestication has altered genetic architecture by comparing the genetic architecture of 18 domestication traits in maize and its ancestor teosinte using matched populations. We observed a strongly reduced number of QTL for domestication traits in maize relative to teosinte, which is consistent with the previously reported depletion of additive variance by selection during domestication. We also observed more dominance in maize than teosinte, likely a consequence of selective removal of additive variants. We observed that large effect QTL have low minor allele frequency (MAF) in both maize and teosinte. Regions of the genome that are strongly differentiated between teosinte and maize (high F_{ST}) explain less quantitative variation in maize than teosinte, suggesting that, in these regions, allelic variants were brought to (or near) fixation during domestication. We also observed that genomic regions of high recombination and regions near transcription factors explain a disproportionately large proportion of heritable variance both before and after domestication. Finally, we observed that about 75% of the additive variance in both teosinte and maize is "missing" in the sense that it cannot be ascribed to detectable QTL and only 25% of variance maps to specific QTL. This latter result suggests that morphological evolution during domestication is largely attributable to very large numbers of QTL of very small effect.

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P69

Transcriptional reprogramming of defense related genes and pathways associated with Tar Spot disease in corn

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Tar Spot is a foliar disease of corn, caused by the obligate biotrophic pathogen Phyllachora maydis Maubl, and has recently emerged as an economic concern for corn production in the United states. Tar spot disease symptoms appear as small, raised, irregular-shape black lesions scattered on the surface of leaves, stalks, and husks of corn. Almost nothing is known about the molecular basis of the P. maydis-Zea mays pathosystem. In this study, we have carried out an RNA-Seq based study to analyze differential gene expression in susceptible (S), moderately susceptible (MS) and non-susceptible (NS) corn NAM (Nested Associated Mapping) parental lines under P. maydis infestation in natural field condition. Phenotypic observation of twenty-four NAM parental lines showed wide range of variation for tar spot resistance. Results suggested that four NAM lines, namely IL14H, MO17, W22, and W22:CRW1 are susceptible to P. maydis whereas TX303, KY21, and HP301 are moderately susceptible to P. maydis. In contrast, CML52, CML69 and CML103 are non-susceptible to P. maydis. Comparative transcriptomic analysis of susceptible, moderately susceptible and non-susceptible NAM lines revealed differentially expressed genes (DEGs) patterns associated with Tar spot. Further validation of the prominent genes associated with Tar spot was performed by qPCR. Significantly, our findings imply the existence of prominent genes and pathways associated with Tar Spot which will provide fundamental knowledge regarding poorly understood P maydis-Zea mays pathosystem.

Funding acknowledgement: United States Department of Agriculture (USDA)

P70 @KviveManiStha

Uncovering the genetic architecture of protein bound amino acids in maize kernels using GWAS combined with gene co-expression network analysis

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Seeds are a major source of protein in both human and livestock, however, seeds of major staple crops are deficient in several essential amino acids (EAA) which can lead to severe malnutrition, even if one's calories requirements are met. Many attempts to increase the EAA has demonstrated only limited success since seed can rebalance their amino acids composition even when major changes are introduced in their proteome. However despite the tight regulation within any given genotype seed amino acid composition display extensive natural variation. The latter can be utilized to uncover the genetic basis of the seed amino acids regulation and may identify new targets for seed amino acids biofortification. Hence we performed genome wide association study (GWAS) on 81 protein bound amino acids (PBAAs) – absolute levels, relative composition and derivative traits based on the amino acids metabolic pathways. Our analysis yielded 364 unique significant SNPs resulting in 1978 candidate genes from a 200kb window spanning the SNPs. To whittle down the candidate genes we have used an orthogonal dataset of a publicly available seed developmental gene coexpression dataset. Our underlying assumption was that genes that are part of the genetic architecture of the seed PBAA will likely to co-express in modules enriched in metabolic processes or protein related metabolism. We performed WGCNA with 1978 genes and did modules enrichment. The most significant enriched biological processes found were translation, cellular amino acid biosynthetic process and cellular respiration. Our results strongly suggests that the genetic variation in translational machinery itself can be involved in the natural variation of protein bound amino acid levels and composition. To date most seed amino acids biofortification relied on targeting metabolic pathway, TF or specific storage proteins. However our data suggests that new venue should be explored towards the manipulation of the translational machinery itself.

Funding acknowledgement: Missouri EPSCoR

P71

Unmanned aerial system image analysis for key physiological traits in maize

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Success in plant breeding relies on the ability to accurately estimate heritable traits for selection. This can be accomplished by genomic predictionmodels, but accurate traits are still necessary to train models. Currently, genotyping is no longer a barrier; rather, the main challenge is to break through the limitations of traditional phenotyping to rapidly acquire traits that can be used for modeling and prediction. Compared with traditional measurements, high-throughput phenotyping approaches are cheaper, fasterand less affected by human bias. In this study, we collected high-resolution color imagery and multi-spectral data using unmanned aerial systems (UAS) as carriers. Using features from the images we intend to extract features to estimate multiple quantitative traits, including plant height, plant number, canopy closure, and normalized difference vegetation index. Images were collected across multiple growth stages (early, vegetative, reproductive, mature) which will allow us to dynamically trace the growth and developmental differences among genotypes and enrich the dimension of these traits. Our aerial data will be modeled against hand-collected traits andused for genetic mapping, genomic prediction, and parameterizing crop growth models to integrate component traits and predict yield. Combining UAS platforms with image analysis provides a reliable, cheap and high efficiency method to capture large-scale phenotypic traits in the field.

Funding acknowledgement: IMPACTS NSF Research Training; Michigan Corn; Iowa Corn; MSU Plant Resilience Institute

P72

Untangling environmental determinants of crop performance combining genomic selection methods and weather data.

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Genomic prediction models have shown to be a powerful tool for predicting crop performance when applied to scenarios where the environmental conditions of the testing set are same than those from the training set (i.e., CV2: incomplete field trials and CV1: predicting newly developed lines). However, the performance of these models is highly affected when the environmental stimuli from the testing set is different to the training set. In these cases, the predictive ability decreases drastically reaching in some cases zero or even negative correlation units. We developed a method that examines the mean crop performance (yield and days to pollen) of populations of maize hybrids as function of variable time windows. The data used here is obtained from the Genomes To Fields (G2F) Initiative. Using only the environmental mean performance of environments in the training set, we conducted a search to identify the most informative window of time of crop season for predicting these means for 14 environmental covariates. Then, we use the same windows of time of these covariates for the environments in testing set for performing predictions of tested and untested genotypes in unobserved environments via the reaction norm model. The obtained results showed that the predictive ability was improved between 150-180% for the case when (1) we target the prediction of already-tested genotypes in unobserved environments (CV0) and (2) between 200-250% when predicting untested genotypes in unobserved environments (CV00). These results strengthen future hypotheses regarding the expansion of the proposed approach to other crops and traits across multiple breeding stages.

Funding acknowledgement: United States Department of Agriculture (USDA)

P73 @wvu_ahenderson

Use it or Lose it: Diversity in salt response following the domestication of sorghum landraces

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Soil salinity negatively impacts plant function, development, and yield. *Sorghum bicolor* is a staple crop known to be drought tolerant, to have adapted to a variety of conditions, and to contain significant standing genetic diversity, making it an exemplary species to study phenotypic and physiological variation in salinity tolerance. In our study, a diverse group of sorghum landraces and accessions was first rank-ordered for salinity tolerance and then individuals spanning a wide range of response were analyzed for foliar proline and ion accumulation. We found that, while proline is often a good indicator of osmotic adjustment and is historically associated with increased salt tolerance, proline accumulation in sorghum reflects stress-response injury rather than acclimation. When combining ion profiles with growth responses and stress tolerance indices, the variation observed in tolerance was similarly not a sole result of Na^+ accumulation, but rather reflected accession-specific mechanisms that may integrate these and other metabolic responses. When we compared variation in tolerance to phylogenetic relationships, we conclude that the most parsimonious explanation for the variation observed among accessions is that salinity tolerance was acquired early during domestication and was subsequently maintained or lost in diverged lineages during improvement in areas that vary in soil salinity. To characterize the genetic underpinnings of variation in salinity tolerance, we used a recombinant inbred line population derived from the wild *S. propinquum* and domesticated *S. bicolor* (Tx7000, landrace durra) to identify candidate loci.

Funding acknowledgement: United States Department of Agriculture (USDA)

P74

Whole-genome prediction for tocochromanol levels in maize grain: accuracy comparison among different models, traits, and populations

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Tocochromanols, collectively called vitamin E, are important target traits for biofortification of maize grain, given that they play a variety of important roles in human health and nutrition. Tocochromanols are highly heritable traits, with most of the large-effect loci genetically controlling these traits resolved to the level of a single gene. However, the genes underlying other smaller effect loci remain unknown. Whole-genome prediction (WGP) based selection, which incorporates both large- and small-effect loci across the genome, is a promising method to increase gains from selection for tocochromanol grain traits. In this study, we examined the potential of WGP for nine tocochromanol traits in maize grain by utilizing the of maize Ames panel (n = 1451) and Goodman-Buckler panel (n = 246) s of diverse inbred lines. Prediction accuracies of BayesB and Bayesian ridge regression (BRR) were compared within and between panels. The within-panel cross-validation results from the Ames panel showed that BayesB had a minor advantage over BRR. Prediction abilities ranged from 0.49 to 0.57 for BRR and 0.55 to 0.75 for BayesB in the Ames panel, while ranging from 0.26 to 0.52 for BRR and 0.27 to 0.52 for BayesB in the Goodman-Buckler panel. The results from WGP between panels also suggested BayesB as a better model, as the prediction ability from Ames to Goodman-Buckler in BayesB was greater than that in BRR by 0.09, and that from Goodman-Buckler to Ames was greater by 0.03. Transcriptome-based prediction will be explored alone and in combination with SNP marker data to further enhance prediction accuracy. These models are expected to connect regulatory variation between populations to help enhance prediction accuracy of tocochromanol traits in maize grain.

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P75

3D genome architecture coordinates trans and cis regulation of differentially expressed ear and tassel genes in maize

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Maize ears and tassels are two separate types of inflorescence which initiate by similar developmental processes but gradually develop distinct architectures. However, coordinated trans and cis regulation of differentially expressed genes determining ear and tassel architecture within the 3D genome context is largely unknown. We identify 56,055 and 52,633 open chromatin regions (OCRs) in developing maize ear and tassel primordia using ATAC-seq and characterize combinatorial epigenome features around these OCRs using ChIP-seq (for H3K4me3, H3K9ac and H3K27me3 chromatin marks), Bisulfite-seq (for DNA methylation) and RNA-seq datasets. Our integrative analysis of coordinated epigenetic modification and transcription factor (TF) binding to OCRs highlights the cis and trans regulation of differentially expressed genes in ear and tassel controlling inflorescence architecture. We further systematically map chromatin interactions at high-resolution in corresponding tissues using in situ digestion-ligation-only Hi-C (DLO Hi-C) method. The extensive chromatin loops connecting OCRs and genes provide a 3D view on cis and trans regulatory modules responsible for ear and tassel specific gene expression. Our comprehensive epigenome annotations and 3D genome maps serve as valuable resource, and provide a deep understanding of the complex regulatory mechanisms of genes underlying developmental and morphological diversities between maize ear and tassel.

Funding acknowledgement: National Science Foundation (NSF)

P76

Ds mutagenesis of the benzoxazinoid gene cluster on Chromosome 4S

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Benzoxazinoids (BX's) are indole-derived secondary metabolites that provide protection from fungal pathogens and insects in maize. The genes encoding the core BX pathway (*Bx1 -Bx5)* reside in a tightly-linked gene cluster on Chromosome 4S. The protein encoded by *Bx1* initiates BX biosynthesis by converting indole-3-glycerol phosphate to indole. Indole is subsequently oxidized by *Bx2*, a cytochrome P450, followed by sequential enzymatic reactions encoded by the genes *Bx3 - Bx14* that synthesize a variety of derivative compounds which vary in their toxicity to insects. A *Dissociation (Ds)* knockout mutation in *Bx1* was identified in the maize inbred W22 that nearly abolishes synthesis of BX metabolites, but *bx2::Ds* mutants continue to produce significant levels of some downstream BX toxins relative to wild-type plants. The mechanism underlying this unexpected finding is unclear. Other P450's in the pathway (i.e. *Bx3, Bx4, or* Bx5) could conceivably use the indole provided by *Bx1* as a substrate, but these enzymes have been demonstrated to show substrate specificity in yeast. Currently, no *Bx4 or* Bx5 mutant lines exist, preventing the further characterization of these genes. A targeted *Ds* mutagenesis screen was recently initiated to create novel *bx::Ds* alleles in this gene cluster. This approach was taken because *Ds* preferentially reinserts into genetically-linked loci, and the creation of higher-order *Bx* mutant lines by identifying recombinants is technically unfeasible due to the tight genetic linkage between these genes. To date, three novel *bx1::Ds* insertion alleles have been isolated after screening approximately 1200 gametes, demonstrating the economy, speed, and practicality of this approach. Characterization of *bx1::Ds bx2::Ds* double mutant lines is currently underway. Results from this study should provide some insight into the need for *Bx* P450 genetic redundancy and could further refine the biochemical mechanisms involved in this important maize defense pathway.

P77

A set of maize mutant lines with single *Ds-GFP* insertions spread throughout the genome

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The availability of a mutant line in which a single gene has been disrupted gives biologists a powerful tool in understanding that gene's action. Our NSF-PGRP-funded project has generated and sequence-indexed a large collection of marked *Ds-GFP (Ds*)* transposon insertions in transgenic maize by taking advantage of next-generation sequencing (NGS) technologies. A set of 16,000 *transposed Ds** (*trDs**) elements was selected for mapping to the reference genome using a strategy that exploits unique sequences in the *GFP*-marked *Ds** element to specifically amplify maize sequences adjacent to the *trDs** element (*dsg* sites), thereby avoiding amplification of junctions from endogenous elements. Of the 16,000 *dsg* sequences, 14,184 (~90%) were unequivocally indexed to a specific cell in the 3-D pool and mapped to the maize genome, illustrating the efficiency of NGS and 3-D pooling for sequence-indexing a large collection of *Ds** insertions. All the information on sequence-indexed *dsg* sites, including their matching transposant lines, are shared via a web browser hosted at Montclair State University (http://acdsinsertions.org). We have set up a MaizeGDB-compatible relational database for the sequence-indexed transposant lines by using the freely available MySQL software. The user interface includes web searching forms written in Java and BLAST search tools. All the stocks are available from the Maize Genetics Co-op. Although the resource has only been presented at meetings, the Co-op has already received requests for 518 *dsg* stocks and the Dooner lab has filled 50 additional requests for stocks before they became available from the Co-op. Successful use of these stocks has already been reported by N. Garcia, who identified an insertion in a highly conserved TTI2 cochaperone gene that produced a germless phenotype (PNAS 2017), and by students in John Fowler's lab, who have identified insertions in pollen-expressed genes that affect paternal transmission (Maize Genetics Conference 2019).

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P78

DNA methylomes of the NAM founders

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Phenomena such as genomic imprinting, paramutation, and transposon cycling demonstrate that variation in gene expression can have epigenetic causes. DNA methylation is a strong indicator of the epigenetic state as well as functional status of genetic elements in the genome. The recently completed assembly of the 25 maize NAM founder genomes (https://nam-genomes.org/) provides an opportunity for accurate methylation analysis of these genomes to explore relations between DNA methylation and gene expression. We are producing 20X coverage, 150nt paired-end methylome sequencing libraries from the complete set of NAM founders as well as B73 using the NEB EM-seq method, combined with mRNA-seq of each in three replicates. We are using the data to explore methylation variation between genomes and its relation to gene expression variation and to annotate putative cis-regulatory elements, which we will release to the maize community with the raw data as soon as they are curated.

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P79

Distal tandem repeats of the *Pl1-Rhoades* allele share paramutation and enhancer-like properties

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In maize, paramutations result in meiotically-heritable regulatory changes of certain alleles including *Pl1-Roades* which encodes a transcription factor required for anthocyanin production. A strongly-expressed *Pl1-Rhoades* allele (denoted *Pl-Rh*) is suppressed in *trans* when combined with a transcriptionally and post-transcriptionally repressed *Pl1-Rhoades* allele (denoted *Pl′*), and both alleles are subsequently sexually transmitted in a repressed state. Mutant screens have identified at least sixteen loci whose functions are *required to maintain repression (rmr)* of *Pl′*. Known RMR proteins include subunits of a plant specific DNA-dependent RNA polymerase (Pol IV) and others that produce 24 nucleotide (24nt) RNAs which, in *Arabidopsis*, facilitate repressive chromatin modifications. Although structural analyses of other maize alleles subject to paramutation identify distinct direct repeats as a common feature, the mechanisms and specific sequences that subject alleles paramutation remain largely unknown. Here we report that a region ~14kb downstream of the *Pl1-Rhoades* coding sequence conferring strong expression and paramutation behavior consists of five bidirectionally transcribed direct repeats that loop to interact with the promoter. Both Pol II transcription and RNA levels from these repeats correlates with *Pl1-Rhoades* expression. In addition, Pol IV-dependent 24nt RNAs are produced from unique sequences within the repeats indicating RMR proteins operate at this feature, possibly directing changes in nucleosome positioning observed between *Pl-Rh* and *Pl′* states. The relationship between these repeats and *Pl1-Rhoades* parallels that of seven direct repeats ~100kb upstream of the *B1-Intense* (*B1-I*) allele which promote both strong expression and paramutation. How these repeats act as long-distance enhancers that facilitate paramutations remains an open question. We are currently identifying additional molecular features distinguishing alternate *Pl1-Rhoades* enhancer states and characterizing the processes controlling these regulatory transitions to determine how grass species generate, maintain, and transmit meiotically-heritable epigenetic regulatory variation.

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P80

Epigenetic regulation of the BTF3-like transcription factor in drought stressed maize seedlings

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Transcription factors (TFs) are important regulators of the transcription of genes involved in plant growth and development, as well as responses to environmental stimuli. Like many crop plants, *Zea mays* (maize) is often subjected to extreme environmental stresses, such as drought, extreme cold and high salinity, that negatively impact crop yield. Characterizing the molecular changes that occur at TF genes under environmental stress conditions could allow us to manipulate these responses to improve crop yield under stress. While many studies have identified the expression changes of drought-responsive genes, little is known about the environmentally-induced epigenetic modifications associated with their transcription. In this study, we used the Drought Stress Gene Database (Drought DB) and the Maize Genetics and Genomics Database (MaizeGDB) to identify maize orthologs of plant genes known to be transcriptionally responsive to drought stress. Using two independent techniques, we identified drought stress-induced epigenetic variations (DNA methylation) in the promoter region of the maize Basal Transcription Factor 3 (ZmBTF3) gene. We are characterizing the sequence contexts of these methylation patterns in relation to a gene-proximal transposable element and the potential regulation by the MOP1-mediated RdDM pathway. This study will help us gain a better understanding of how the maize BTF3-like gene is regulated through epigenetic modifications.

Gene / Gene Models described: *ZmBTF3*; Zm00001d013999
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P81

MOP1-mediated epigenetic regulation of ABA-induced transcriptional responses in maize.

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Plants are often subjected to extreme environmental conditions and must adapt rapidly. The phytohormone abscisic acid (ABA) accumulates under abiotic stress conditions, signaling transcriptional changes that trigger physiological responses. Epigenetic modifications are also required to facilitate transcription, particularly at genes exhibiting temporal, tissue-specific and environmentally-induced expression. In maize (*Zea mays*), MEDIATOR OF PARAMUTATION 1 (MOP1) is required for progression of an RNA-dependent epigenetic pathway that regulates transcriptional silencing of loci across the genome. MOP1 function has been previously correlated with genomic regions adjoining particular types of transposable elements and genic regions, suggesting that this regulatory pathway functions to maintain distinct transcriptional activities within genomic spaces, and that loss of MOP1 may modify the responsiveness of some loci to other regulatory pathways. As critical regulators of gene expression, MOP1 and ABA pathways each regulate specific genes. To determine if loss of MOP1 impacted ABA-responsive gene expression in maize, *mop1-1* and *Mop1* homozygous seedlings were subjected to exogenous ABA and RNA-sequencing. A total of 3,242 differentially expressed genes (DEGs) were identified in four pairwise comparisons. Overall, ABA-induced changes in gene expression were enhanced in *mop1-1* homozygous plants. The highest number of DEGs were identified in ABA-induced *mop1-1* mutants, including many transcription factors; suggesting multifaceted regulatory scenarios including direct and indirect transcriptional responses to genetic disruption (*mop1-1*) and/or stimulus-induction of a hierarchical, cascading network of responsive genes. Additionally, a modest increase in CHH methylation at putative MOP1-RdDM loci in response to ABA was observed in some genotypes, suggesting that epigenetic variation might influence environmentally-induced transcriptional responses in maize.

Gene / Gene Models described: *mop1*; Zm00001d003378
Funding acknowledgement: National Science Foundation (NSF), Start-up funds from the University of Washington Bothell School of STEM

P82

Non-Mendelian segregation of maize embryo-lethal mutants: Genetic or epigenetic?

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We are identifying maize genes that play crucial roles in maize embryo development by genetic screening for EMS-induced embryo-specific (emb) mutants altered in embryo morphogenesis. We will present pedigree lineage results obtained with the UND-9 mutant, which was produced by EMS treated W22 pollen placed on B73 silks. The resulting kernels were planted and the progeny were self-pollinated, producing ears that were screened for emb mutant embryos. One of these progeny (GG22-5@) was the source of UND-9. Progeny plants grown from this ear (NN365-374) were self-pollinated. The resulting ears segregated normal appearing kernels with emb embryos at frequencies of 5.0%, 8.0%, 13.0%, 26.9% and 44.5%, as well as other frequencies. The heritability of these non-Mendelian ratios and transmission frequencies through the male (pollen) and female (embryo sac) gametophytes was examined by double pollination of ears (selfing of half of an ear and crossing by B73 onto the other half) and out crossing of pollen onto B73. The resulting progeny were all self-pollinated and scored for segregation of kernels with emb embryos. A summary of this scoring follows: 1) Both the low frequency and the high frequency segregation trait is heritable through three generations of transmission; 2) The abnormal segregation frequencies transmit equally well through the male and the female gametophytes, and when compared with the progeny of selfing. Conclusions: The abnormally low segregation frequency may reflect the presence of a linked duplicate locus on the B73 chromosome homolog; but this does not explain the large variability in the low segregation frequency. Because the emb is a recessive trait, its reduced transmission is not likely to have an epigenetic cause. The abnormally high segregation frequencies may result from an epigenetic mechanism that silences the normal allele with varying frequency.

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P83

Packaging centromere-specific integrase into proteinaceous nanoparticles

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The centromeric retrotransposon (CR) of maize offers a natural system to package DNA and insert it into a discrete location present on every eukaryotic chromosome. *In vivo*, CR Gag proteins self-assemble into a virus-like particle (VLP) with a proteinaceous shell that encloses CR proteins and ssRNA. Following reverse transcription, the CR integrase (IN) inserts the resulting dsDNA into the centromere. Autonomous VLP assembly plus centromere-specific IN activity provide a potential avenue for *in vitro* packaging of transgenes that may increase their stability following transformation and allow targeted integration. Advantages of centromere targeting include a lack of recombination activity in that chromosomal region that allows the stacking of multiple traits. To determine the feasibility of using an *in vitro* CR system for future work in transgene delivery, constructs containing CR Gag covalently (fused to C-terminus) or noncovalently (E/K-coil) linked to the CR IN were designed, expressed in *Escherichia coli*, assembled into VLPs under various conditions and analyzed under the electron microscope. Gag VLPs (d=~20-30 nm) form in 0.1, 0.5, 1.0 and 2.0 M NaCl. Interestingly, Gag covalently linked to the integrase without the addition of a linker appears to form VLPs of similar size. This may indicate a cargo capacity and VLP T-number conducive to a 1:1 Gag:IN ratio and/or prevailing strength of Gag-Gag over IN-IN interactions. The results of this study suggest that enzymes of up to at least 56 kDa can be concentrated within the CR VLP in a 1:1 Gag:Enzyme ratio, ensuring the presence of enzyme within every VLP.

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P84

Parental RNA polymerase IV affects hybrid performance

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Maize RNA polymerase IV (RNAP IV) affects genome-wide RNAP II-based transcription[1] and defines developmental expression profiles of specific alleles[2-4]. RNAP IV also mediates and maintains transcriptional repression of alleles undergoing paramutation[5] – a meiotically-heritable change in gene regulation facilitated by *trans*-homolog interactions[6]. Paramutation behaviors of certain *red1* and *purple plant1* alleles represent clear examples of single locus heterosis[7,8], fueling speculations that similar behaviors might contribute to hybrid vigor[8,9]. Results here show that RNAP IV absence in B73 parents negatively impacts heterotic traits of B73/Mo17 hybrids. Hybrid plant height gains are contributed by RNAP IV function in either parent yet only maternal RNAP IV affects heights of non-hybrid offspring. Remarkably, significant hybrid grain yeilds were dependent on maternal RNAP IV. Understanding the genomic features that recruit RNAP IV and the various roles both RNAP IV and RNAP IV-generated 24 nucleotide RNAs play in defining heritable regulatory variation therefore promises novel opportunities for predicting and controlling biomass production. 1. Erhard *et al.* 2015 *Genetics* | 2. Parkinson *et al.* 2007 *Dev Biol* | 3. Erhard *et al.* 2009 *Science* | 4. Erhard *et al.* 2013 *Plant Cell* | 5. Hollick *et al.* 2005 *Genetics* | 6. Hollick 2017 *Nat Rev Genet* | 7. Styles and Brink 1969 *Genetics* | 8. Hollick and Chandler 1998 *Genetics* | 9. Kermicle and Alleman 1990 *Development*

Gene / Gene Models described: *r1, pl1*; GRMZM5G822829, GRMZM2G701063
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P85

Population-level analysis reveals the widespread occurrence and phenotypic consequence of DNA methylation variation not tagged by genetic variation in maize

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DNA methylation can provide a source of heritable information that is sometimes entirely uncoupled from genetic variation. However, the extent of this uncoupling and the roles of DNA methylation in shaping diversity of both gene expression and phenotypes are hotly debated. Here, we investigate the genetic basis and biological functions of DNA methylation at a population scale in maize. We perform targeted DNA methylation profiling for a diverse panel of 263 maize inbred genotypes. All genotypes show similar levels of DNA methylation globally, highlighting the importance of DNA methylation in maize development. Nevertheless, we identify more than 16,000 differentially methylated regions (DMRs) that are distributed across the 10 maize chromosomes. Genome-wide association analysis with high-density genetic markers reveals that over 60% of the DMRs are not tagged by SNPs, suggesting the presence of unique information in DMRs. Strong associations between DMRs and the expression of many genes are identified in both the leaf and kernel tissues, pointing to the biological significance of methylation variation. Association analysis with 986 metabolic traits suggests that DNA methylation is associated with phenotypic variation of 156 traits. There are some traits that only show significant associations with DMRs and not with SNPs. These results suggest that DNA methylation can provide unique information to explain phenotypic variation in maize.

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P86 @BioJlynn

Single-locus immunoprecipitation proteomics (SLIP) analysis of proteins interacting with the *b1 tandem repeat* paramutation sequence

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The control of gene expression by *cis*-regulatory DNA sequences is a shared genomic feature across the kingdoms of life. The maize *booster1* gene (*b1*) is a mechanistic model for control of gene expression from a distal *cis*-element. Two epialleles of *b1* produce distinct tissue-specific pigmentation phenotypes and have been well studied over the past 60 years. The *B-Intense* (*B-I*) allele is highly transcribed, while the *B'* is lowly transcribed. When combined in the same the nucleus, *B'* paramutates *B-I*, converting the *B-I* allele to *B'*. Enhancement and paramutation of *b1* requires a hepta-tandem repeat sequence (*b1TR*) located ~100kb upstream of the *b1* TSS. Differences in DNA-methylation and chromatin structure has been observed at the *b1TR* in *B'* and *B-I* plants. It is likely that enhancement and silencing of *b1* each rely on a distinct complement of proteins that may interact directly with the *b1TR* and modify local chromatin structure. To identify *b1TR*-interacting proteins, we have developed a single-locus immunoprecipitation proteomics (SLIP) approach that utilizes a transgenic copy of the *b1TR* adjacent to a GAL4-Upstream-Activation-Sequence to capture *b1TR* chromatin proteins using mass-spectrometry. By comparing proteins identified at the *b1TR* with proteins identified at non-specific control sequences, we can narrow our search for putative *b1TR*-interacting proteins associated with enhancement, silencing, and paramutation. Using our method, we have generated a list of proteins that may play a role in gene regulation through interaction with the *b1TR*. Many of these proteins lack functional annotation or have not been shown to function as epigenetic regulators. Follow up experiments are being performed to confirm direct interaction with the *b1TR* and to determine the function of these proteins in *b1* paramutation. The identification of proteins interacting directly with the *b1TR* will increase our mechanistic understanding of paramutation and provide targets for discovery of paramutation loci.

Gene / Gene Models described: *b1*; Zm00001d000236
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P87

Teosinte pollen drive: A novel meiotic drive system in *Zea mays*

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Mendel's law of equal segregation maintains that alleles of any given locus will be inherited in the progeny with equal probability. In principle, faithful completion of meiosis and subsequent gametogenesis enforces this outcome. However, it has become increasingly clear that these rules are violated in a diverse range of eukaryotes. Meiotic drive refers to a type of intragenomic conflict whereby certain loci bias their transmission at the expense of competing gametes. Such phenomena have been observed in a wide variety of organisms including fungi, plants, and mammals. Because these systems tend to operate at the reproductive level, often in the absence of deleterious somatic effects, they can have rapid and profound effects on genome composition and the evolution of natural populations. Here, we identify and characterize a novel meiotic drive system occurring in the pollen of hybrids between *Zea mays* and *Zea mays ssp. mexicana*. By combining a simple and highly scalable method for the sequencing of individual pollen grains with other genetic, genomic, and molecular techniques, we have characterized the general mechanism underlying the drive phenotype and further dissected the relative contributions of each component.

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P88 @SigmaFacto

The divergence of maize NAM founders revealed by syntenic LTR retrotransposons

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Assembly of the 26 maize nested association mapping (NAM) population founder lines provides an opportunity to analyze transposable elements (TEs) in these diverse genotypes. Using the Extensive de-novo TE Annotator (EDTA) pipeline, we annotated both structurally intact and fragmented TEs in each of the genomes, including LTR retrotransposons (LTR-RTs), TIR elements, and Helitrons. Overall, 88.5% of the genomes were annotated as TE sequences, while LTR-RTs contribute more than 75%. The frequent insertional activity of LTR-RTs creates thousands of genetic footprints, and the age of each LTR-RT can serve as timed markers that continuously record the genomic divergence of maize lines. By resolving the syntenic status of LTR-RTs, we propose a model-free method for unbiased estimation of genomic distance that is missed in SNP-based methods. We then estimated the regional genetic distance across the genome, which effectively recovers the shared evolutionary history between NAM founders. Further estimation of genomic divergence between maize and teosinte genomes revealed large-scale introgression during the domestication and improvement of maize.

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P89

The impact of Terminal Inverted Repeat (TIR) elements in maize genomes

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Terminal Inverted Repeat (TIR) elements are Class II DNA TEs which transpose by "cut and paste" mechanism. In maize, there are five major superfamilies of TIR elements: *hAT* (DTA), *CACTA* (DTC), *PIF/Harbinger* (DTH), *Mutator* (DTM), and *Tcl/Mariner* (DTT). These TEs play important roles in genome structure and evolution, including generating allelic diversity, regulating gene expression and inducing structural variation. TIR elements can undergo standard transpositions of a single TE, and may also undergo alternative transpositions, which involve the termini of two distinct elements. Alternative transpositions of the maize *Ac/Ds* family can generate diverse genome rearrangements including duplications, deletions, inversions and translocations. In previous work, we developed TIR-Learner, a new annotation pipeline for TIR elements. Here, we aim to identify active TIR elements in maize genomes that may induce genome instability at specific loci via alternative transpositions. Annotated TIR elements were searched against protein database and the Conserved Domain Database (CDD); TEs that carry conserved transposase domains were selected as candidate autonomous elements. Expression of these candidate elements was then queried by analysis of published RNA-seq data. The results showed that some putative autonomous TIR elements are expressed at the transcription level. These potential active elements were then used to search published genome sequence for TE configurations capable of undergoing alternative transposition events. The results may identify loci that are unstable and prone to undergo rearrangements. These results provide new insight into the impact of TIR elements on maize genome structure and genome evolution.

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P90

The role of RNA polymerase IV in effecting heritable regulatory changes

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Paramutation is a behavior in which one parental allele at a given locus facilitates a meiotically heritable change at the other. This behavior occurs at specific alleles of multiple maize loci encoding transcriptional activators of flavonoid biosynthesis including *red1* (*r1*), *booster1* (*b1*), *purple plant1* (*pl1*), and *pericarp color1* (*p1*). The *Pl1-Rhoades* (*Pl1-Rh*) allele can exist in a highly expressed reference state (*Pl*) or an epigenetically repressed paramutant state (denoted *Pl'*). *Pl1-Rh* alleles in the *Pl'* state often revert to *Pl* in plants homozygous for a null mutation (*rmr6-1*) in a gene encoding the RNA polymerase (RNAP) IV largest subunit indicating that RNAP IV maintains the heritable information specifying *Pl1-Rh* paramutation. Because RNAP IV both sources 24 nucleotide RNAs and controls the heritable regulatory status of *Pl1-Rh*, we hypothesize that RNAP IV, and potentially small RNAs in general, condition the inheritance of genome-wide regulatory information. To test this idea, seedling RNA-Seq profiles of BC_5 progeny from sibling non-mutant (*Rmr6-B73* homozygotes) and *rmr6-1* mutant fathers were compared to identify heritable RNAP IV-dependent effects. RNA abundances of 95 genes were either significantly enriched or depleted in the progeny of mutant *rmr6-1* fathers as compared to progeny of non-mutant fathers. These differences point to alleles, like *Pl1-Rh*, whose dysregulation in the absence of RNAP IV might persist through meiosis. To control for possible haploinsufficiency or linkage drag effects, RNA-Seq profiles of near-identical heterozygous *rmr6-1* BC_5 progeny from sibling *rmr6-1* mutant and heterozygous fathers were also compared. Future studies aim to determine the role of environmental factors in effecting heritable changes at *Pl1-Rh* and other alleles identified by RNA-Seq profiling as Bernard Mikula showed that the extent of heritable changes brought about by paramutations occurring at *r1* is influenced by the environment during early development.

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P91 @mcstitzer

Transposable element accumulation reduces fitness in maize

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Transposable elements (TEs) are mobile DNA sequences found in all eukaryotic genomes. Their abundance scales linearly with genome size, and in maize, they make up the majority of DNA in the genome. The abundance and position of individual TE copies differs extensively between maize individuals, but it is unclear the degree to which these differences in TE content affect plant phenotypes and fitness. In organisms with smaller genomes, like yeast and Drosophila, experimental evidence for the deleterious impact of TEs on fitness is unequivocal. Yet, for larger genomes with many more TEs, it is unclear whether these same fitness costs exist. Here, we use recombinant inbred lines from two maize biparental mapping populations phenotyped in 4 to 14 environments to measure the impact of TE copy number and polymorphism on fitness related traits. From whole genome assemblies of parental lines, we project TE copy number to genotyped recombinant inbred lines (RILs), and associate this TE copy number to phenotypes. After correcting for parental ancestry, we find that TE copy number is negatively associated with fitness-related traits like seed number and plant height. There is no correlation for traits not directly tied to fitness, such as leaf width. Notably, movement of TEs during the generations of self-fertilization required to generate these populations may contribute to the deleterious cost of TEs, as bursts of new transposition are evident in whole genome resequencing of advanced generation RILs. TE copy number is also associated with changes in expression of host genes, most often dysregulation outside of parental values. Our findings suggest that even though the maize genome has evolved to tolerate its TE passengers, TEs still act as parasites with dramatic consequences.

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P92 @PaulSharu

Transposon-induced inversions activate tissue-specific gene expression in maize

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Genomic rearrangements caused by alternative transpositions of multiple copies of *Activator (Ac)* DNA transposon present close together can disrupt gene function, change gene expression and form chimeric genes. We have isolated an allele *p1-wwB54* which has a partially deleted *p1* with insertion of *Ac* and *fAc* (*fractured Ac*) elements and an intact *p2* gene. We study the molecular structures of genomic rearrangements such as Composite Insertions, Deletions and Inversions caused by alternative transposition of these elements. The *p1* and *p2* genes are expressed in different tissue types. *p1* is expressed in kernel pericarp and cob glumes whereas *p2* is expressed in anther and silk, but not kernel pericarp and cob. The kernel pericarp is white due to non-functional *p1* gene. From *p1-wwB54,* we identified 19 independent inversions that change the position of a *p1* enhancer and activate expression of *p2* in the kernel pericarp, resulting in red kernel color. Structures of these inversions were resolved using PCR and Southern blotting, revealing presence of multiple copies of the *p1* enhancer in most cases. Multiple copies of enhancer also correspond to darker red kernel phenotype compared to alleles with single copy enhancers. We are currently testing how the multiple *p1* enhancers may be interacting among each other and with the *p2* promoter from varying and long distances to activate tissue specific gene expression. For animations of Alternative Transposition, see; http://thomasp.public.iastate.edu/transposition.html

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P93 @sna8

Uncovering imprinting of PAV genes and transposable elements using whole genome assemblies

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Genomic imprinting, or parent-of-origin biased gene expression, has been reported in the endosperm of many plant species. Imprinted genes exhibit preferential expression of the maternal or paternal allele and are often associated with allele-specific variation for DNA methylation or histone modifications. While some genes have conserved imprinted expression patterns across plant species, there are also examples of genes with imprinting status that varies across different haplotypes within the same species, leading to the question of how genetic variation and epigenetic variation interact to influence gene expression patterning. Assessing how genomic imprinting arises has been limited by the technical difficulties associated with calling allele-specific expression using a single reference genome where only loci that are shared between genotypes and contain SNPs that distinguish alleles can be assayed. To overcome this limitation, we performed RNA-seq on reciprocal crossed hybrid endosperm between maize genotypes with complete, de-novo genome assemblies. By mapping to whole genome assemblies of both parents and comparing expression across reciprocal crosses, we were able to identify imprinted expression of both shared and non-shared portions of the genome. This allowed the identification of imprinting for several genes with present-absent variation (PAV) among genotypes. This approach also enabled the analysis of imprinting at transposable elements. We find > 100 imprinted transposable elements, over half of which show presence/absence variation in genome content between genomes. Interestingly, while genes show both maternally-biased and paternally-biased patterns of imprinting, the vast majority of imprinted transposable elements exhibit maternally-biased expression. By analyzing examples variable imprinting across genotypes, we are beginning to define the mechanisms that underlie parent-specific expression patterns in the endosperm.

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P94

Why CR2s are more prominent in domesticated maize than teosinte

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Maize centromeres are characterized by multiple independent neocentromere formations near the ancestral centromeres after domestication, or in approximately the past 10,000 years. These neocentromeres were subsequently colonized by CR2 elements, a subfamily of evolutionarily related centromeric retrotransposons (CR). Hufford et al. 2012 first described CR2 abundance in domesticated maize being much higher than in teosinte. A total of 1,746 CR elements were extracted from the B73 RefGen_v4CEN assembly and analyzed. SNP analysis of CR2 elements over time revealed expansion of a single haplotype in the past 100,000 years. It is unclear if this is due to early selection of a single centromere, exceptionally high expression of this element, improved transposition frequency, or genetic drift. Different recombinant CR2 elements with target site duplications suggest recombination during transposition, which complicates phylogenetic analyses.Hufford, Matthew B et al. “Comparative population genomics of maize domestication and improvement.” Nature genetics vol. 44,7 808-11. 3 Jun. 2012, doi:10.1038/ng.2309

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P95

Zygosity-dependent transgene silencing in maize

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Much of the basic molecular mechanisms of small RNA (sRNA)-mediated gene silencing have been elucidated in *Arabidopsis* using transposable elements (TE) (reviewed in Liu and Chen, 2016 Mol Plant (6) 826-836; Cuerda-Gil and Slotkin, 2016 Nat Plants 2:16163). These findings are generally applicable to transgene silencing in crops such as maize (Sidorenko *et al*., Nat Plants 2017 3(11):875-884). However, specific cases of transgene silencing in maize do not always follow examples of TE inactivation from *Arabidopsis*. Here, we investigated a case of zygosity-dependent non-heritable transgene silencing in maize. In our study we tested frequency and severity of transgene silencing in homozygous parents and their hemizygous progeny, resulting from outcrosses to a wild type tester. Molecular analyses revealed that transgene silencing in parental plants was associated with increased production of sRNAs. Interestingly, after an outcross, transgene silencing was released and this correlated with reduced sRNAs abundances. Release of transgene silencing in progeny of outcrosses suggests that transition between homozygous and hemizygous plants crosses a threshold, possibly production of aberrant RNAs (Liu and Chen, 2016 Mol Plant (6) 826-836), that triggers transgene silencing.

P96

Comparative genome sequence analysis of teosinte and maize to characterize resistance to the pathogen *Ustilago maydis*

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Ustilago maydis (*U. maydis*), the causal agent of corn smut, is an important agricultural pathogen. Significant yield loss of approximately $1 billion in the United States is incurred annually due to *U. maydis*. The lack of resistant maize cultivars necessitates the identification of new sources of resistance. Teosinte, a wild progenitor of maize, has demonstrated resistance to various diseases and insects. We identified two maize-teosinte near-isogenic lines (NILs) from a maize (B73)-teosinte (*Zea parviglumis*) introgression population that were resistant to *U. maydis*. Genotypic analysis of the two resistant NILs identified a 3.9 Mbp teosinte introgressed region that is present only in the two resistant NILs. Since teosinte has not been sequenced, comparative analysis of the teosinte and maize genome will enable us to identify the region in the teosinte genome that is contributing to the resistant phenotype in two NILs. The goals of this study are to: 1) Perform whole-genome sequencing of the teosinte parent, and 2) Utilize the teosinte sequence to identify the similarities and differences of the genes in the 3.9 Mbp region of the two NILs, teosinte and maize. The sequence analysis of the parent teosinte is ongoing. Data from this work will identify the R-genes and defense-related genes in two NILs. The selected R-genes and defense-related genes can be potentially used to improve resistance to *U. maydis*.

Funding acknowledgement: United States Department of Agriculture (USDA)

P97

Increased expression of antifungal and insecticidal flavonoid phytoalexins in specialty maize lines

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The long-term goal of the maize and sorghum sustainability project at Penn State University is to identify novel phytochemicals with properties that deter pests and pathogens. It has been shown that the sorghum flavonoids called 3-deoxyanthocyanidins (3-DAs) have antifungal activity against *Colletotrichum sublineola*. A recent study from our laboratory demonstrated that sorghum plants defective in 3-DA biosynthetic pathway are also susceptible to corn leaf aphid (CLA). The 3-DA accumulation in sorghum is regulated by *yellow seed1* (*y1)*, an MYB transcription factor and an orthologue of maize *pericarp color1* (*p1*). We developed transgenic maize lines expressing sorghum *y1*. These transgenics (named as *ZmY1*) phenocopied maize *p1* allelic patterns. Further, we demonstrated that these *ZmY1* plants are resistant to Southern corn leaf blight (*Cochliobolus heterostrophus*) and anthracnose caused by *Colletotrichum graminicola.* We tested *ZmY1* lines for insect herbivory using fall armyworm (FAW) (*Spodoptera frugiperda*) caterpillars, a serious insect pest worldwide. Our preliminary results with *ZmY1* plants showed significant mortality of FAW caterpillars as compared to the near-isogenic line without *y1* transgene. Encouraged by these results, we initiated a breeding program for developing high flavonoid maize lines. Results obtained from 3-DA overexpressing breeding lines and their interactions with FAW caterpillars will be presented.

Gene / Gene Models described: *p1,*; Zm00001d028854
Funding acknowledgement: United States Department of Agriculture (USDA), NE-SARE

P98

Using antimorph *Ae1-5180* as an alternative to *ae1* to increase amylose content in maize endosperm

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Starch can be either highly branched (amylopectin) or largely unbranched (amylose). Branch formation is catalyzed by starch branching enzymes. The primary starch branching enzyme in maize endosperm is starch branching enzyme IIB (SBEIIb), which is encoded by *ae1* (*amylose extender 1*). In most Midwestern dents, the endosperm starch is about 75% amylopectin and 25% amylose. The amylose content increases up to 50% when *ae1* is homozygous recessive. An antimorph, (*Ae1-5180*) was identified (Stinard *et al.*, 1993) that acts in a dominant fashion to eliminate SBEIIb. The essential portion of this mutant allele is a *Mutator1* (*Mu1*) element flanked by an inverted duplication of the 3' portion of the *Ae1* gene. Based on the restriction map by Stinard *et al.*, we developed an approximate predicted sequence for Ae1-5180 using the known sequences of the wildtype ae1 gene and Mu1 transposon. Using this, we designed PCR primers targeted to amplify regions within a 3kb XhoI restriction fragment that appears crucial for the dominant action of *Ae1-5180*. The primers amplify two DNA bands from samples expected to have *Ae1-5180*, but none from B73. The sequence of the lower molecular weight band matches with 181 bp of *Mu1* and 364 bp of *Ae1* (545 bp). The higher molecular weight band also possesses 181 bp of *Mu1*, but includes 438 bp of *Ae1* (619 bp). The additional 74 bp includes a restriction site for NotI, which was predicted from the original restriction map. Sequencing results indicate that these primers reliably detect the presence or absence of the Ae1-5180 allele. Also, the insertion site of Mu1 in Ae1-5180 is now exactly defined.

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P99

A dominant mutation in the DNA-binding domain of ZmARF28 alters subcellular location and dimerization

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The phytohormone auxin influences many plant developmental processes. Auxin signaling has been well-studied in a number of species including *Arabidopsis thaliana* and *Zea mays* and components of the canonical nuclear auxin signaling pathway have been identified. Members of a large transcription-factor family at the center of the nuclear auxin signaling pathway, the AUXIN RESPONSE FACTORS (ARFs), fall into three classes. Class A ARFs are canonical transcriptional activators with defined genetic and molecular functions. The class B and C ARFs are predicted transcriptional repressors, but their molecular roles are less well understood.A single point mutation in the DNA-binding domain (DBD) of the maize Class B ARF, ZmARF28, has been identified as the causal mutation in the dominant *Truffula (Trf)* mutant. The *Trf* mutation affects leaf and tassel development in maize. The point mutation converts a serine to an asparagine near the dimerization surface of the ZmARF28 DBD.We have generated the same mutation in the Arabidopsis ortholog of *ZmARF28*, *AtARF2*, and expressed it transiently in Arabidopsis protoplasts and *Nicotiana benthamiana*. This point mutation, arf2T298N, affects cytoplasmic localization *in vivo*. In addition to altered sub-cellular localization, arf2T298N dimerization is increased in the nucleus, a requirement for auxin responsive transcription. Taken together these results suggest a possible explanation for *Trf* whereby ZmARF28 localization and activity are altered. We will follow up these studies with DNA-binding assays and genetic analysis in Arabidopsis to determine the molecular and biochemical functions of ARF2 and the *Trf* mutation.

Gene / Gene Models described: *ZmARF28, AtARF2*; GRMZM2G006042, AT5G62000.1
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P100

ABA-mediated differences in drought-related traits in maize: Is *ABA 8'-hydroxylase 4* the causal gene?

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Drought is one of the most important causes of maize production loss in the US, where losses up to 37% were recorded for the years 1981 to 2016 (Li et al. 2019). Intensified by climate change, there is an urgent need for high yielding maize lines with improved water use efficiency and drought tolerance. Based on near isogenic lines (NILs), we showed that multiple drought-related traits, including water-use-efficiency (WUE), stomatal conductance, stomatal density, and abscisic acid (ABA) content, are controlled by a genomic segment on chromosome 7 (Avramova et al. 2019). The genetic co-localization of trait associations may be explained by tight linkage of several causal genes in the region, or pleiotropic effects of several or one gene. Based on a NIL carrying the target region and the lowest donor parent background, we initiated fine mapping. Recombinants were used to study the genetic co-localization of drought related traits, their interplay and trade-offs, and to identify their respective causal gene(s). Nine homozygous recombinant NILs carrying overlapping segments in the target region were selected for in-depth genotyping (600K array) and phenotyping. Physiological (e.g. WUE, stomatal characteristics) and metabolic (e.g. ABA) measurements pointed to four recombinants sharing a small donor parent segment associated with all the traits. Among the genes in this segment, ABA 8'-hydroxylase 4 (ZmAbh4) is the most promising candidate gene for ABA-mediated differences in stomatal conductance and we generated CRISPR/Cas9 mutants for this gene and all members of the ZmAbh gene family. Preliminary results showed increased concentrations of ABA in the leaves of plants homozygous for a mutation in ZmAbh4, but further analyses are needed to show whether ZmAbh4 is one of the causative genes for the observed phenotypes.

Gene / Gene Models described: *Abh4*; GRMZM2G065928
Funding acknowledgement: Deutsche Forschungsgemeinschaft (DFG)

P101

Assessing the impact of mutated AUX/IAA proteins in maize inflorescence development

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Auxin hormone signaling is essential for the proper development of plants. *BARREN INFLORESCENCE 4* (*BIF4*) is a maize gene that encodes an AUXIN/INDOLE-3-ACETIC ACID (AUX/IAA) repressor protein within the nuclear auxin signaling pathway. Along with the *BIF1* gene, which also encodes an AUX/IAA protein, *BIF4* plays an essential role in inflorescence formation during development. These AUX/IAA proteins experience auxin induced degradation that allows the activation and expression of downstream genes. The degron domain within *BIF4* is a highly conserved sequence responsible for the rate of protein degradation. AUX/IAA degradation rate has been previously shown to affect root and shoot developmental progression in Arabidopsis. This research focuses on the characterization of three maize transgenic constructs expressing different versions of *BIF4* fused to the fluorescent protein VENUS: VENUS-BIF4, VENUS-BIF4 m1, and VENUS-BIF4 AAA. All three *BIF4* proteins are expressed by a native *BIF4* promoter. VENUS-BIF4 retains the degron domain sequence of a wild type maize plant, and thus is expected to have the natural auxin-induced degradation rate. VENUS-BIF4 m1 is a stabilized mutant that does not degrade in the presence of auxin. VENUS-BIF4-AAA is an intermediate mutant that has a reduced rate of degradation, when tested in a yeast synthetic system. There are two main objectives of this research: i) to determine by confocal microscopy the expression changes of the three BIF4 proteins during inflorescence development; and ii) to determine the phenotypic effects of the three distinct proteins on the development of tassels and ears.

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P102

A mitochondrial PPR protein affects the editing of cox2 and nad2 and kernel development in maize

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Maize is one of the most important food crops and kernel size is an important component of yield. Here we isolated an EMS mutant (*zm3047*) with defects in kernel development and identified the underlying gene. We confirmed the function of this gene by complementation test. Loss of *Zm3047* function leads to severely arrested kernel, reduced 100-kernel weight, and embryo lethality. The *Zm3047* gene encodes a PLS-type pentatricopeptide repeat (PPR) protein that targets to mitochondria, and participates in the C-to-U editing of mitochondrial *nad2* and *cox2* transcripts, which are subunits of the complex I and complex IV respectively. The loss of editing at *nad2* and *cox2* affects the assembly and activity of these two complexes. Additionally, yeast two-hybrid experiments suggest that the Zm3047 protein interacts with ZmDYW2 and ZmMORF8, two components of the mitochondrial editosome. Our results indicate that Zm3047, ZmDYW2 and ZmMORF8 possibly assembled into a RNA-editosome to be required for editing of *cox2* and *nad2*, and kernel development in maize.

Funding acknowledgement: National Natural Science Foundation

P103

Characterization and mapping of a cytochrome P450 mutant affecting plant architecture in maize

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Plant height is an important trait associated with lodging resistance and grain yield. Dwarf mutants are good materials for elucidating the regulatory mechanisms of plant growth and development. Here we isolated a maize dwarf mutant *d12* from a chang7-2 Ethylmethane sulfonate (EMS) mutagenesis population. It has a pleiotropic effect on plant growth that vary across different genetic backgrounds. Homozygous *d12* mutant in the chang7-2 background exhibited reduced plant height and internodes length, short leaves, and less tassel branch numbers. Scanning electron microscopy observation of the longitudinal sections of the fourth internode revealed that the cell elongation was significantly inhibited in *d12* compared with wild type. Quantification of endogenous brassinosteroids (BRs) showed that the brassinolide (BL) level of *d12* mutant were significantly lower than that of wild type plants, while castasterone (CS) was somewhat higher in *d12*. Since BRs are growth promoting phytohormones controlling cell elongation, we speculate that *d12* mutants may have defect in BR synthesis process. Genetic analysis suggested that the mutant traits were controlled by a single recessive gene. Identification of the *D12* gene was performed by map-based cloning using F2 plants from a cross between *d12* and B73. The site of the *d12* mutation was narrowed down to the region between two Insertion/deletion polymorphism makers, which located on the short arm of maize chromosome 3. Sequencing of the candidate genes in these region revealed a G to A change in a cytochrome P450 gene, which may function in BRs synthesis. The single SNP mutation result in a premature termination of translation at codon 494. The tissue-specificity of *D12* expression indicated that its transcript constitutionally accumulated in all the tissues, and relatively higher in leaf, tassel and internodes. Our research have provided opportunities for the analysis of the role of brassinosteroids in regulating maize plant architecture.

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P104 @sdhungana

Characterization and mapping of the *carbohydrate partitioning defective60* mutant in maize

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Carbohydrate partitioning is the process by which sugars, primarily sucrose, synthesized in the photosynthetic source tissues (mature leaves) are mobilized to non-photosynthetic (sink) tissues, such as roots, seeds, and developing organs. To identify genes controlling carbohydrate partitioning, we identified a number of mutants, termed *carbohydrate partitioning defective (cpd)*, which overaccumulate starch and sugars within their leaves. One such recessive mutant, *cpd60,* hyperaccumulates starch, sucrose, glucose, and fructose in its leaves, and displays stunted growth, reduced fertility, leaf chlorosis, and accumulation of anthocyanins in the mature leaves. The rate of export of C-14 labeled sucrose from mature source leaves was also found to be reduced in the mutants. Furthermore, ectopic lignin depositions were observed in the phloem tissues of mature mutant leaves. In addition, three more alleles of *cpd60* have been identified and are being characterized to elucidate the function of the gene. The mutation responsible for the *cpd60* phenotype has been mapped to the lower arm of Chromosome 1 by Bulked Segregant Analysis (BSA). By using polymorphic markers and whole genome sequencing based approaches, we have mapped the causative mutation to an intergenic region. The identification of the gene responsible for the *cpd60* phenotype will provide new insights into the genetic regulation of sugar metabolism and allocation in maize. With this knowledge, we can translate our understanding of carbohydrate partitioning to other crop species, such as sorghum and sugarcane, for genetic improvements to increase food yield and biofuel production.

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P105

Comparative metabolomics and fluxomics to identify steps limiting oil synthesis

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The demand for seeds rich in oil has increased considerably due to the rapid growth of human consumption, animal feed, and biofuel production. In maize kernels, the endosperm and embryo are the main sites for synthesis and accumulation of starch and oil, respectively. Thus far, breeding efforts to achieve elevated oil content resulted in smaller endosperm which is the main component of the kernel, therefore leading up to 50% smaller grain size and lower. The key to increasing oil content in corn kernels without affecting the grain yield may rely on directly altering embryo metabolism by redirecting carbon towards fatty acid synthesis. To achieve this goal, a deep understanding of the metabolic pathways leading to triacylglycerol (TAG) production in maize embryo is required.
In plants, *de novo* fatty acid synthesis (FAS) takes place in the plastid, and requires carbon (acetyl-CoA), energy (ATP), and reducing power (NADH and NADPH). ^{13}C-Metabolic Flux Analyses previously highlighted that the synthesis of NADPH by the plastidic malic enzyme was limiting FAS in the embryos of a maize inbred line, LH59, low in oil (34%, w/w). We hypothesize that maize kernels accumulating more oil may have changed their carbon metabolism to circumvent the limitation in NADPH provision. To test this hypothesis, the intracellular metabolite levels were compared between maize embryos from two different maize lines, ALEXHO S K SYNTHETIC (Alex) and LH59, which accumulate 48 and 34% of oil, respectively. Then a ^{13}C-Metabolic Flux Analysis in Alex embyos was performed to build a map of carbon flow through central metabolism that was then compared to the one for LH59 embryos. This study quantified the rerouting of carbon through specific metabolic pathways in order to achieve more oil production. This combination of innovative tools will pave the way for controlling seed composition in important food crops.

P106

Connecting genotypes to molecular phenotypes: Metabolite-based genome-wide association studies and multi-omic co-regulation analyses drive gene annotation

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Biochemistry is the predominant language and mechanism by which plants interact with other organisms. In response to attack by microorganisms, maize undergoes massive reorganization at the transcriptome and proteome level to produce among the most complex array of inducible and often protective specialized metabolites yet described in a single species. To broadly capture regulatory loci and specific biosynthetic genes responsible, we leveraged the Goodman diversity panel, metabolomics, transcriptomics, genome-wide association studies (GWAS) and combined Mutual Rank global co-expression analyses to connect genes to biosynthetic enzymes and metabolites. Liquid chromatography (LC) and gas chromatography (GC) coupled with mass spectrometry (MS) results thousands of distinct analytes in single tissues and time points. Correlation analyses rapidly group metabolites into shared pathways capturing closely-related yet previously unknown biochemicals. In the absence of significant GWAS SNPs, single and bi-omic Mutual Rank analyses can powerfully connect metabolites to biosynthetic genes. Metabolite mapping and co-regulation analyses produce outstanding gene candidates spanning terpene synthases, 5a-steroid reductases, cytochrome P450s, and components of amino acid, oxylipin and phenylpropanoid pathways. Candidates were selected for genetic analyses and biochemical confirmation using *in vitro* enzyme assays and/or transposon insertion mutants to prove endogenous relationships. Our goal is the creation of a maize specialized metabolome database combined with transcriptome libraries and association studies to powerfully drive gene annotation that connects field relevant biochemical phenotypes to genotypes.

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P107

Cross-talk between biochemical defense pathways in *Arabidopsis thaliana*

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Cellular metabolism is a complex set of interwoven interactions. That complexity challenges the prediction of how metabolism responds to change. To try to increase our ability to predict how single-gene changes result in global metabolic shifts, we studied how changing expression in the indolic glucosinolate chemical defense pathway in Arabidopsis thaliana influences production in the related aliphatic glucosinolate pathway. Aliphatic glucosinolates are synthesized in a pathway parallel to the indolic pathway and share a number of enzymes (*SUR1, GGP1, UGT74B1*). Research on the aliphatic pathway has shown that aliphatic-specific and shared gene knockdowns have no control over the indolic pathway. However, by knocking down the activity of each step in the indolic pathway and measuring the production of all glucosinolates via High Performance Liquid Chromatography (HPLC), our results suggest cross-talk between these pathways.
Preliminary results reveal that when the gene *GTSF9* of the indolic pathway is knocked down, the production of aliphatic glucosinolates is reduced, but the production of indolic glucosinolates is not affected. These results are puzzling given that *GSTF9* is not thought to be shared between the pathways like the enzymes *SUR1* and *GGP1*. Indeed, previous research suggests *GSTF9* is predicted to act in the aliphatic pathway exclusively. To further study this pathway cross-talk, we will extract and sequence RNA from single-gene knockouts of the genes in the indolic pathway to track how changes in gene expression of individual enzymes change global patterns of expression. These results will allow us to begin to understand the genetic mechanisms that mediate cross-talk between these pathways.

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P108

Cross-talk between biochemical defense pathways in *Arabidopsis thaliana*

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Cellular metabolism is a complex set of interwoven interactions. That complexity challenges the prediction of how metabolism responds to change. To try to increase our ability to predict how single-gene changes result in global metabolic shifts, we studied how changing expression in the indolic glucosinolate chemical defense pathway in Arabidopsis thaliana influences production in the related aliphatic glucosinolate pathway. Aliphatic glucosinolates are synthesized in a pathway parallel to the indolic pathway and share a number of enzymes (SUR1, GGP1, UGT74B1). Research on the aliphatic pathway has shown that aliphatic-specific and shared gene knockdowns have no control over the indolic pathway. However, by knocking down the activity of each step in the indolic pathway and measuring the production of all glucosinolates via High Performance Liquid Chromatography (HPLC), our results suggest cross-talk between these pathways.
Preliminary results reveal that when the gene GTSF9 of the indolic pathway is knocked down, the production of aliphatic glucosinolates is reduced, but the production of indolic glucosinolates is not affected. These results are puzzling given that GSTF9 is not thought to be shared between the pathways like the enzymes SUR1 and GGP1. Indeed, previous research suggests GSTF9 is predicted to act in the aliphatic pathway exclusively. To further study this pathway cross-talk, we will extract and sequence RNA from single-gene knockouts of the genes in the indolic pathway to track how changes in gene expression of individual enzymes change global patterns of expression. These results will allow us to begin to understand the genetic mechanisms that mediate cross-talk between these pathways.

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P109

Dedicated farnesyl diphosphate synthases in maize produce isoprenoids for growth or defense.

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Farnesyl diphosphate synthases (FPS) are enzymes that produce the 15-carbon isoprenoid farnesyl pyrophosphate that serves as a substrate for a wide range of compounds important for growth and development. In maize farnesyl pyrophosphate is also a precursor for antifungal sesquiterpenoid phytoalexins (zealexins). Maize has three FPS genes that our study shows are differentially regulated in response to fungal infection. Analysis of CRISP/Cas9 mutants in the three genes revealed that one of the FPS genes is predominantly responsible for zealexin production in response to infection with the fungal pathogen Fusarium graminearum. Furthermore, mutations in a different FPS gene resulted in severe developmental phenotypes including dwarfism and reduced photosynthetic capacity suggesting it was important for the production of developmental related isoprenoids. These findings indicate that maize has evolved dedicated FPSs to provide precursors for isoprenoid compounds important for growth or defense that may help it mitigate growth vs. defense tradeoffs.

Gene / Gene Models described: ; GRMZM2G098569,GRMZM2G168681,GRMZM2G147721
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P110

Development of an amenable system for site-specific addition to a maize chromosome

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Currently, transformed maize is produced by random integration into plants of transgenes, which have variable expression and require an extended crossing scheme to make combinations. Here, we describe the initial steps in the development of a gene stacking system. First, a target landing pad was transformed that allows the selectable marker to be removed by Cre recombinase. Then, amendment constructs can be targeted to the landing pad using complementary attachment sites for ϕC31 Integrase that is expressed in the recipient line. Two amendment constructs were made that should enable sequential modification of the integrated landing pad by utilizing complementary attP and attB sites, which can be acted upon by ϕC31 Integrase. The amendment constructs contain cargo and a promoterless selectable marker which, upon successful recombination with the landing pad, restores expression of the selectable marker. After recovery of a transformed landing pad, the selectable marker was removed by exposure to Cre recombinase and confirmed by sequence analysis. Then, the modified target landing pad was combined with ϕC31 Integrase. Bombardment of constructs that activate expression of DsRed upon integration and of an amendment construct revealed targeted integration, which was confirmed by PCR across the recombination site followed by sequencing. The results demonstrate the feasibility of targeted transformation and an initial demonstration of the functionality of the gene stacking system.

Funding acknowledgement: National Science Foundation (NSF)

P111

Discovering the gene regulatory networks that govern the phenylpropanoid pathway in maize

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Gene regulatory networks (GRNs) are central to all cellular processes. Deciphering GRNs at the molecular level is key to understanding and manipulating important agronomic traits for improved food and fiber production. These GRNs are comprised of many transcription factor (TF) DNA interactions and remain poorly understood. Our research has focused on understanding the GRNs that govern the production of phenolic compounds which are among the most diverse and widespread of specialized plant compounds and underly many important agronomic traits. For this objective, we previously developed the GRASSIUS database (www.grassius.org) and the maize TFome as community resources for the discovery of protein DNA interactions (PDIs) underlying GRNs in maize (Burdo *et al*., The Plant Journal. 2014 80:356-66). The maize TFome was successfully deployed to discover more than 1100 PDIs governing the regulation 54 phenylpropanoid genes in maize (Yang *et al.,* 2017. Mol Plant, 10:498–515). More recently, we performed a comprehensive bioinformatics analysis of the maize genome and mRNA-seq datasets to reveal new aspects of the genes involved in phenylpropanoid, monolignol, and flavonoid production in this important crop (Gomez-Cano et al., Plant Science 2020 Vol 291, Article 110364, In press). Here we combine insights from these previous studies with new experimental results to further expand the GRN governing the phenylpropanoid pathway in maize. Our focus is on the understanding the regulation of the phenylpropanoid genes that are most highly expressed during maize development and discovering regulatory overlap that may exist. We also are expanding our understanding of this GRN by conducting Y1H screens to identify TFs that regulate the genes encoding the top 20 TFs previously shown to bind phenylpropanoid promoters in maize. This project is funded by NSF grant IOS-1733633

Funding acknowledgement: National Science Foundation (NSF)

P112

Discovery and genetic validation of regulatory variants for nitrogen-response traits in maize

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Our research group has investigated the genetic basis for the tremendous growth response of maize hybrids to nitrogen (N) fertilizer. This program has developed the infrastructure to monitor N-responsive phenotypes at field and population scales, leveraged community resources for functional genomics research, and exploited novel genetic variation for N-responsive traits. Phenotyping methods, some performed in collaboration with other groups, have used agronomic measures, developmental phenotypes, metabolite profiling, expression profiling of mRNAs and small RNAs, and promoter-reporter transgene activity. Nearly 7000 plots and hundreds of genotypes, including a core set of 25 diverse hybrids and their inbred parents, have been phenotyped over 16 years. Approaches to gene discovery such as QTL mapping and gene expression profiling have identified hundreds of potential candidate genes that are statistically correlated with nitrogen responses. A number of these candidates have been validated using genetic experiments, where either mutant lines, near-isogenic line pairs, or transgenic lines have been confirmed to alter N-responsive traits in field trials. We present here evidence for four N-responsive genes. Both UniformMu and recent CRISPR/Cas9 multiplex editing have confirmed the NRT1.1 transporter as a QTL for nitrogen utilization. Another UniformMu mutant in the MADS-box gene *ZAP1*, which showed strong N responses in developing B73 leaves, delays vegetative senescence and increases biomass yields. Near-isogenic lines derived from residual heterozygosity in NAM lines validated the impact of allelic variation in the *L-asparaginase* gene on nitrogen remobilization and grain protein concentration. Finally, transgenic lines that overexpress the maize *HVA22* gene specifically in maize endosperm have elevated grain protein concentration, which validates the largest effect QTL we have identified for that trait. Collectively, our findings demonstrate that despite the complex genetic architecture of nitrogen-response phenotype, it is possible to identify single genetic variants that modulate N-responsiveness in maize.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Department of Energy (DOE)

P113

Discovery of *Anthocyanin3*: A negative regulator of the maize flavonoid pathway

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Anthocyanin3 (*A3*) is a recessive intensifier of maize anthocyanin pigments in vegetative tissues and tassels. Previous work has deduced that the *a3* element works at the level of transcription and may be an inhibitory transcription factor when functional. The locus involved with the *a3* intensification phenotype has been pinpointed to a region approximately 8 cM downstream from *Anthocyanin1* (*A1*). The goal of this study was to discover the gene involved with the *a3* phenotype and to characterize the effect the gene has on anthocyanin synthesis. A transposon-tagging approach was employed to generate a *de novo* mutation of *A3*. A genetic stock with a *Dissociation* (*Ds*) element insertion in *A1* was mobilized by *Activator-immobile* and testcrossed to the *a3-reference* stock 320N. One plant exhibited the dark purple plant phenotype. Inverse PCR of purple plant DNA using nested *Ds*-specific primer sets pinpointed the insertion to the promoter of MYBR97, an R3-MYB-like protein with homology to CAPRICE in Arabidopsis. This Arabidopsis homolog is a well-studied MYB-repressor protein involved with the flavonoid pathway. Quantitative PCR was performed on pools of wild type and mutant *a3-ref* lines from an F_2 population crossed to B73. Transcript levels of many anthocyanin biosynthetic genes increased in lines that did not express *A3*. Enhancing pigmentation has implications on breeding for natural colorants in the grain. When *a3-ref* was crossed to purple pericarp landrace Apache Red Cob in a large population, linkage to *a3-ref* in this population did not increase pigment concentration. Overall, the discovery of the *A3* gene corroborates the important role of R3-MYB repressors on fine-tuning the expression of secondary metabolite pathways and suggests that there is still much left to learn about the anthocyanin pathway in maize.

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P114

Discovery of modules involved in the biosynthesis and regulation of maize phenolic compounds

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Phenolic compounds are among the most diverse and widespread of specialized plant compounds and underly many important agronomic traits. We conducted a comprehensive bioinformatics analysis of the maize genome and mRNA-seq datasets to reveal new aspects of the genes involved in phenylpropanoid, monolignol, and flavonoid production in this important crop. Remarkably, just 19 genes accounted for 70% of the overall mRNA accumulation of these genes across 95 tissues, indicating that these are the main contributors to the flux of phenolic metabolites. Eighty genes with intermediate to low expression play minor and more specialized roles. Remaining genes are likely undergoing loss of function or are expressed in limited cell types. Phylogenetic and expression analyses revealed which members of gene families governing metabolic entry and branch points exhibit duplication, subfunctionalization, or loss of function. Co-expression analysis applied to genes in sequential biosynthetic steps revealed that certain isoforms are highly co-expressed and are candidates for metabolic complexes that ensure metabolite delivery to correct cellular compartments. Co-expression of biosynthesis genes with transcription factors discovered connections that provided candidate components for regulatory modules governing this pathway. Our study provides a comprehensive analysis of maize phenylpropanoid related genes, identifies major pathway contributors, and novel candidate enzymatic and regulatory modules of the metabolic network. Our findings have recently been published in Plant Science 2020 Vol 291, Article 110364. This project is funded by NSF grant IOS-1733633

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P115

Enhanced flavan-4-ols corn diet protects intestinal barrier function in mice

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The protective role of a plant-based diet, rich in phytochemicals, against chronic diseases has emphasized the importance of developing crops with enhanced nutritional value. Maize is one of the most important crops worldwide, however, the long-lasting selection for high-yield and other agronomic traits has compromised phytochemicals in commercial lines. Flavonoid compounds well known for their antioxidant activity is a promising trait to be re-introduced into elite maize lines. In this study, we determined the temporal gene expression, metabolite profiles and antioxidant capacity of four maize near-isogenic lines (NILs) with varied anthocyanins and phlobaphenes accumulation. Here we report that the accumulation of anthocyanins and phlobaphenes conferred significantly higher antioxidant capacity (p

Funding acknowledgement: United States Department of Agriculture (USDA)

P116

Evolution of the cardenolide pathway in *Asclepias syriaca*

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The cardenolide pathway is an important plant defense system of *Asclepias syriaca*, milkweed, against herbaceous predators like the *Danaus plexippus*, monarch butterfly. Cardenolides target the sodium-potassium ATPase in cell membranes and are toxic to various organisms. However, the cardenolide's toxic effects are inhibited by *D. plexippus* through amino acid substitutions that confer resistance to the insect. Evolution of the cardenolide pathway is of interest due to the insect's resistance to the system's toxicity. Monarch butterflies store cardenolides to build up resistance to the milkweed toxicity. There is evidence that monarchs choose plants on which to lay their eggs that have a moderate amount of cardenolides that will both provide chemical protection but will not kill their offspring. Here we study how the cardenolide synthesis pathway has evolved in two closely related species *A. syriaca* and *A. speciosa*. *A. syriaca* produces higher quantities of cardenolides than *A. speciosa* and we will discuss the differences in pathway structure and molecular evolution of the genes in this pathway. We will also compare the pathways to other cardenolide-producing species to determine how this pathway has changed over evolutionary history.

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P117

Functional characterization of cis-regulatory variation underlying drought tolerance in maize

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Maize provides a tremendous amount of natural variation on a genetic and phenotypic level. However, utilizing this vast diversity requires an in-depth knowledge about the connection between genotype and phenotype. A huge proportion of the functional genetic variation is found in non-coding regions, where we lack the genetic code to decipher their effects. In order to solve this problem, we developed F1-mediated identification of non-coding cis-element-impacting SNPs (FIND-CIS), a method that combines transcription factor (TF) foot printing, an omni-hybrid approach and a local association pipeline. This method enables us to pinpoint functional variance involved in the phenotypic plant response. Facing the challenges of climate change, one of the most important and complex traits is the reaction to drought stress. For the FIND-CIS analysis we grew 29 hybrids between B73 and an inbreed line from the Ames population as mother and father, respectively. Three days after 75% of the plants had reached the developmental stage V4 we withheld watering. We harvested leaf and stem tissue 86 h after the last watering. To characterize the drought response, we measured field capacity (FC) of the soil, relative water content (RWC), fresh weight and apical growth of well-watered and drought stressed plants. We identified the hybrids B73 x Oh43 and B73 x A619 as the most drought tolerant and the hybrids B73 x CML103 and B73 x Mo18W as the most drought susceptible under our conditions and at that particular developmental stage. Furthermore, the behavior under drought correlated with mRNA levels of known drought responsive genes. Downstream bioinformatical analysis has shown further evidence for the phenotypic outcome of our study linking it to genetic variation. Our approach provides a strong base for a deeper understanding of regulatory elements and their phenotypic effects potentially improving drought tolerance.

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P118 @NateKorth

Genetic analysis of fiber biosynthesis in maize seed and impact on the human gut microbiome

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Microorganisms that inhabit the human gut are essential for life and have been implicated in various health and disease states. Diet is thought to be the greatest driver of configuring the species composition of the human gut microbiome. Despite the connection of diet and the microbiome, crop breeding and improvement strategies have not traditionally been focused on nutritional outcomes that impact the gut microbiome. Arabinoxylan (AOX) is a hemicellulose plant cell wall material that varies structurally between different plant species, some of these structures are utilized by gut microbes post digestion. However, the relationship between the AOX fiber components and microbes that degrade them is largely unknown. We have identified maize lines with transposon insertions that alter genes implicated in different aspects of AOX biosynthesis including xylan backbone elongation, and crosslinking of xylan molecules by ferulic acid and arabinose. Target genes were chosen based on function and expression in the kernels and were identified in the Uniform Mu transposon mutagenesis population developed at the University of Florida. Subsets of first-generation seed were processed in a simulated human digestion. The digested product was introduced individually into *in vitro* microbiome reactions, spanning multiple human subjects. The microbial composition of the microbiome was analyzed by 16S rRNA sequencing. Preliminary results indicate mutations in xylan elongation and ferulic acid crosslinking impacts growth of specific groups of gut bacteria. Characterization of microbial responses to structurally distinct AOX mutants will provide a comprehensive view of the how these complex molecules can influence the microbiome and health.

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P119

Genetic and biochemical deconvolution of pathways controlling maize immunity

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Specialized metabolites provide key layers of biochemical immunity underlying crop resistance; however, challenges in resolving pathways limit applications. To understand maize (Zea mays) terpenoid antibiotics imparting disease resistance we integrated large-scale omic correlations, association mapping, enzyme-structure function studies and targeted mutagenesis. Three zealexin (Zx) gene clusters comprised of four sesquiterpene synthases (Zx1-4) and six cytochrome P450s (CYP) in the CYP71Z (Zx5-7) and CYP81E (Zx8-10) families drive the production of diverse antibiotic cocktails. Gene duplications, ensuring pathway resiliency to single null mutations, are combined with enzyme substrate-promiscuity to create biosynthetic hourglass pathway utilizing diverse substrates to yield a high degree of product complexity. Zx pathway activation mediating pathogen resistance occurs during a dramatic reorganization of >50% of the measurable proteome and suppression of constitutive defense pathways. The elucidated genetic basis of complex biochemical phenotypes driving disease resistance enables innovative strategies for transferring durable chemical immunity between crops.

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P120

Global impacts of genome imbalance on gene expression in maize aneuploids and polyploids

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It has been long known that genome imbalance caused by changing the dosage of individual chromosomes (aneuploidy) has a more detrimental effect phenotypically than by varying the dosage of complete sets of chromosomes (polyploidy). Previous work from the laboratory examined individual genes for effects of changes in chromosomal dosage. The predominant effect was an inverse modulation in trans across the genome but positive modulations were also observed. Here we extend such studies to global studies of such effects. The genome has been examined using a collection of 15 various B-A translocations via RNA-seq studies of maize mature leaf tissue. Within diploids, monosomics, trisomics, and tetrasomics were compared to the normal diploid. The results indicate that significant changes in gene expression occur both on the varied chromosome (*cis*) and the remainder of the genome (*trans*). In general, *cis* genes range from dosage compensation to a dosage effect, whereas for *trans* genes the most common effect is an inverse correlation in that expression decreases with increased doses of chromosome dosage. Comparisons across ploidies show much less modulation. In addition, genes in different functional groups exhibit diverse responses to varied chromosomal dosage. This study provides a novel insight into the underlying molecular mechanisms involved in genomic balance and how regulatory dosage effects operate. Funding from NSF IOS-1545780.

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P121 @ChristinaAFin

Insight into sweet corn starch biosynthesis using gene expression analysis

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Sweet corn (*Zea mays* var. *saccharata*) has high sugar content due to mutations in the biosynthetic pathway that convert sucrose to starch in the endosperm. The pathway is controlled by several genes, but *sugary1* (*su1*) and *shrunken2* (*sh2*) are the main genes explored in commercial sweet corn. The *su1* allele is a leaky mutation in isoamylase, a starch branching enzyme, while *sh2* is a null mutation of the endosperm ADP-Glucose-Pyrophosphorylase (AGPase). Besides these key genes, starch biosynthesis is not well characterized. Moreover, *sh2* plants still have approximately 25% of wildtype endosperm starch content. The objective of this study is to further elucidate the regulation of starch biosynthesis and understand the source of this residual starch production in the endosperm of sweet corn. An RNAseq experiment was performed on near-isogenic lines (NILs) (wt, *sh2*, and *su1*) in seven genetic backgrounds. Kernels were harvested in blister-stage, milk-stage, and dent-stage to study transcriptome dynamics over time. In order to specifically study endosperm expression, RNA extractions were performed on dissected kernels. Differential expression and coexpression network analyses were conducted and indicate complex changes in expression in starch biosynthesis genes as well as genes not previously included in the pathway. We see background effects on the transcriptome independent of the mutation. Elucidating the consistent transcriptional responses to these mutants as well as background-specific effects will help unravel the steps in, and regulation of, starch biosynthesis, and potentially provide targets for mutation to create sweeter sweet corn varieties.

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P122

Insights into the genetic architecture of the *ga1* locus in maize genotypes

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The maize *ga1* locus controls cross incompatibility between field corn and popcorn varieties. The *Ga1-s* haplotype mediates its effect through pollen- and silk-specific factors that interact to enable correct pollen tube growth and fertilization. Two pectin methylesterase genes, *ZmPme3* – a candidate gene for the silk function (Moran Lauter et al., 2017) and *ZmGa1P*, the gene encoding the pollen function (Zhang et al., 2018) were identified at the *ga1* locus. We examined the genetic architecture of the *ga1* locus within field corn and popcorn lines by creating self and pairwise dot plots of the locus from assembled maize genome sequence data. A self-dot plot of the 1.1 Mb region surrounding the *ga1* locus in B73 (which lacks the *Ga1-s* functions) showed a pattern characteristic of tandem duplications. A homology analysis of these duplicated regions in field corn lines revealed ~ 58 *ZmPme3* and ~24 *ZmGa1P* non-functional homologs with deletions, insertions and/or in-frame stop codons. Pair-wise dot plots of seven inbred lines lacking *Ga1-s* function indicate high collinearity in this region, including conservation of the tandemly repeated Ga1-associated sequences. A dot-plot of B73 vs Hp301 (without and with *Ga1-S* function, respectively) on the other hand displays low collinearity. We hypothesize that the origin of these tandem duplications is non-homologous recombination. The presence of non-functional copies of genes at the *ga1* locus in field corn lines studied suggests that the *Ga1-s* function was present in ancestral maize varieties. The functional alleles may have conferred an evolutionary disadvantage in some conditions, resulting in their inactivation in most maize lines.

Funding acknowledgement: United States Department of Agriculture (USDA)

P123

Investigating the role of a Beta-glucosidase in the regulation of senescence in maize

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Senescence is a complex physiological process representing a major event in the plant development. Delayed senescence (a.k.a stay-green) is an important agronomic trait in maize and other cereals. Genetic, molecular, and metabolic determinants of stay-green trait are not well understood. Our recently concluded systems genetic analysis of senescence (Sekhon et al., Plant Cell, doi/10.1105/tpc.18.00930) indicated an important role of a beta-glucosidase gene, *ZmBGLU42*, in specifying stay-green phenotype. We hypothesize that ZmBGLU42 regulates senescence by modulating the sugar-mediated signaling. Characterization of natural alleles of *ZmBGLU42* supports our hypothesis. To further confirm this hypothesis, we are currently studying the role of *ZmBGLU42* in senescence induced due to lack of sink. Through detailed phylogenetic analysis, we have characterized the beta-glucosidases in maize, rice, and Arabidopsis. Our ongoing research is also focused on characterizing the role of *ZmBGLU42* ortholog in Arabidopsis through analysis of T-DNA insertion lines. Results from these experiments will be presented. These investigations will generate novel insights into the role of sugar-signaling in regulation of senescence. Understanding the role of beta-glucosidases may allow fine-tuning the onset of senescence to enhance net carbon yield and stress resilience in maize and other cereals.

Funding acknowledgement: National Science Foundation (NSF)

P124

Marker-assisted introgression of *hydroxylase, lycopene-ε-cyclase* and *opaque2* favourable alleles for reconstitution of nutritionally enriched PMH1 and Buland maize hybrids

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The present investigation is undertaken to introgress favourable alleles for high β-carotene in elite inbred lines viz. QLM 11, QLM 12, QLM 13 and QLM 14 which are parental lines of maize hybrids *viz*. Buland and PMH1. The gene specific markers for *o2*, *crtRB1* and *lcyE* were employed for foreground selection of favourable allele in backcross population. Introgressed progenies possessed high concentration of proA (9.21-12.44 µg/g), compared to 2.45-4.41 µg/g in the recurrent parents. In addition, endosperm protein quality showed increased tryptophan % in the inbreds ranged from 0.6 to 3.8%. The 54 RPMH1 hybrids were generated by crossing BC_2F_3 lines (QLM 13 as a seed parent and QLM 14 as a male parent) with different combinations. Hybrids have been evaluated during the *kharif* season 2018. The 32 RBuland hybrids were generated by crossing BC_2F_3 lines (QLM 11 as a seed parent and QLM 12 as a male parent) and 39 RPMH1were again generated from BC_3F_4 lines (QLM 13 as a seed parent and QLM 14 as a male parent) with different combinations. Both reconstituted hybrids have been evaluated during the February-June, 2019 for yield parameters at Ludhiana and Gurdaspur. The promising 5 versions of RBuland and 11 versions of RPMH1 were selected and were reconstituted again. These are being further evaluated at three locations viz. Ludhiana, Gurdaspur and Delhi. The reconstituted RPMH1 and Buland hybrids recorded significant enhancement of endosperm tryptophan % protein ranged from 1.03-3.47% and β-carotene varied from 9.29-11.40 µg/g. These elite, high-yielding RPMH1 and RBuland hybrids with improved protein quality will be released for commercial cultivation, and hold significant promise for improving nutritional security.

Funding acknowledgement: MINISTRY OF SCIENCE & TECHNOLOGY DEPARTMENT OF BIOTECHNOLOGY MINISTRY OF SCIENCE & TECHNOLOGY, GOVERNMENT OF INDIA

P125

Metabolomic analysis of maize nodal root growth under precisely controlled water deficit

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In maize and other cereals, most water is acquired by whorls of nodal roots that develop sequentially from subterranean stem nodes rather than by seedling roots. Nodal roots frequently must emerge and elongate through very dry topsoil to access water at depth. The molecular genetic mechanisms of nodal root elongation maintenance at tissue water potentials that inhibit leaf and stem growth remain largely uncharacterized. We utilized a split-root system to separately impose precise, constant soil water potentials on seedling and nodal roots of the maize inbred line FR697. A water deficit regime was designed to simulate field drought conditions with dry topsoil surrounding the nodal roots and greater water availability within the seedling root compartment. Under these conditions, FR697 exhibited a classical drought stress phenotype, including reduced leaf expansion, an increased root:shoot biomass ratio, and osmotic adjustment within the root tip. However, node 2 root elongation was completely maintained, in contrast to both node 1 and seedling primary roots. To investigate the molecular basis of root growth maintenance, kinematically defined regions of 1) meristematic, 2) rapidly elongating, and 3) decelerating root tip growth zones were harvested from water deficit-treated and control plants. Soluble metabolomes were generated from each of these tissues. Surprisingly, metabolomic responses to water deficit, including accumulation of multiple classes of compatible osmolytes, were broadly conserved between node 2 roots and comparable regions of the seedling primary root tip despite the large observed difference in growth maintenance. Intriguing disparities were observed in a subset of amino acid derivatives. These physiological and metabolomic data have been integrated with transcriptomic data of equivalent tissue samples to identify candidate genes for targeted mutagenesis.

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P126

Multi-omics dissection of cuticular lipid production on maize silks

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The plant cuticle is comprised of a cutin polymer matrix infused with and coated by long-chain non-polar and amphipathic lipids that together form a protective layer against environmental stresses. The cuticular lipids on maize silks are comprised of very-long-chain fatty acids (VLCFAs), and VLCFA-derivatives including aldehydes and hydrocarbons. These metabolites are linked via enzymatic reactions as presumed precursors, intermediates, and end-products for cuticular lipid biosynthesis. This pathway is sensitive to genetic background, reflected by varying lipid metabolome compositions between inbreds B73 and Mo17, especially for abundance of VLCFA precursors and hydrocarbon products. To elucidate the genetic (B73 vs Mo17) impact on cuticular hydrocarbon biosynthesis, we queried the spatio-temporal profiles of silk transcriptomes and cuticular lipid metabolomes that capture the developmental progression and the environmental transition as silks emerge from the husks. A suite of multivariate analyses tailored for dissecting unbalanced high-dimensional omics data with small sample sizes was used to identify candidate transcripts predictive of particular metabolome compositions. These computational approaches include partial least square regression (PLSR) and sparse-PLSR to identify candidate genes with either high or low to moderate expression levels, respectively. In addition, modified random forest approaches examined the associations between individual metabolites and clusters of genes. From these approaches, an aggregate transcript-metabolite network and resultant candidate gene lists were generated. Candidate genes included expected players in hydrocarbon production and extracellular lipid transport, as well as novel, uncharacterized genes that are potentially involved in these processes. Notably, this suite of computational approaches is broadly applicable for identification and characterization of cause-and-effect relationships accessible through multi-omics data sets

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P127

Mutation of *ZmDMP* enhances haploid induction in maize

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Doubled haploid(DH) breeding based on in vivo haploid induction has led to a new approach for maize breeding. All modern haploid inducers used in DH breeding are derived from the haploid inducer line Stock6. Two key quantitative trait loci, *qhir1* and *qhir8*, lead to high-frequency haploid induction. Mutation of the gene *MTL/ZmPLA1/NLD* in *qhir1* could generate a ~2% haploid induction rate(HIR); nevertheless, this mutation is insufficient for modern haploid inducers whose average HIR is ~10%. Therefore, cloning of the gene underlying *qhir8* is important for illuminating the genetic basis of haploid induction. Here, we present the discovery that mutation of a non-Stock6-originating gene in *qhir8*, namely, *ZmDMP*, enhances and triggers haploid induction. *ZmDMP* was identified by map-based cloning and further verified by CRISPR–Cas9-mediated knockout experiments. A single-nucleotide change in *ZmDMP* leads to a 2–3-fold increase in the HIR. *ZmDMP* knockout triggered haploid induction with a HIR of 0.1–0.3% and exhibited a greater ability to increase the HIR by 5–6-fold in the presence of *mtl/zmpla1/nld*. *ZmDMP* was highly expressed during the late stage of pollen development and localized to the plasma membrane. These findings provide important approaches for studying the molecular mechanism of haploid induction and improving DH breeding efficiency in maize.

Gene / Gene Models described: *ZmDMP*; GRMZM2G465053
Funding acknowledgement: The National Key Research and Development Program of China—Maize heterosis utilization technology and strong heterosis hybrids breeding (2016YFD0101200, 2016YFD0101003 and 2018YFD0100201) and the Modern Maize Industry Technology System (CARS–02–04).

P128

Predicting sugar and starch in mature sweet corn kernels

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Sweet corn quality is dependent on sugar and starch content in the kernel at eating stage. Sweet corn lines with higher sugar levels at eating stage have a tendency for poor germination and emergence suggesting a trade-off between eating and seed quality. However, the relationship between eating stage constituents and mature kernel composition is not well understood. Quantifying sugar and starch levels analytically is time-consuming and expensive. To enable lower cost phenotyping, we developed a calibration for single kernel near infrared reflectance (skNIR) spectroscopy to predict sugar and starch in mature sweet corn kernels. A panel of 100 sweet corn varieties was assembled consisting of equal parts *sugary1* (*su1*) and *shrunken2* (*sh2*) mutant backgrounds. Single-kernel NIR spectra were collected for 300 kernels. Sugar and starch were quantified using enzymatic analytical chemistry. Surprisingly, mature *su1* and *sh2* kernels have extensive overlap of sugar and starch levels suggesting other genetic factors contribute to the accumulation of these constituents in mature kernels. Predictive models were developed with Partial Least-Squares (PLS) regression of 67 genotypes. The models were then validated with the remaining 33 genotypes. Predictions using skNIR have a standard error of prediction for sugar of 33.2 mg g^{-1} fw and standard deviation of 49 mg g^{-1} for for *su1* and *sh2* types. This error was substantially below the standard deviation of the populations suggesting that the PLS model can be used to bin kernels into high, moderate, and low sugar groups. For starch, our PLS model had a standard error of prediction of 36.5 mg g^{-1} fw and standard deviation of 54 mg g^{-1} for all genotypes. These results suggest that skNIR could be a rapid way of assessing sweet corn sugar content, which can help better define the relationships between eating quality, post-harvest shelf life, and mature seed germination.

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P129

QTL mapping for maize starch content and candidate gene prediction combined with mutation analysis

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Increasing maize kernel starch content may not only lead to higher maize kernel yields and qualities, but also help meet industry demands. By using the intermated B73 × Mo17 population, QTLs were mapped for starch content in this study. A major QTL Qsta9.1 was detected in a 1.7 Mb interval on chromosome 9 and validated by allele frequency analysis in extreme tails of a newly constructed segregating population. According to genome-wide association study (GWAS) based on genotyping of a natural population, we identified a significant SNP for starch content within the ORF region of GRMZM5G852704 (AP2-EREBP-transcription factor 31, ereb31) colocalized with QTL Qsta9.1. In addition, we found a mutation mutator insertion in the upstream of ereb31 transcription factor through the newly constructed ChinaMu mutant library, and we will conduct functional verification in the next step. Our studies combined GWAS, traditional QTL mapping, GWAS and phenotype mutation analysis to investigate the functional roles mechanism for controlling underlying seed starch content in maize.

Gene / Gene Models described: *ereb31*; GRMZM5G852704
Funding acknowledgement: National key research and development program of China (2017YFD0102005), Natural Science Foundation of Jiangsu Province, China (BK20160582), National Natural Science Foundation of China (31601315)

P130

RAMOSA3 determines inflorescence branching and interacts with nuclear RNA binding proteins in maize

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Meristem fate in maize is modulated by metabolic enzymes called trehalose-6-phosphate phosphatases (TPPs). A prime example of this is the loss of maize TPP *RAMOSA3* (*RA3*), which leads to reduced meristem determinacy and more inflorescence branching. However, how *RA3* regulates meristem determinacy remains enigmatic. Our recent study found a lack correlation between TPP enzymatic activity and branching phenotypes. Interestingly, RA3 localizes to speckles in cytoplasmic and nuclear compartments, suggesting its function may be associated with regulation of RNA processing and transcription. Mass spectrometry-based targeted proteomics found that RA3 can associate with two closely related RNA binding proteins, whose molecular function is largely unknown. Consistently, bimolecular florescence complementation assays revealed that their association occurs in the nucleus, forming speckles, and implying a potential regulatory function in meristem determinacy. Our ongoing genetic and biochemical analysis aims to uncover the biological meaning of this association in modulating meristem determinacy. These findings provide a potential novel mechanistic link between metabolic signals and gene regulation in inflorescence architecture.

Gene / Gene Models described: ; GRMZM2G014729, Zm00001d002781, Zm00001d025981
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P131

REL2 acetylation in plant pathogen interactions

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Plant pathogens are some of the most devastating crop stressors. Protein acetylation has emerged as a major post-translational modification that modulates many different cellular processes, including plant immunity and stress responses. Acetylation and deacetylation alter the state of defense gene promoters, promoting susceptibility or resistance. *Cochlibolus carbonum*, Northern Leaf Spot, produces an effector molecule called HC-Toxin that functions as a histone deacetylase inhibitor, which is required for pathogen virulence. ZmREL2, ramosa1 enhancer locus, encodes a transcriptional corepressor that is homologous to the TOPLESS (TPL) gene in Arabidopsis. Furthermore, expression of ZmREL2 in Arabidopsis rescues developmental defects in tpl mutants. The TPL family acts as corepressors in many different pathways including auxin (TPL-IAA-ARF) and jasmonate (TPL-JAZ-MYC2) signaling. We identified a site of lysine acetylation on REL2 using global acetylome profiling of corn plants treated with HC-Toxin or infected with *C. carbonum*. We have found that rel2 mutant corn plants are susceptible to *C. carbonum* infection, unlike their B73 counterparts, which demonstrates that this gene is directly related to plant immunity. In addition, using Yeast Two Hybrid assays, we have shown that mutations of REL2 that mimic acetylation result in reduced interaction of REL2 with transcription factors containing DLN and RLFGV repression motifs. Finally, we confirmed using luciferase corepression assays that REL2 acts as a corepressor. Furthermore, REL2 acetylation null mutations abolish the repression activity of REL2. Ultimately the goal of this work is to elucidate how hyperacetylation impacts the biological activity of REL2 and its roles in plant pathogen interactions.

Gene / Gene Models described: *REL2*; Zm00001d024523
Funding acknowledgement: National Science Foundation (NSF)

P132

SWEETs and SUTs are regulated by distinct and overlapping transcription factors suggesting functional redundancy as well as gene specific functions in carbohydrate partitioning

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Carbohydrate partitioning describes the process of how sugars (typically sucrose) produced during photosynthesis are distributed from source organs (primarily leaves) to sink organs (e.g., roots, stems, flowers, and fruits) where these sugars are catabolized, anabolized, or stored. In maize, phloem loading in source organs and unloading in seeds requires crossing the apoplasm between the mesophyll and companion cells in leaves and between companion cells and parenchyma cells in developing kernels. Two families of transporters—one that imports sucrose and one that exports—are responsible for this activity. The Sucrose Transporter (SUT) gene family encodes sugar-proton symporters and it is thought that the SUTs work in conjunction with the SWEET transporters (Sucrose Will Eventually be Exported Transporters), which function as passive efflux proteins. Discerning how carbohydrates are distributed within a plant is a key step in both understanding and potentially manipulating the role it plays in plant development, drought tolerance, and crop yield. Furthermore, multiple pathogens, both bacterial and fungal, increase the transcription levels of several SWEETs indicating that they are co-opted during pathogenesis. Using a yeast one hybrid screen, we have begun to reveal the regulatory network that governs the expression of SUTs and SWEETs. Our findings suggest that many of these transporters, directly or indirectly, share the same regulatory network as genes involved in energy metabolism, sucrose synthesis, and carbon partitioning. Furthermore, we observed overlap in transcription factors that regulate SWEETs and SUTs which is consistent with the functional redundancy observed in SWEET13a,b, and c mutants (Bezrutczyk et al., 2018). Finally, every SUT or SWEET examined was regulated by several non-overlapping transcription factors suggesting gene specific functions as well.

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P133

Single cell methylome analysis of maize meiocytes

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Crossing-overs (COs) are responsible for the reciprocal exchange of genetic material between chromosomes during meiotic recombination. The newly formed unique genotypes created as a result of this process contribute to the adaptive potential increase in the next generation. The number of CO events in maize is limited to one or two per chromosome per meiosis, and CO locations differ substantially among meiocytes. One of the main factors determining CO location along chromosomes is chromatin structure, which is controlled by epigenetic mechanisms, such as DNA methylation. In fact, we found that CO sites can be unambiguously predicted based primarily on DNA methylation patterns. In addition, we found that DNA methylation mutants can exhibit altered CO landscape. DNA methylation is very dynamic during plant ontogenesis and differs according to cell type. Furthermore, DNA methylation patterns have been shown to change during male germline development. Given these results, we hypothesize that specific patterns of DNA methylation during meiosis may be unique among meiocytes and responsible for the meiocyte-to-meiocyte CO location differences. The goal of our current research is to examine differences in DNA methylation at the level of single meiocytes in maize. To date, we developed a method for single meiocyte isolation at specific meiotic stages for whole-genome methylation analysis in maize and optimized the most critical steps in the whole-genome amplification process required as a part of the analysis. We anticipate that our results will shed more light on the understanding of mechanisms controlling CO formation in plants.

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P134

Sweet sorghum stalk strength oscillates in a diurnal pattern

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Global grain yields are reduced by 5-20% annually due to stalk lodging: a complex phenomenon wherein the stalk falls over before harvest. The purpose of this project is to identify and evaluate the morphological and compositional phenotypes responsible for improved stalk lodging resistance. Using sweet sorghum as a model, a newly developed phenotyping device capable of measuring the force required to lodge grain stalks was used to lodge over 500 stalks of sorghum over the course of 48 continuous hours. Naturally occurring diurnal variation in turgor pressure (here proxied by water potential which was observed to range between 0.1MPa – 2.5 MPa), temperature, and relative humidity were observed to impart slight, yet significant effects on the mechanical attributes associated with stalk lodging resistance in fully mature sweet sorghum stalks. As data analysis continues, over two dozen additional parameters are being analyzed for individually lodged stalks in order to explore how multiscale material properties might interact to determine stalk strength.

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P135

Symbiont-induced systemic resistance to pathogens relies on oxylipins other than jasmonic acid for long distance signaling.

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While multiple long distance signal molecules were identified for pathogen-triggered systemic acquired resistance, mobile signals for symbiont-induced systemic resistance (ISR) remain unknown. There is increasing evidence that oxylipin signals other than JA have roles in ISR. Here, we report on the identification of xylem-resident 13- and 9-oxylipins as mobile ISR signals by metabolite and transcriptome profiling of maize LOXs and OPR mutants colonized by the beneficial fungus *Trichoderma virens* or fungal mutants lacking ISR peptide signals. We found that OPDA/JA/GLV-producing 13-LOX, *LOX10*, and the 9-LOX, *LOX12*, are required for *Trichoderma*-induced ISR. Rather than showing increased resistance to pathogens, *Trichoderma*-colonized *lox10* and *lox12* mutants became more susceptible. Oxylipin profiling of xylem sap from diverse maize/*Trichoderma* genotype combinations exhibiting contrasting ISR responses identified 12-OPDA and a 9-LOX-derived α-ketol, KODA, as molecular signals activating ISR. Transfusion with sap supplemented with 12-OPDA or KODA enhanced resistance against foliar infection in a dose-dependent effect. Surprisingly, *Trichoderma* induced ISR in JA-deficient mutants, suggesting that JA is not required for ISR. Transcriptome analysis of *T. virens*-treated maize roots revealed upregulation of 12-OPDA signaling, but downregulation of JA signaling. Thus, OPDA and KODA, not JA, are required for activation of *Trichoderma*-induced ISR.

Gene / Gene Models described: *lox10, lox3, opr7, opr8*; Zm00001d033623, Zm00001d053675, Zm00001d032049, Zm00001d050107
Funding acknowledgement: United States Department of Agriculture (USDA)

P136

Systems genetic analysis of source-sink regulated senescence reveals novel candidate genes and pathways

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Senescence is a complex biological process controlled by a number of internal and external factors. We are particularly interested in understanding the role of source-sink communication and identify the underlying molecular mechanisms. To this end, through systematic characterization of the natural diversity, we have identified novel candidates underlying source-sink regulated senescence (SSRS). We have also conducted time-course transcriptome and metabolite analysis of SSRS sensitive and resistant inbred lines. Our results reveal dynamic changes in transcriptome underly the SSRS. The identified candidate genes represented interesting phenomena including proteolytic processes, autophagy, and sugar metabolism. The metabolic analysis revealed that SSRS is associated with differential accumulation of sugars, cytokinin activity, and hexokinase activity. We are currently examining the role of candidate genes related to some of key processes identified from our study including proteolytic activity and autophagy using Arabidopsis mutants. Development and characterization of rice and maize mutants for the candidate genes is also underway. Results from these ongoing experiments will be presented. Identification and characterization of novel genes and pathways will pave the way for enhanced mechanistic insights into the regulation of senescence.

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P137

The NIN-like protein 5 (ZmNLP5) transcription factor is involved in modulating the nitrogen response in maize

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Maize exhibits marked growth and yield response to supplemental nitrogen (N). Here, we report the functional characterization of a maize NIN-like protein ZmNLP5 as a central hub in a molecular network associated with N metabolism. Predominantly expressed and accumulated in roots and vascular tissues, ZmNLP5 was shown to rapidly respond to nitrate treatment. Under limited N supply, compared with that of wild-type (WT) seedlings, the *zmnlp5* mutant seedlings accumulated less nitrate and nitrite in the root tissues and ammonium in the shoot tissues. The *zmnlp5* mutant plants accumulated less nitrogen than the WT plants in the ear leaves and seed kernels. Furthermore, the mutants carrying the transgenic *ZmNLP5* cDNA fragment significantly increased the nitrate content in the root tissues compared with that of the *zmnlp5* mutants. In the *zmnlp5* mutant plants, loss of the ZmNLP5 function led to changes in expression for a significant number of genes involved in N signalling and metabolism. We further show that ZmNLP5 directly regulates the expression of nitrite reductase 1.1 (*ZmNIR1.1*) by binding to the nitrate-responsive *cis*-element (NRE) at the 5′ UTR of the gene. Interestingly, a natural loss-of-function allele of *ZmNLP5* in Mo17 conferred less N accumulation in the ear leaves and seed kernels resembling that of the *zmnlp5* mutant plants. Our findings show that ZmNLP5 is involved in mediating the plant response to N in maize.

Gene / Gene Models described: *ZmNLP5, ZmNIR1.1*; GRMZM2G042278,GRMZM2G079381
Funding acknowledgement: National Key Research and Development Program of China (Grant No. 2016YFD0101002), the National Natural Science Foundation of China (No. 31271728), the Natural Science Foundation of Jiangsu Province, China (BK20160582)

P138

The carotenoid-deficient albescent1 mutant results from lesions in tandemly-duplicated putative plastid terminal oxidase-coding genes

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Maize *albescent1* (*al1*) is a light-sensitive, pale-leaf, pale-endosperm mutant first described in 1929 by Phipps and renamed *al1* in 1935 by Emerson. Mapping and allelism tests conducted by Phil Stinard at the Maize Genetics Cooperation Stock Center localized *al1* mutant alleles to chromosome 2 in the vicinity of a putative plastid terminal oxidase (PTOX)-coding gene, homologous to genes underlying the Arabidopsis *IMMUTANS* and tomato *Ghost* mutants. Work in Stephen Moose's lab confirmed UniformMu alleles in the putative PTOX were associated with *albescent1*-like phenotypes and that PTOX expression was altered in some alleles of *al1* curated by the Stock Center. We have continued characterization of a series of stock center alleles and Mutator alleles of the *al1* mutant showing a range of phenotypic severity. As part of this effort, Stock Center alleles have been introgressed into the W22 background to aid in phenotypic analyses and comparisons between alleles. The *al1* locus contains two, tandemly-duplicated putative PTOX-coding genes separated by less that 100,000 bases. Genetic analysis indicates that mutations in either gene may be sufficient to cause the *al1* phenotype. Partial loss of PTOX expression leads to accumulation of phytoene and carotenoid deficiency because plastoquinone-9 (PQ9), a required cofactor for phytoene desaturation, cannot be sufficiently reduced when PTOX is limited. Insufficient carotenoid biosynthesis leads to photooxidation of chlorophyll and bleaching when grown in high light. The most severe alleles of *al1* appear to result in non-viable seeds. Previous work on *white seeding 3* (*w3*), a mutant blocked in homogentisate solaneysl transferase unable to synthesize PQ9, revealed that *w3* mutants retained the ability to produce small amounts of carotenoids. Examination of *w3 al1* double mutants showed a heightened carotenoid-deficient phenotype with extreme vivipary. This finding indicates that PTOX may serve as an electron acceptor for PDS in the absence of PQ9.

Gene / Gene Models described: *al1, w3*; GRMZM2G102349, GRMZM2G010555
Funding acknowledgement: United States Department of Agriculture (USDA)

P139

The crossbanded maize mutant *Carbohydrate partitioning defective4* exhibits attenuated sugar transport and partial phloem occlusion

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Efficient partitioning of photosynthates in the form of sucrose from source leaves to distal sink tissues is essential for maize growth, development, and reproduction. In order to identify novel genes involved in carbohydrate partitioning, we have mapped and characterized a suite of *carbohydrate partitioning defective* mutants that exhibit characteristic patterns of leaf chlorosis and growth inhibition as a result of impaired sucrose export from source leaves. *Carbohydrate partitioning defective4* (*Cpd4*) is a semi-dominant, EMS-induced mutation. *Cpd4* leaf laminae exhibit distinctive diurnal crossbanding in the heterozygote and uniform chlorosis and stunted growth in the homozygote. Chlorotic sectors develop over the course of several weeks and hyperaccumulate starch and soluble sugars. Phloem sieve element occlusions comprised of callose and lignin are observed within chlorotic sectors, and long-distance sucrose transport is impaired, suggesting that these occlusions may inhibit carbohydrate partitioning. In developing leaves, callose occlusions precede both the sink-source transition and carbohydrate accumulation and occur in etiolated seedlings independently of light exposure. Although the callose occlusions are broadly similar to those reported for *Carbohydrate partitioning defective1* (*Cpd1*), *Cpd4* occlusions occur later in leaf development and are absent from roots, suggesting a unique mechanism. Furthermore, although *Cpd1*; *Cpd4* double heterozygotes exhibit a strong synergistic *carbohydrate partitioning defective* phenotype, a synergistic effect on callose accumulation is not observed, and the two mutations are neither allelic nor syntenic paralogues. *Cpd4* maps to a single locus on Chromosome 9S, and efforts are underway to identify the causal polymorphism by whole genome sequencing.

Funding acknowledgement: National Science Foundation (NSF)

P140

The essential role of an RNA Binding Motif Protein 48 (RBM48) in U12 splicing is conserved between maize and humans

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Maize RNA Binding Motif Protein 48 (RBM48) is an essential splicing factor required for the splicing of minor U12-type introns. U12-type introns share unique structural features and are processed by a distinct minor spliceosome. These introns derive from the last eukaryotic common ancestor and constitute ~0.5% of all introns in species with a minor spliceosome. Mutations that result in aberrant splicing of U12-type introns have been reported to cause developmental defects in both plants and animals; however, the biological relevance of a distinct minor group of introns is not well understood. We recently reported a maize *rbm48* mutant that displayed severe defects in endosperm development and genome wide aberration in splicing of primarily U12-type introns. Since the mis-splicing of U12-type introns in *rbm48* mutants impacts expression of many minor intron-containing genes (MIGs), it negated our efforts to identify genes contributing to the mutant phenotype. In this report we generated a CRISPR/Cas9-mediated RBM48 functional knockout (RBM48 FunKO) in human K-562 cells and demonstrated that the essential role of RBM48 in U12 splicing is conserved between maize and humans. With comparative RNA-seq analysis we have identified candidate orthologous MIGs between maize and human that display aberrant splicing of U12-type introns. Mutations in the vast majority of these MIGs have been reported to cause developmental defects in both plants and animals. These studies indicate that RNA-seq comparison of orthologous mutations of U12 splicing factors between distantly related species could provide a novel strategy to not only identify candidate genes impacting mutant phenotypes, but also lead to the elucidation of conserved gene regulation and cellular processes required for normal growth and development.

Funding acknowledgement: National Institutes of Health (NIH), National Science Foundation (NSF)

P141 @carrie_f_olson

The genomic and metabolic consequences of hybridization between two milkweed species (*Asclepias*)

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Between 30-70% of flowering plant have hybridization in their history, but the effect that hybridization has on metabolic pathways is unknown. Here we are studying the extent and history of hybridization between two species of milkweed that grow in the Western (*Asclepias speciosa*) and Eastern United States (*A. syrica*). In addition to different geographic locations, these species have diverged in a number of important phenotypes, including: production of secondary metabolites, flower morphology, and drought resistance. In the central US, these species have come into secondary contact, sometimes forming hybrids. We have found evidence of transgressive segregation for floral morphology in the region of sympatry suggesting advanced hybrids. We submitted 100 collections from three transects across the hybrid zone and will discuss the extent and history of hybridization in these species.

Funding acknowledgement: National Institutes of Health (NIH), National Science Foundation (NSF)

P142 @carrie_f_olson

The genomic consequences of hybridization between two milkweed species (*Asclepias*)

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Funding acknowledgement: National Institutes of Health (NIH), National Science Foundation (NSF)

P143

The silk cuticular lipid landscape of the Wisconsin Diversity Panel: Impacts of genotype, environment, and GxE interactions

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The cuticle is the outermost barrier between aerial portions of the plant and the external environment, protecting the plant from both biotic and abiotic stresses. The cuticle is synthesized by the epidermis and consists of a cutin polymer matrix infused with and coated by non-polar, amphipathic cuticular lipids that vary in composition depending on species, organ, and developmental stage. Further, plants can display strong metabolic responses to environmental stress. For example, the cuticle on maize silks, which is comprised primarily of very long chain hydrocarbons and fatty acids, is responsive to the environment; the portions of silks that are exposed to the external environment display compositionally different metabolic profiles as compared to the husk-encased portions. To assess the impacts of genotype, environment, and GxE interactions on cuticular lipid composition on silks that have emerged from the encasing husk-leaves, we profiled the cuticular lipid metabolomes of emerged silks from a phenologically restricted subset of 468 maize inbreds of the Wisconsin Diversity Panel grown in three environments (Iowa State University 2016, 2017; University of Minnesota 2016). Moreover, 40 inbreds representing the breadth of diversity in the larger panel were assessed in an additional five environments. Collectively, we have identified that there is a significant effect of genotype, environment, and GxE interactions on cuticular lipid profiles, namely for total surface lipid abundance and proportional abundances of saturated and unsaturated hydrocarbons, as well as for many individual constituents. To further assess the impacts of environment, we are applying multivariate approaches to identify weather parameters that may influence specific cuticular lipid traits.

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P144

Transcriptome remodeling by the hypersensitive response and associated regulatory variation in maize

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The plant hypersensitive response (HR) is a subcategory of programmed cell death that dramatically remodels metabolism and gene expression in response to an infection threat. The maize gene *Resistance to Puccina sorghi1* is encoded within a complex of nucleotide binding–leucine- rich repeat loci, alleles whichsemi-dominantly confer constitutive activation of HR. The *Rp1-D21*allele initiates HR in the absence of pathogen permitting measurements of metabolism and gene expression during HR without the interpretive complexity of a second organism. Prior genetic mapping experiments localized genetic variants that modify the exaggerated form of HR conferred by *Rp1-D21*. We have now identified expression level QTL, metabolism changes, and allele-specific gene expression patterns that both place of modifying loci in the HR response pathway and illuminate the molecular mechanisms responsible for NLR-symptom severity in this HR model. The *Rp1-D21* allele was maintained as a heterozygote in the inbred H95 and F1 progeny from crosses to this line were used to make congenic paired materials with divergent HR phenotypes. When the mutant was crossed into enhancing genetic backgrounds, it resulted in many thousands of DGE as well as a greater amplitude of expression difference in *Rp1-D21*F1s from HR enhancing backgrounds. Using F1 crosses to the most divergent HR severity subpopulation of the NAM RIL, B73 x NC350, as well as crosses between parent lines we identified genetic factors controlling maize HR-induced expression quantitative trait loci (eQTL). Remarkably the number of eQTL during HR was dramatically greater than in wildtype hybrids and two loci known to modulate HR severity were trans-regulatory QTL altering the expression of thousands of genes each. Allele specific expression was used to confirm and distinguish cis-eQTL from linked trans-acting QTL in both wildtype and mutant F1 plants using variation between H95 and either the B73 or NC350 alleles.

Gene / Gene Models described: *Rp1*; Zm00001d023317
Funding acknowledgement: National Science Foundation (NSF), Department of Energy (DOE)

P145

Understanding the biomechanical and metabolic basis of stalk strength in maize using novel phenotyping and statistical modeling

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Stalk lodging in maize (*Zea mays* L.) severely compromises productivity. Progress in genetic improvement of stalk lodging has been limited due to a lack of reliable and field-deployable phenotyping approaches. To this end, our team of plant biologists, biomechanical engineers, and mathematicians is developing and deploying new phenotyping tools. Recently, we reported the development of DARLING (Device for Assessing Resistance to Lodging IN Grains) to assay the stalk strength of field-grown plants (Cook et al., 2019; doi.org/10.1186/s13007-019-0488-7). To identify key metabolic and biomechanical determinants of stalk strength, we analyzed variation in a set of 16 hybrids derived from 8 diverse inbreds, each crossed with a stiff-stalk (B73) and a non-stiff stalk (Mo17) inbred line. Measurement of stalk bending strength with DARLING revealed substantial phenotypic variation among hybrids. We are currently examining various additional biomechanical properties of these stalks using both novel and existing phenotyping approaches. Additionally, we are generating high-resolution near-infrared spectral data. These data will be combined using novel Bayesian generalized linear mixed effects models to identify the most informative phenotypes underlying stalk strength. Identification of most informative component traits underlying stalk strength would allow us to begin to generate a comprehensive picture of the genetic architecture of lodging resistance.

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P146 @jferarp

Variation in C_4 photosynthetic pathways over the maize life cycle

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C_4 photosynthesis is a convergent trait that has evolved in multiple plant lineages to concentrate CO_2 around Rubisco in specialized bundle sheath cells. Historically, the pathway has been characterized into three different subtypes based on the decarboxylase involved, although recent work has provided evidence that some plants can use multiple decarboxylases. For example, maize uses NADP-malic enzyme as the primary C_4 pathway, but as much as 25% of carbon is shuttled using the phospho*enol*pyruvate carboxykinase (PEPCK) pathway. Having multiple C_4 pathways could be advantageous in balancing energy and reducing equivalents between bundle sheath and mesophyll cells and in decreasing the size of the metabolite gradients between cells. In addition, flexibile partitioning of carbon through the sub-pathways may better accommodate changing environmental conditions or source to sink demands on growth. In *Cleome gynandra*, for example, the enzyme activity of C_4 decarboxylases fluctuates with different stages of leaf development; however it is unclear if the pathway flexibility is an innate aspect of leaf development or an adaptation to the leaf microenvironment. In this project, maize plants were characterized for variation in its two C_4 pathways at nine plant ages throughout the life cycle and for two positions in the canopy. Measurements were taken for photosynthetic traits using a LI-COR 6800 gas exchange instrument and collected samples were used to measure gene expression, enzyme activity, chlorophyll, soluble protein, and composition of amino acids, sugars and sugar phosphates. Variation was observed in these traits for both leaf age and canopy position, reflecting the ability of C_4 pathways to adapt to changing microenvironments and the leaf life cycle.

Gene / Gene Models described: ; GRMZM2G001696, GRMZM2G085019
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Department of Energy (DOE)

P147

What's glutamate signaling got to do with maize plant architecture?

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Glutamate receptors (GLRs) are homologs of mammalian ionotropic glutamate receptors (iGluRs) that act as cation channels and transport Ca^{+2} ions across the membranes. Plants have a large family of GLRs with 17 members in Maize and 20 in *Arabidopsis;* however, the intriguing question is, what do these receptors do. Some recent findings implicate them in processes as diverse as long-distance wound signaling and sperm chemotaxis (Ortiz-Ramírez et al. 2017; Toyota et al. 2018). Our study demonstrates that they also have a role in shaping plant architecture. We identified a missense mutation in one of the 17 maize GLRs in a partially dominant dwarfing mutant that we named D13. This mutant appeared in an M1 population of EMS-mutagenized inbred B73. Besides the reduced height, an additional phenotype of D13 is the accumulation of anthocyanins in juvenile leaves, a trait that is also more severe in homozygous condition. Several features of D13 are enthralling. First, it is relatively unstable and shows variable levels of severity. Second, it is easily impacted by the genetic background, with some inbreds such as Mo17 completely suppressing the phenotype. The mechanism behind dwarfing in D13 remains unknown, but the RNA-seq analysis rules out the involvement of GA and BRs, two phytohormones that have been shown to play a major role in regulating height in plants.

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P148

"Idling effect": a working model for anther elongation arrest in *ZmMs33* mutant

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ZmMs33 encodes a sn-2 type glycerol-3-phosphate acyltransferase (GPAT), its loss-of-function mutant leads to genic male sterility (GMS) and anther elongation arrest. However, the underlying mechanism of abnormal anther elongation in *ZmMs33* mutant remains obscure. Here, we found Ocl4 encoded by another maize GMS gene can bind *ZmMs33* promoter and ZmMs33 is a functional GPAT with C16:0-CoA as one of its substrates. The lipidomics measurements showed that total amounts of cutin and wax, as well as majority of detected cutin and wax monomers, were decreased in mutant. Genes function in key metabolism steps of fatty acid (FA) synthesis, elongation and unsaturation, as well as wax and cutin biosynthesis were down-regulated in mutant anther, while genes in FA catabolism and triglyceride synthesis were up-regulated in transcriptomes. Besides, significantly high levels of reactive oxygen species (ROS) were observed in *ms33-6038* mutant anther, which may lead to activated branch chain amino acids catabolism trigged by carbon starvation in mutant. MAPK signaling pathway and antioxidative responses were activated in *ms33-6038* mutant anther viewed by transcriptome data. We also found that most of the cell elongation positive regulators were up-regulated and negative regulators were down-regulated in mutant. Further comparative transcriptomic analysis revealed that the expression alterations of genes involved in cell elongation, carbon starvation stress response, photosynthesis, primary metabolism, ROS metabolism and oxidative stress response were specific in *ms33-6038* mutant compared to transcriptomes of GMS mutants *ms30*, *ocl4*, *mac1* and *ms23*, respectively. Taken current results together, we present a proposed working model called "Idling effect" for anther elongation arrest in *ms33-6038* mutant: although gene involving in cell elongation were up-regulated at expression level, anther cell elongation were inhibited at metabolism level by reduced energy level and increased ROS amount introduced by *ZmMs33* function defection.

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P149

Adherent1 is a 3-Ketoacyl CoA synthase required for maize cuticle development and organ separation

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In land plants all aerial epidermal cells are covered by the cuticle, an extracellular hydrophobic layer. The cuticle represents a primary barrier between cells and the external environment, provides protection against abiotic and biotic stresses, and prevents organ fusion during development. The main components of the cuticle are cutin and cuticular waxes. Here we report the characterization of a classic mutant of maize called *adherent1* (*ad1*), first described a century ago, and we show that *AD1* encodes an enzyme involved in the deposition of cuticular waxes on the epidermis of leaves and inflorescences. In *ad1* mutants, epidermal fusions can occur between organs in both juvenile and reproductive stages. These results suggest that *AD1* plays an important role in establishing epidermal properties required to maintain proper organ separation throughout maize development. We evaluated the amount and composition of cuticular waxes in wild type and ad1 plants using gas chromatography-mass spectrometry (GC-MS) and gas chromatography coupled to a flame ionization detector (GC-FID), respectively. *ad1* mutants had reduced amounts of cuticular wax components with acyl chains longer than 31 carbons, suggesting a key role for *AD1* in the formation of wax compounds with more than 31 carbons. Cuticular wax biosynthesis is precisely regulated at the transcriptional level. Accordingly, *AD1* is strongly expressed in the epidermis of different developing organs and is subjected to direct transcriptional regulation by the MYB transcription factor FUSED LEAVES1 (FDL1), which in turn controls a series of additional genes involved in cuticle formation. Altogether, our results identify a major pathway of cuticle biosynthesis essential for the development of maize plants.

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P150

Analysis of differential gene expression in the upper and lower meristems of maize

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All flowers are the products of floral meristems, which give rise to the floral organs and ultimately are consumed by organogenesis. In maize, male and female flowers (florets) are present on separate inflorescences; the tassel produces male flowers and the ear produces female flowers. In both inflorescences, spikelet meristems give rise to an upper floral meristem (UFM) and lower floral meristem (LFM). In tassels, carpels abort and both FMs develop to maturity, resulting in spikelets with two male florets. In the ear, stamens arrest and the LFM aborts, resulting in spikelets with a single female floret. Early floral development in both FMs appears similar, although development of the LFM is delayed relative to the UFM. Previous data, however, indicate that gene regulatory networks differ between the UFM and LFM. To further examine gene expression in the UFM and LFM, we used laser-capture microdissection coupled with RNA-seq and identified approximately 600 genes differentially expressed between the UFM and LFM in ears. The UFM is enriched for genes involved in protein metabolism, carbohydrate/nucleotide transport, and sugar response/transport, whereas the LFM is enriched for genes involved in hormone regulation, amino acid metabolism, and cell wall modification. Based on these results, we further investigated cell wall composition and sugar accumulation in developing florets. Interestingly, pectin is dramatically reduced in the LFM compared to the UFM, consistent with increased expression of pectin degradation genes in the LFM. Starch accumulated at the boundary between the UFM and LFM, suggesting that sugar distribution is carefully regulated during floral development. Thus, these data suggest unexpected differences in cell physiology between the UFM and LFM, which may have broader roles in cell fate and development.

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P151

Analysis of *stunter2* and *stunter3*, maize maternal effect mutants with reduced kernel size

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Regulation of growth and development of seeds in plants is largely controlled by the haploid female gametophyte through gene expression following meiosis. *stunter2* (*stt2*) and *stunter3 (stt3)* are novel maize mutants that disrupt proper development of the female gametophyte, which ultimately affects seed development post fertilization. These two mutants phenocopy *stunter1 (stt1)*, a previously characterized maize mutant with viable but reduced embryos and endosperms and small female gametophytes. *stt2* and *stt3* embryo sacs are smaller, with smaller central cells and fewer antipodal cells than wild type. Additionally, both mutants exhibit reduced transmission through the male gametophyte. Like *stt1*, *stt2* and *stt3* pollen grains are smaller, and the mutations negatively affect pollen tube germination. Post-fertilization, both embryo and endosperm development are delayed, with *stt2* and *stt3* exhibiting disruptions in the development of the basal endosperm transfer layer (BETL), which facilitates nutrient transport to the developing seed. Consistent with disruption of the BETL, *stt3* exhibits reduced amylose content in the endosperm compared to wild-type sibling endosperm. Whereas *stt2* may be allelic to *stt1*, *stt3* is unlinked and represents a unique lesion. These mutants will help elucidate mechanisms for maternal control of seed development in maize.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P152

Beyond the protoplast: Isolating and analyzing fixed cells for single-cell research

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The digestion of the cell wall is a major hurdle for single-cell research in plants as it requires substantial protocol optimization and produces morphologically homogenous and highly fragile protoplasts. Here we provide a protocol for single-cell research using maize anthers fixed prior to cell wall digestion. We found that fixed cells could be subjected to harsher conditions than protoplasts while maintaining RNA of sufficient quality for short read sequencing. Fixation also preserved cellular morphology – a distinguishing feature of many plant cell types useful for automated cell sorting or manual isolation. In addition, the fraction of cellular release from fixed tissue samples was much higher than that of popular protoplasting protocols. We found that the fixed cell protocol can be broadly applied to a variety of taxa and tissues with little optimization and should thus facilitate single-cell research in plant biology.

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P153

Building an academic/industry collaboration to improve transformation of recalcitrant maize inbreds

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Morphogenic genes such as *Baby boom* (*Bbm*) and *Wuschel2* (*Wus2*) have been used to produce transgenic plants from recalcitrant maize inbred lines (Lowe et al., 2016, Plant Cell 28: 1998; Lowe et al., 2018, In Vitro Cell Dev Biol-Plant 54: 240). This method (named QuickCorn), developed by Corteva Agriscience, is fast and less genotype-dependent than traditional maize transformation. Here we report an effort in evaluating the robustness of the protocol by comparing transformation frequencies of three public maize inbred lines obtained by multiple researchers in two different laboratory settings. The goal is to identify factors contributing to protocol reproducibility for newly trained researchers when transforming public maize genotypes. *Agrobacterium*-mediated transformation was used to infect immature zygotic embryos (IZEs) of B73, Mo17 and W22, three important but historically recalcitrant inbred lines. Total frequencies of regenerated events (# rooted plantlets per 100 infected IZEs), transgenic events (# transgenic events per 100 infected IZEs) and quality events (# events with single copy integration and morphogenic gene excised per 100 infected IZEs) were calculated. Our preliminary comparison show that all three recalcitrant public inbred lines can be transformed, but with varied frequencies. W22 had the highest response with an average ~40% regeneration rate. Average regeneration rates of B73 and Mo17 were ~7% and ~4%, respectively. Average transformation frequencies were ~14% for W22, and ~4% for both B73 and Mo17. Importantly, the results of all four researchers (two with previous QuickCorn experience and two with little QuickCorn experience) were comparable. This shows that compared to traditional transformation methods, the QuickCorn method does not demand highly trained researchers, making it more amenable for academic laboratories. While not directly tested in these experiments, healthy immature ears at the correct developmental stage, correctly prepared media, and properly handled explant materials appear to be fundamental to protocol reproducibility.

Funding acknowledgement: National Science Foundation (NSF), Crop Bioengineering Center of Iowa State University (CBC)

P154

Characterization of brassinosteroid deficient semi-dwarf mutant1, modulating shoot architecture in maize

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Shoot architecture is a key determinant of grain yield in maize. Among the major plant growth regulators, brassinosteroids (BRs) affect multiple developmental processes and plant architecture traits including organ size, sex determination and leaf angle. However, the genetic mechanisms by which BRs regulate plant architecture traits in maize remain poorly understood. We recently identified and characterized a recessive, EMS-induced maize mutant, which we tentatively named *brassinosteroid deficient semi-dwarf mutant1* (*bds1*), with a semi dwarf stature due to compressed internodes. Internode number is unaffected. *bds1* mutants also have short leaf sheaths and twisted leaf blades and display a partial tassel-seed phenotype in the Mo17 background and reduced tassel branch number in B73. The mutants also exhibited a photomorphogenic phenotype in the dark i.e. failed mesocotyl and shoot internode elongation. Treatment of wildtype plants with the BR biosynthesis inhibitor propiconazole mimicked the *bds1* mesocotyl phenotype. Using map-based cloning and whole genome sequencing, we localized *bds1* to a small region on chromosome 3S containing a point-nonsense mutation in a gene encoding a cytochrome P450 enzyme, and confirmed the gene cloning with a second *bds1* transposon-induced allele. The *bds1* gene appears to be a paralog of the rice BR biosynthetic gene *CYP90D2/D2*. In a feeding experiment, the *bds1* mutant reduced mesocotyl length was partially rescued with exogenous BL. Metabolite accumulation quantification and other genetic analysis is underway. Based on these results, we propose that *bds1* is a novel BR biosynthetic gene that modulates shoot architecture in maize.

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P155 @plantsdonttweet

Characterization of cytokinesis3 using bulked segregant analysis and live cell imaging

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Cell division is key to proper growth and development in multicellular organisms. In plants, the division plane is established early in the cell cycle and is marked by proteins that remain at the division site until the new cell wall is built. The final steps of cell division are executed in cytokinesis by the phragmoplast, a cytoskeletal and vesicular structure, that lays down the new cell wall. While key players important to cytokinesis have been reported, many more remain to be identified. Severe defects in cytokinesis often result in embryo lethality, making this a difficult process to study. Here, we describe cytokinesis3 (cyto3), a recessive cytokinesis mutant generated through ethyl methanesulfonate (EMS) mutagenesis. cyto3 is a mutant with minor cytokinesis defects and no misoriented divisions. Only 1-3% of cyto3 mutant cells have cytokinesis defects characterized by so called 'cell wall stubs' and mutant plants make typical ears and tassels. In addition, we characterize cyto3 using live cell imaging of fluorescently tagged mitotic microtubule structures and division plane markers. Finally, we used bulked segregant analysis coupled to whole genome re-sequencing to map the interval containing cyto3.

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P156

Characterization of maize trehalose-6-phosphate synthases (ZmTPSs) and their functions in controlling inflorescence architecture

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In plants, trehalose, a non-reducing disaccharide, is associated with developmental and stress signaling, however the mechanisms are unclear. Trehalose is synthesized in a two-step process. First, trehalose-6-phosphate synthase (TPS) catalyzes trehalose-6-phosphate (T6P) from glucose-6-phosphate and UDP-glucose, then trehalose-6-phosphate phosphatase (TPP) dephosphorylate T6P to trehalose. The classic maize mutant *ramosa3* (*ra3*) has increased inflorescence branches, and our studies revealed that RA3 encodes a catalytic TPP, however its enzyme activity is not responsible for controlling branching. To further explore the molecular mechanism of RA3's "moonlighting" functions in controlling inflorescence architecture, we screened for RA3 interactors. An IP-MS experiment found that RA3 interacts with ZmTPS1, a noncatalytic TPS. In addition, *zmtps1* mutants enhanced *ra3* phenotypes, suggesting the interaction between ZmTPS1 and RA3 is biologically significant. On-going work is applying cell, molecular and biochemical approaches to determine how ZmTPS1 regulates RA3 function. Interestingly, a yeast-2-hybrid experiment found that ZmTPS1 also interacts with two catalytic TPSs, ZmTPS14 and ZmTPS11. Our working model is that the noncatalytic ZmTPS1 might bind and affect the enzyme activity of a catalytic TPS complex in maize. To test this model, we generated CRISPR mutant alleles of these catalytic ZmTPSs, and double and triple *tps1, tps11* and *tps14* mutations are being generated. These mutants will be used to characterize the function of noncatalytic and catalytic maize TPS. In addition, other approaches will be applied to test RA3 interactions with catalytic and noncatalytic TPS proteins, to further understand the enigmatic role of trehalose signaling in plants.

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P157

Cloning an ear length QTL reveals ethylene as a developmental signal controlling kernel number in maize

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Maize is the worlds' most productive cereal crop, in part due to its large ears with many hundreds of kernels. The ear develops by a stereotypical pattern of meristem divisions; the inflorescence meristem produces spikelet-pair meristems which branch to form spikelets, which terminate with the production of florets. Therefore, meristematic activity in the inflorescence determines the number of florets and seeds. Recent insights have revealed genes important for the number of kernel rows, but control of the number of kernels per row, and ear length, is poorly understood. In a QTL population, we determined the developmental basis for ear length variation to be due to spikelet potential, not spikelet number per se. In other words, long ear lines do not make more spikelets, but more of their spikelets form seeds. We cloned a QTL, *qEL7,* underlying this trait, and found it encodes an ethylene biosynthetic enzyme, 1-aminocyclopropane-1-carboxylate oxidase2 (ACO2), and we confirmed its function by gene expression, enzyme kinetics assays and transgenic validation. Silencing ZmACO2 lines resulted in ~15-24% increase in ear length, and ~12-27% increase in kernel number per row. RNA-seq analysis of our QTL NILs identified candidate downstream regulators, including *BARREN INFLORESCENCE4* and AP2/EREBP transcription factors such as *INDETERMINATE SPIKELET1* and *BRANCHED SIIKLESS1*. We also identified a 7bp indel containing a TGACG motif in the *ZmACO2* promoter as a candidate polymorphism controlling *ZmACO2* expression. This motif is a candidate binding site for a bHLH transcription factor encoded by *BARREN STALK1* (*BA1*). *ZmACO2* and *BA1* are expressed in similar domains, and *ZmACO2* expression decreases dramatically in *ba1* mutants, suggesting *BA1* is an upstream regulator of *ZmACO2*. Our studies provide direct evidence for a role of ethylene in the regulation of spikelet meristem fate, kernel number and ear length, and provide a tool to improve grain yield by modifying ethylene levels.

Gene / Gene Models described: *ZmACO2*; Zm00001d020686
Funding acknowledgement: National Science Foundation (NSF)

P158

Control of subcellular localization of a KNOX transcription factor in rice.

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In plant shoot meristems, class I knotted1-like homeobox transcription factors (KNOX TFs) prevent cells from differentiation, whereas these TFs are down-regulated in a group of cells from which leaves or internodes differentiate. Although transcriptional regulation of KNOX genes is well-studied, it is unknown whether their activity is controlled at a post-translational level. We found that a rice KNOX TF, Oryza sativa homeobox1 (OSH1), was excluded from nuclei in callus when ectopically expressed using a maize ubiquitin promoter, but accumulated in the nucleus during de novo shoot meristem formation. In vegetative shoot meristems, OSH1 localized both in the nucleus and cytosol but the non-nuclear accumulation became evident during stem differentiation, suggesting that the exclusion of OSH1 from nuclei associates with cell differentiation. Deletion and chemical treatment studies revealed that OSH1 has at least three distinct regions involved in nuclear export, and that its export is mediated by an exportin dependent manner. These results raise a possibility that the nuclear import/export pathways play an important role in controlling KNOX TF activities in shoot meristems.

Gene / Gene Models described: *OSH1*; LOC_Os03g51690
Funding acknowledgement: JSPS

P159

Dissecting genetic networks regulating embryo-endosperm relative size in maize

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Understanding mechanisms that determine organ size is a fundamental theme in plant development. In addition, ratio of embryo and endosperm size is an important determinant of grain composition. Overall seed size is known to be predominantly regulated by maternally expressed genes, and many genes regulating seed size have been identified. However, zygotic mechanisms that determine relative size of cereal embryo and endosperm, but not overall seed size, are little understood. So far, only three genes, including two identified by our group, have been implicated in regulation of relative size of embryo and endosperm. In the rice *giant embryo* (*ge)* mutant, loss of function of a *CYP78A13* P450 gene conditions grains with larger embryo and smaller endosperm. We have identified BIGE1, a *trans*-Golgi localized MATE transporter that coordinates embryo and endosperm size in maize. Similar to rice *ge* mutant seeds, loss of *Big embryo1 (Bige1)* causes enlargement of embryo at the expense of the endosperm. In contrast to *ge* and *bige1*, mutations in the *Shohai1* (*Shai1*) gene encoding RWP-RK domain transcription factor have the opposite effect of enhancing endosperm size at the expense of the embryo. Intriguingly, the genetic analyses of *bige1* and *shai1* revealed non-autonomous functions of these genes in which the endosperm genotypes affected on the embryo phenotypes, implicating communication from endosperm to embryo in regulation of the relative size of these two organs. To identify additional genes controlling relative size of maize embryo and endosperm, we screened UniformMu population and isolated candidate mutants with altered relative size of embryo and endosperm. Further genetic analysis revealed that these mutants represent nine independent loci. Detail characterizations of the mutants are underway.

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P160

Dissecting maternal and zygotic control of biotin-dependent signaling required for epidermal cell fate specification in embryogenesis

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Development of seeds in plants depends on the orchestration of maternal and zygotic programs. Because zygotic developmental programs initiate immediately after fertilization, maternal factors are often assumed to have limited role in plant embryogenesis. Nevertheless, supply of critical factors to the embryo is under maternal control leaving open the possibility of maternal influence on embryogenesis. Here, we analyzed the rice *globular embryo 5* (*gle5*) mutation which fails to maintain epidermal cells during early embryogenesis. *GLE5* encodes bifunctional key enzyme, BIO1/3, that catalyzes two successive steps in biosynthesis of the water-soluble vitamin, biotin. Biotin is pivotal to all kinds cells because it is a cofactor of carboxylases required for lipids or amino acids metabolism. We hypothesize that *gle5* prevents protoderm maintenance by disrupting synthesis of lipids especially very long chain fatty acids (VLCFA). Surprisingly, *gle5* does not affect endosperm development including normal aleurone differentiation and lipid accumulation. Moreover, biotin content was nearly normal in mutant endosperm. We inferred that endosperm acquired sufficient biotin from maternal tissue. Analysis of a hypomorphic mutation in the maize *gle5* ortholog confirmed that both embryo and endosperm obtain part of their biotin requirement from maternal sources. Morphological and transcriptome analysis of developing rice *gle5* embryos indicated that maternal biotin is sufficient to support embryogenesis through the initiation of epidermal cell differentiation at about 3 days post-pollination, whereas the zygotic biotin pathway is essential for maintenance of epidermal cell fate through later stages of embryogenesis. Intriguingly, loss of epidermal cell fate is associated with perturbation of protoderm specific transcription factors, *HD-ZIP4*, as well as specific *KCS* genes implicated in key VLCFA biosynthetic steps. We propose that specific VLCFA functions as a signal to maintain the gene regulatory network controlling epidermal cell fate. Overall, we demonstrated the essential function of water-soluble vitamin, biotin, in embryo protoderm maintenance and coordination of maternal and zygotic programs of seed development.

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P161

Duplicate transcription factor genes GT1 and VRS1 repeatedly evolve roles in growth repression

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Developmental genes may diverge and evolve new roles following whole genome duplication. For example, MADS box genes have undergone functional diversification following whole genome duplication. Another set of developmental genes that may have evolved new roles following duplication is the class I HD-ZIPs. These transcription factors play various roles in growth repression across the flowering plants, suggesting the repeated recruitment of these genes to regulate repression. In the grasses, class I HD-ZIPs GRASSY TILLERS1 (GT1) and SIX-ROWED SPIKE1 (VRS1) are ancient duplicates and are important domestication genes involved in repressing both reproductive and vegetative meristems. In maize, GT1 inhibits lateral bud growth and represses the carpel in tassel florets. In barley, VRS1 represses the carpels in the lateral spikelets. Here, I ask how these growth repression regulators evolved following whole genome duplication. I hypothesize that whole genome duplication has allowed these genes to be repeatedly recruited for new roles in growth repression. Using both existing mutants and CRISPR/Cas9-edited lines, I profiled the phenotypes of gt1 and vrs1 mutants in maize and Brachypodium distachyon (Brachypodium). These mutants exhibit loss of apical dominance in both Brachypodium and maize, but also show derepression of floral structures in maize. Gene expression localization analyses further illuminate how these two gene lineages have diverged following whole genome duplication. I am also dissecting the regulation of these genes using CRISPR/Cas9 genome editing in both maize and Brachypodium. Uncovering how these genes were recruited following whole genome duplication will enable us to better direct plant development for increased crop yield.

Gene / Gene Models described: *GT1, VRS1*; Zm00001d028129, Zm00001d021934,Bradi1g71280,Bradi1g23460
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P162

Effects of heat stress during pollen development

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Major shifts in the duration and intensity of ambient temperature affects plant development and reproduction. In maize, pollen development is especially sensitive to abiotic and biotic stresses(1). Using the *Leaf Collar Method*, we tracked pollen stages allowing us to study and dissect environmental responses on discrete pollen developmental stages(2). To understand how heat stress impact individual developmental stages during pollen development, we imposed a moderate (35°C/25°C day/night) heat stress treatment on maize plants at the tetrad, unicellular, bicellular and tricellular stages. During the tetrad stage we observed a strong variation in the basic metabolic pathways, resulting in reduced starch content, decreased enzymatic activity, and thus generating germination-defective pollen, ultimately leading to sterility(3). At the unicellular stage, heat stress strongly affected pollen viability and pollen tube growth, resulting in severe sterility and reduced seed set. The bicellular stage appeared less sensitive to heat stress. We explored the responses to heat stress and identified a set of genes including transcriptional regulators with a potential role to mitigate the effect of heat stress during pollen development. To functionally characterize differentially expressed candidate genes, we are currently generating maize CRISPR-Cas9 lines to study their function during pollen development under normal conditions and heat stress. Engineering such candidate genes could potentially help in the future to improve thermal resilience in crop plants.

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P163

Elucidating the regulatory networks underlying architectural pleiotropy between tassel branching and leaf angle

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Plant architecture is central to yield and has been at the core of crop domestication and improvement. In maize and other cereals, inflorescence branching and leaf angle are important architectural traits that contribute to planting density and yield potential. Interestingly, several classic maize mutants show disruptions in both traits, suggesting a core regulatory network underlies pleiotropy between them. Here, we investigate regulatory modules that contribute to architectural pleiotropy between tassel branching and leaf angle in maize by defining gene regulatory networks that function in lateral organ boundaries to promote development of these morphologically distinct organs. Using a set of mutants with specific defects in one or both traits, we generated comprehensive, context-specific co-expression maps that describe ligule and tassel branch development. Mutants included *liguleless1* (*lg1*), *liguleless2* (*lg2*), *wavy auricle blade 1* (*wab1*), *Wab1-R* (dominant allele), *ramosa1* (*ra1*), *ramosa2* (*ra2*), *feminized upright narrow* (*fun*), *BRASSINOSTEROID-INSENSITIVE1* (*bri1-RNAi*) and *BR-INSENSITIVE2* (*bin2-RNAi*) all introgressed into B73, and were compared to B73 normal plants. Plants were grown in environmentally controlled chambers and precisely-staged tassel primordia were hand-dissected at two stages: right before and after first primary branches initiated. Two stages capturing early ligule development were also collected from mutants with leaf angle defects. RNA-seq data from all samples were integrated into co-expression networks, which were extended to include publicly available transcription factor occupancy maps for important developmental regulators, chromatin accessibility maps and natural variation to identify novel genes and regulatory elements underlying diversity in leaf angle and tassel branching phenotypes. We also phenotyped over 500 diverse maize lines for these and other relevant architecture traits for use in multi-trait GWAS models, which are leveraged to identify polymorphisms associated with architectural pleiotropy. Collectively, these data provide novel insight into regulatory mechanisms controlling important architecture traits that can be used for crop improvement.

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P164

Establishing the timing of haploid genome activation in pollen precursors using allele-specific single-cell RNA-seq

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Plants alternate between multicellular haploid and diploid generations. During pollen development, it is unclear when the transcriptome converts to mono-allelic expression following meiosis. This is of significant relevance to genetics and evolution because it defines the period that recessive mutations can be subjected to haploid selection. We followed cell differentiation throughout meiosis and pollen development with single-cell RNA-sequencing (scRNA-seq). Using a hybrid between the A188 and B73 inbred lines, we were able to identify when transcription switches from biallelic (diploid-derived) to monoallelic (haploid-derived) expression in individual microspores. The bulk of the transcriptome remained biallelic for over a week after meiosis, indicating that transcripts synthesized from the diploid genome persist long into the haploid stage. This lag period was followed by a rapid conversion to near-complete monoallelic expression. Allele-specific scRNA-seq on mature pollen grains can also be used to directly infer cross-over positions on each chromosome. This work provides an extensive time-course of gene expression throughout pollen development in maize and narrows the timing with which haploid selection may act in pollen precursors.

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P165

Exploring genetic mechanisms repressing endosperm proliferation in maize

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The maize endosperm supports embryo development, seed germination, and early seedling growth. Early endosperm development consists of syncytial nuclear divisions, followed by cellularization and mitotic proliferation. Around 8-12 days after pollination (DAP), endosperm cells are specified into at least four cell types and are repressed for mitotic proliferation. Prior reports suggest that endosperm tissue culture is only possible for a few field-inbred genotypes. Endosperm cells from the A636 inbred can be grown in tissue culture as explants from early developmental stages prior to 10 DAP. We tested endosperm proliferation in four field-inbred corn and five sweet corn lines. At 9 DAP, only A636 and the sweet corn, Ia453-sh2, show robust endosperm proliferation indicating that these two genotypes could be used for genetic analysis of endosperm proliferation. Recent studies on two RNA splicing factors, *Rgh3* and *Rbm48*, showed that efficient minor intron RNA splicing is critical for the normal repression of endosperm cell proliferation in this tissue culture assay. Both *rgh3* and *rbm48* mutants have robust endosperm proliferation for mature 14-18 DAP explants, which do not grow when sampled from normal endosperm. To explore the genetic mechanisms repressing endosperm proliferation, we screened 38 *rough endosperm* (*rgh*) mutants through endosperm tissue cultures at 13-14 DAP. Six mutant lines, *rgh*-53*, *rgh*-59*, *rgh*-117*, *rgh*-414*, *rgh*-1081* and *rgh*-1083,* had a strong endosperm proliferation phenotype. RT-PCR comparison of mutant endosperm cultures showed minor intron RNA splicing defects in both *rgh*-59* and *rgh*-414*, while the others showed no evidence of RNA splicing defects. Fine mapping located *rgh*-117* on chromosome 4 and *rgh*-414* on chromosome 10. Histological sections of *rgh*-117* and *rgh*-414* kernels at 11 and 17 DAP demonstrated severe embryo development defects. We are on the progress to identify genes that repress the endosperm cell proliferation during the endosperm development.

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P166

FCP1 peptide signaling is involved in leaf development in maize and SAM bifurcation in *arabidopsis*

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Plant development depends on the stable pool of pluripotent stem cells, which are maintained by CLAVATA (CLV)-WUSCHEL (WUS) signaling pathways. This pathway coordinates stem cell proliferation with differentiation in shoot apical meristem (SAM). Interestingly, a similar pathway appears to be conserved in the vascular meristem, where it has been shown that CLE41/TDIF - TDR/PXY regulates WOX4 in Arabidopsis. We identified FEA3, encoding a leucine-rich repeat receptor-like protein in maize. FEA3 functions in regulation of stem cell proliferation by responding to ZmFCP1. Here, we found that the ZmFCP1 is involved in vascular differentiation as well as SAM proliferation. The trans-activated Zm*FCP1* overexpression driven by *ZmWUS1* promoter or *ZmYABBY14* promoter suppressed vascular development, resulting in a dramatic reduction in total number of vascular in maize leaf. And also we found that AtCLE27, a functional AtFCP1, has a conserved function as a regulator of SAM in *Arabidopsis*, *Atcle27* mutants generated by using the CRISPR/Cas9 system show fasciation and bifurcation of SAM in *Ler*. However, this phenotype seems to require an unknown element in *Col*.

Funding acknowledgement: National Research Foundation of Korea (NRF, 2018R1D1A1B07047711 and 2018R1A4A1025158)

P167

Genetic interactions of the maize *large scutellar node1* (*lsn1*) mutant with auxin and other plant growth hormones

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A recessive maize mutant, *large scutellar node1* (*lsn1*), was isolated and described to have aberrant seedling development (Landoni et al. 1999). The *lsn1* seedling has a short primary root with a fasciated or flattened tip and severe vascular defects in leaves. If able to grow to maturity, *lsn1* has persistent root defects, retarded shoot development and reduced fertility. The root and vascular defects of *lsn1* point to a possible connection with the plant growth hormone, auxin. In order to investigate this connection, genetic interactions of *lsn1* with auxin biosynthesis, transport and signaling mutants were analyzed. Contrary to what were expected, the phenotypes of the respective double mutants showed mostly epistatic and additive interactions which are an indication that the *lsn1* functions upstream of auxin during root development and independently of auxin during reproductive development. To investigate if *lsn1* is involved in other hormonal pathways, interactions with ethylene, cytokinin, brassinosteroid and gibberellic acid are being investigated using mutants, reporter lines and chemical approaches. We further mapped the causative gene for the *lsn1* mutant using genomics and bioinformatics to a 3 Mbp region in chromosome 8, bin 4. Investigation of the functions of *lsn1* will lead to a better understanding of how hormonal crosstalk regulates organ development in maize.

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P168

Identification of novel auxin pathway proteins regulating maize root development

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In maize, root development is an important part of organogenesis and changes could have an effect on drought resistance and yield. Auxin, a phytohormone, is fundamental in root organogenesis. We hypothesized that auxin pathways may be enriched in maize root tissues and mined the maize root atlas proteome data to test this idea. From this bioinformatic analysis we identified several auxin candidate proteins with specific expression patterns across cortex, stele, elongation zone and the root apical meristem. Using a reverse genetics approach we have identified three new loss-of-function mutants in auxin pathways with altered root growth and development, including an AUXIN RESPONSE FACTOR (ZmARF27) and two putative PINFORMED-LIKE (PILS) auxin transporters (PILS2 and PILS6). These mutants display overlapping and distinct root phenotypes in both the presence and absence of auxin. ZmARF27 is a Clade A ARF, which is hypothesized to act as an transcriptional activator and regulate genes essential to root development. Using 5 day-old and 10 day-old seedlings we have quantified early root traits including primary and seminal root length, lateral roots and formation of root hairs. This mutant screen provides an inroads into linking auxin signaling and/or transport during root morphogenesis. Future characterization of these novel root mutants will fill current knowledge gaps regarding how auxin response factors and efflux carriers control root development in maize and test the hypothesis that altered root architecture impacts yield in maize.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P169

Improved maize leaf transformation using *Babyboom* (*BBM*) and *Wuschel2* (*WUS2*)

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Transformation of maize and other monocots, traditionally, has been carried out using immature embryos as the primary explant. However, the production of a consistent supply of immature embryos is not only labor- and time-consuming but also space-intensive, as a large amount of greenhouse space has to be dedicated to growing plants for obtaining the immature embryos. As a viable alternative, we have developed an *Agrobacterium*-mediated transformation protocol for leaf-base tissue from maize seedlings. When tissues from wild-type maize leaf-base segments were transformed with an *Agrobacterium* strain carrying a T-DNA constitutively expressing the fluorescent marker ZsGREEN and the selectable marker HRA, no transgenic sectors were recovered. However, when the leaf segments were transformed with a T-DNA that included a strongly-expressed maize *Babyboom* (*BBM*) gene plus maize *Wuschel2* (*WUS2*) gene, they produced robustly growing transgenic calli. The transgenic calli gave rise to vigorous, fertile T0 plants, following excision of the *BBM* and *WUS2* gene cassettes. Using this combination of morphogenic gene cassettes (*BBM* under the control of the maize Ubiquitin-1 promoter and *WUS2* under the regulation of the NOS promoter) we have successfully transformed many Pioneer maize inbreds from different heterotic groups. We produced hundreds of regenerated transgenic plants from PHH5G, PHI-Flint and PH85E (each plant an independent transgenic event) that were grown to maturity in the greenhouse. Further improvements to the method have increased the abundance and growth rate of somatic embryos that develop from transformed leaf segments. Using this method, we have successfully demonstrated leaf-base transformation in numerous cereals including pearl millet, sorghum, switchgrass, rice, and the maize public inbred W22.

P170

Investigation the axial cell wall chemistry of two sorghum stalk varieties with high and low lignin contents

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Sorghum stalk lodging can significantly reduce harvest yields and economic loss for farmers. Therefore, understanding the properties of the stalks (mechanical properties, cell wall chemistry, cell morphology, etc) and how it influences lodging would be useful in developing new breading lines that are resistant to lodging. In this study two sorghum varieties (brown midrib mutant (*bmr*) with reduced lignin content and unmodified wild type (WT)) were investigated. In relation to understanding and correlating crop lodging to cell wall chemistry the sorghum stalks were evaluated at the nodes and internodes for their axial variation of chemical composition by various techniques (FTIR, XRD, lipids, lignin and carbohydrate contents, Py-GCMS, and 2D ^{1}H-^{13}C NMR). The carbohydrate analysis showed that nodes and internodes of the two varieties in the axial direction have different carbohydrates,and lignin contents. 2D NMR showed that the lignin is rich in b-O-4 linkages, however, phenylcoumaran, dibenzodioxocin, and resinol linkages were not detected. The aromatic regions for bmr highlights that the syringyl units were not visible, but for the WT, syringyl units were able to detected in the spectrum in all nodes and internodes. This work shows that differences in chemical composition of the stalks could influence its mechanical properties and lodging behavior.

Funding acknowledgement: National Science Foundation (NSF)

P171

LIGULELESS2 and friends

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liguleless2 (*lg2*) is a classic maize mutant which affects both the leaf and the tassel (male inflorescence). In *lg2* mutants the boundary between the lower (sheath) and upper leaf (blade) regions is lost, resulting in an upright leaf, and tassel branch number is severely reduced. This pleiotropic phenotype suggests that LG2 is a key regulator of boundary specification during both leaf and floral development. Despite the *LG2* gene being cloned in 1998 by the Freeling lab, we know very little about the activity of LG2, a grass specific BZIP transcription factor. We have been using genetics, transcriptomics and proteomics to identify the LG2 network in both the leaf and tassel. Here we will present our latest findings on LG2 interactors and gene regulation.

Gene / Gene Models described: *LG2*; GRMZM2G060216, Zm00001d042777
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P172 @cropinnovation1

Maize transformation and editing resources at the Wisconsin Crop Innovation Center – highlight on LH244

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A goal of the Wisconsin Crop Innovation Center is to develop and offer transformation and gene-editing services in elite, commercially relevant germplasm and cultivars in crops to facilitate discovery and technology transfer. Towards this goal, we have licensed technology from Corteva and Japan Tobacco that is being used to develop standard processes in ex-PVP dent inbreds and elite sweet inbreds. In this poster we highlight advances in inbred LH244. LH244, an elite B73-type Stiff Stalk inbred line, has recently become publicly available due to the gracious donation by Bayer Crop Science of early release of ex-PVP and patent rights, and will be supported by release of a genome sequence by Bayer. LH244 was a primary transformation and discovery platform within Monsanto and is suitable for efficient transformation. We have had initial transformation success with reporter constructs in LH244 using a modified B104 protocol with bialaphos selection, and a callus-based protocol with paramomycin selection. Test gene-editing and customer constructs are currently being introduced into LH244 using these approaches and vectors that have been successful in previous projects. Details on maize service offerings, and updates on technology advances can be found at cropinnovation.cals.wisc.edu.

Funding acknowledgement: National Science Foundation (NSF)

P173

Mapping and characterization of the *tls4* mutant in maize reveals potential role for endocytosis in auxin-regulated tassel development

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The *tassel-less4 (tls4)* mutant in *Zea mays* (maize), generated by ethyl methane sulfonate (EMS) mutagenesis, exhibits defective reproductive development in the male inflorescence (tassels). Bulked segregant analysis (BSA) and next generation sequencing (NGS) data were used to map *tls4* to bin 4.10 on chromosome 4. Further fine mapping was performed using simple sequence repeat (SSR) markers and derived cleaved polymorphic sequence (dCAPS). The current mapping region (179 kbp) contains an EMS signature mutation within a gene known to be involved in endocytosis which is the strongest candidate gene at present. To validate this candidate gene, an endocytosis assay was conducted, revealing potential endocytosis defects in *tls4*. The phenotypic traits of *tls4* are characteristic of defects related to the plant growth hormone auxin, which regulates organogenesis. To investigate a possible connection of *tls4* with auxin, a double mutant analysis with an auxin transport mutant, *barren inflorescence2* (*bif2*) was carried out, revealing a synergistic interaction in tassels, further suggesting a possible role of the *tls4* gene in membrane trafficking. This research reveals a promising candidate gene for *tls4* and a potential role for endocytosis in regulating auxin related reproductive development of maize. Upon further confirmation, this research will expand the understanding of the auxin genetic network and will aid in generating maize crops with modified tassels which can be grown to maximize crop production.

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P174

Microautophagy of storage proteins in maize aleurone cells

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In the cereal endosperm, starchy endosperm cells accumulate storage proteins (mostly prolamins) and starch whereas the peripheral aleurone cells store oils, storage proteins, and specialized metabolites. Although both aleurone and starchy endosperm cells synthesize prolamins, they employ very different pathways for their subcellular trafficking. Starchy endosperm cells accumulate prolamins in protein bodies within the endoplasmic reticulum (ER), whereas aleurone cells deliver prolamins to vacuoles *via* an autophagic mechanism that does not depend on the canonical ATG8 (AUTOPHAGY RELATED 8)-conjugation pathway. We found that ER prolamin accretions in aleurone cells come in close contact with the vacuolar membrane and then are engulfed directly into vacuoles *via* microautophagy. Microautophagy is the least characterized form of autophagy at both cellular and molecular levels. In plants, the molecular machinery orchestrating microautophagy is poorly known and can be ATG8-dependent or ATG8-independent. We performed RNA-sequencing analysis of aleurone and starchy endosperm tissues at 18 and 22 days after pollination and performed mass spectrometry using the vacuolar membrane-enriched fractions of aleurone cells to identify factors mediating microautophagy of storage proteins. By tagging the candidate proteins with mCherry and co-expressing them with tonoplast markers in Arabidopsis leaf protoplasts, we found several of candidate proteins are associated with the vacuolar membrane and delivered into the vacuole in autophagic compartments.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P175 @carolynplants

Microtubules at the cell cortex contribute to division plane positioning in plant cells.

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Division plane positioning is critical for plant, bacterial and animal development. In plants, a cytokinetic microtubule and microfilament structure, the phragmoplast, directs the positioning of new cell wall formation to complete cell division towards a previously specified location called the division site at the cell cortex. The mechanism by which the phragmoplast is guided towards the division site is unclear. Here, we show that microtubules nucleated at the cell cortex and independent from the phragmoplast are assembled during telophase, and organized by their interaction with division site localized microtubule-binding proteins. Cell cortex localized microtubules are added into the phragmoplast to guide phragmoplast positioning. Further, cell cortex localized telophase microtubule arrays are misoriented or absent in a maize mutant with phragmoplast guidance defects called tangled1. This provides a plausible mechanism to link division site localized proteins at the cell cortex to phragmoplast guidance to the division site during plant cell division.

Gene / Gene Models described: *tan1*; GRMZM2G471321,
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P176

NARROWSHEATH controls mediolateral outgrowth of lateral organs at the margins

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The mechanisms whereby lateral organ initial cells are organized from the peripheral zone of the shoot apical meristem (SAM) are poorly understood. The maize gene *NARROWSHEATH1 (NS1)/WOX3* is expressed at the marginal boundary of leaf founder cells in the SAM and in the margins of young leaf primordia, where it mediates mediolateral outgrowth. To investigate the mechanisms of NS1 function we performed ChIP-seq of NS1, followed by laser-microdissection RNAseq of *ns* mutant and wild type leaf primordia margins, to identify NS1 bound and modulated gene targets which include the paralogs *AUXIN RESPONSE FACTOR10* and *25*. Using a comparative approach, we performed reverse genetic analyses in Arabidopsis of the homologous *ARF2* growth repressor in a *prs/wox3* mutant background; this lead to a recovery of the leaf phenotype. Combining the results from the reverse genetic analyses with microscopic analysis of cell division dynamics and the characterization of *NS1* overexpressing plants in maize, the data suggest NS1/WOX3 controls mediolateral outgrowth by direct repression of growth inhibitory genes and indirect promotion of cell division at leaf primordia margins. Intriguingly, the homologous *WOX* genes *WUS1* and *WOX5* are expressed in the organizing centers of the Arabidopsis SAM and root meristem respectively, whereupon these protein products traffic to adjoining cells to activate stem cell identity non-autonomously. In contrast, our previous data revealed that PRS1/WOX3 does not traffic, and these latest data suggest that NS1/WOX3 stimulates primordial cell division in the same margin initial cells where it is transcribed.

Gene / Gene Models described: *NS1*; GRMAM2G069028
Funding acknowledgement: National Science Foundation (NSF)

P177

Reticulon 2 regulates endoplasmic reticulum (ER) turnover and represses ER stress in maize aleurone cells

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Maize aleurone cells accumulate large amounts of storage compounds such as proteins, lipids, and over 70% of the endosperm minerals (phosphate, magnesium, potassium, iron, and calcium). Within the aleurone cells, the endoplasmic reticulum (ER) is central to storage protein and lipid synthesis as well as lipid storage. During the genetic analysis of aleurone development, we discovered three independent mutations in a reticulon gene (*Rtn2*) that drastically affect the autophagic turnover of ER and other organelles in aleurone cells. Autophagy controls the delivery of cytoplasmic components (including organelles) to the vacuole for degradation. The *rtn2* mutant aleurone cells show increased expression of ER stress markers and mis-regulated accumulation of autophagic cargo in their vacuoles. We have found that Rtn2 localizes to the ER and acts as an autophagy receptor by interacting with Atg8 (Autophagy-Related 8), a key component for the progression of autophagy. Rtn2 also binds ER chaperones and its association to both ER chaperones and Atg8 increases during ER stress. Induction of autophagic ER degradation by drug-induced ER stress results in the accumulation of Rtn2-decorated cargo encapsulated within cytoplasmic autophagosomes, and Rtn2-labelled autophagic bodies inside vacuoles. We propose that Rtn2 mediates the autophagic turnover of the ER during ER stress and plays a critical role in ER homeostasis within maize aleurone cells.

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P178 @JDScharwies

Root branching response to moisture gradients

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Root branching is pivotal for plants to gain access to water and nutrients in their environment. While roots grow, they respond to changes in moisture and nutrient availability that will inform their decisions for growth and branching. Lateral roots develop post-embryonically from founder cells at the periphery of the root stele. If moisture is equally distributed around the root, lateral roots will emerge uniformly around the circumference of the parent root. It was observed in maize, Arabidopsis, and rice that non-uniform moisture distribution can be perceived by the growing portion of the root and influence the direction of root branching, a process called hydropatterning. This phenomenon could help plants to be more energy efficient while gaining access to water and nutrients. Two parallel approaches are being taken to characterize the genetic basis of the hydropatterning response in maize: 1.) Recombinant Inbred Lines (RILs) of the Nested Association Mapping (NAM) population B73 x Oh7B (Zea mays) were used for Quantitative Trait Locus (QTL) mapping, since roots of the inbred parents show a significant difference in their hydropatterning response. A QTL associated with the hydropatterning response was discovered and fine mapping is underway. 2.) To cover more genetic diversity, a Genome-Wide Association Study (GWAS) is being performed on the 282 diversity panel from the U.S. National Plant Germplasm System. In this panel, we discovered varying degrees of hydropatterning in different genetic backgrounds. Additionally, lateral root initiation is being studied in the maize inbred line B73, which shows a very strong hydropatterning response. Since auxin promotes lateral root initiation, confocal imaging is being used to study the expression of an auxin efflux transporter fused to a yellow fluorescent protein (ZmPIN1a-YFP). Studying the developmental sequence that leads to hydropatterning will help to understand when moisture gradients are perceived by the root.

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P179

Sequencing and genetic characterization of the *Suppressor of sessile spikelets* semi-dominant mutants in maize

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The maize semi-dominant *Suppressor of sessile spikelets* (*Sos*) mutants, *Sos1, Sos2,* and *Sos3* have defects in the developmental progression of meristems that give rise to the male and female reproductive structures in maize. All three *Sos* mutants display overlapping and unique phenotypes. Common phenotypes include formation of single spikelets in the tassel and the ear resulting in a reduction in kernel row number. In addition, *Sos2* and *Sos3* mutants exhibit variable severe phenotypes including club tassels and ball-shaped ears in *Sos2*, ears and tassels with barren patches in *Sos3*, and sterile single spikelets in both. In order to determine the respective gene responsible for each of these mutants, all three mutants were fine-mapped as well as sequenced via whole-genome sequencing methods. Mapping efforts physically placed each mutation on a different chromosome, supporting that each mutant is the result of an independent mutation. The sequencing results, coupled with phenotypic ratios, also suggested that the variation of severity in the *Sos2* and *Sos3* phenotypes, may be due to genetic modifiers. To better understand the function of the *Sos* mutants in reproductive development, each *Sos* mutant was crossed by known meristem maintenance mutants in the *CLAVATA* pathway. This provided genetic evidence for unique but overlapping functions. Results of double mutant analysis indicated *Sos1* functions in the *td1* and *td1-fea2* signaling pathways and *Sos2/Sos3* function through the *fea2* and *fea2-ct2* pathways. This information is being complemented by RNA-seq analysis of *Sos1, Sos2,* and *Sos3* mutants, to determine the regulatory pathways behind these three different mutants. The results of this combined research, including double mutant and expression analyses, provide a more comprehensive picture of how these genes, and their modifiers, regulate maize reproductive development.

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P180

ShadowAuxin: Optimization of quenching FRET pairs for a fluorescent auxin biosensor

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Auxin is a hormone crucial in nearly every aspect of plant growth and development. Advancing our understanding of auxin signaling in maize and other crop plants may provide needed tools for agricultural scientists to help feed a growing population in the face of global climate change. However, existing tools for detecting and measuring auxin level and location have significant shortcomings that limit their utility in plants. We are working to build and test a novel auxin biosensor, ShadowAuxin, capable of accurate quantification and tracking of auxin in live plants. Our biosensor design relies on the dimerization of two fluorescent proteins capable of Forster Resonance Energy Transfer (FRET) when in close proximity. Our biosensor utilizes a quenching fluorescent protein in the FRET pair; when this quenching FRET pair are held in close proximity by fused dimerization domains, the fluorescent signal should dim. Pilot experiments in yeast cells have shown that this quenching FRET can be measured via fluorescence flow cytometry, and have revealed the importance of dimer-domain choice and carefully controlled protein expression level. A new pair of human-hormone inducible promoters has enabled us to precisely control the expression level of each component of the FRET pair in order to maximize FRET quenching. Next, we aim to increase FRET efficiency by testing new sets of heterodimerization domains. Ultimately, this quenching FRET system will be coupled with an auxin-sensing domain to generate a biosensor with rapid response and wide dynamic range.

Funding acknowledgement: Arnold and Mabel Beckman Foundation

P181

Society for in vitro biology 2020 world congress on in vitro biology

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The Society for In Vitro Biology (SIVB) is holding the 2020 World Congress on In Vitro Biology (sivb.org/meetings) in San Diego, California from June 6-10, 2020 at the Town and Country - San Diego. This year's international Congress, held once every 4 years, is a phenomenal chance to explore the latest research and core principles of *in vitro* plant and animal research on a global scale through daily plenary sessions on *Emerging In Vitro Technologies; Bioethics and Public Policy Benefits in Genome Editing*;*Perspectives in Cannabis and Cannabinoids* and *Frontiers in Single Cell Technologies* in addition to Keynote on *Emergence of Spontaneous Oscillatory Networks from Human Brain Organoids* by Dr. Alysson R. Muotri from UC, San Diego. The program includes topics such as: *Application of Omic's Technology*; *Beyond KOs:Emerging Genome Editing Technologies*; *Exploring Microbiomes: Application to Humans and Agriculture*; *Organoid Models:Windows into Human Disorders*; *Plant Memory:The Importance of Assessing Culture Carry Over Effects During Micropropagation Protocol Development*; and *Plan(t)s for the Future Planet*; and more. In addition to hosting the upcoming 2020 SIVB Congress which provides scientists a venue to keep current in the fields of plant and animal biotechnology, SIVB also supports several additional activities each year that influence those in academia and industry. The society provides information on new technologies to policy makers and government agencies that bases its core in science. SIVB's membership actively educates the next generation of graduate students and postdoctoral researchers, helping them develop and refine skills required for quality jobs in both industry and academia.

P182

Structure-function relationship of the bulliform cell cuticle in the leaf rolling response of maize

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The cuticle is the outer physical barrier of plants, establishing an important interaction interface with the environment. This hydrophobic layer consists of the lipid polymer cutin embedded with and covered by different waxes, providing protection against environmental stresses like desiccation, UV radiation, and pathogen attack. Thickness, structure, and chemical composition of the cuticle vary widely among different plant species, and even within a species, depend on organ identity, developmental stage, and growth conditions. Our project aims to identify the relationship between cuticle ultrastructure, composition and function of bulliform cells as an example of a specialized cuticle likely contributing to the cell's specific function. Bulliform cells are organized in longitudinal rows only on the adaxial side of most grass leaves, and are implicated to play a role in the leaf rolling response upon drought and heat stress. We were able to show increased shrinkage of bulliform cells during dehydration of the leaf in situ, and independently confirmed increased cuticular conductance of bulliform-enriched tissue by dehydration analysis of mutants with increased bulliform cell content and comparison of adaxial and abaxial cuticular conductance. Ultrastructural data showed distinct alterations in cuticular organization dependent on the cell type, which we related to differences in wax and cutin monomer composition analyzed by GC-MS, identifying changes in cutin composition as the main modification of the bulliform cell cuticle. We hypothesize that this cell type-specific cuticle is likely to be more water permeable than pavement cell cuticles, possibly facilitating the function of bulliform cells in stress-induced leaf rolling of grasses.

Funding acknowledgement: National Science Foundation (NSF), DFG (Deutsche Forschungsgemeinschaft)

P183

Study of gene co-expression networks (GCNs) in drought stress response during early endosperm development in maize

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We are studying the drought stress response on early endosperm development. Early endosperm development in maize is a crucial time affected by drought or other abiotic stresses resulting in impacts on kernel quality and yield. Early drought stress affects the sink capacity determination, which cannot be recovered by watering at later stages. Our hypothesis is that drought impacts the number of endosperm cells determined during early endosperm development and ultimately decides sink capacity during the grain-filling phase when storage products accumulate. We are interested to study the phenotypic response to drought stress during early endosperm development. We are also interested to study the altered dynamics of gene expression profiles because of drought stress, which result in the altered phenotype. In this study, plants will be subjected to drought stress conditions for varying periods during early kernel development. Kernels will be fixed for histological analysis. Endosperms will be dissected out, mRNA isolated and RNAseq performed. We aim to analyze the expression data and construct GCNs using Weighted Gene Co-expression Network Analysis (WGCNA) in order to gain insight into how drought stress at different times affects the early endosperm development, including growth, differentiation, and early storage product synthesis and accumulation. We hope to identify a few central regulators that respond to drought stress and control downstream genes to affect the growth and developmental processes or storage product accumulation and storage. We will use WGCNA to address this aim and identify interesting modules and hub genes that control drought stress response in endosperm.

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P184

The *lateral suppressor1* gene encodes a GRAS transcription factor required for axillary meristem development in maize

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Axillary meristems in maize produce the tillers, ears, tassel branches, and spikelets necessary for plant development. A new mutant, *lateral suppressor1* (*las1*), was identified in an EMS population as a novel locus controlling axillary meristem development. The *las1* mutant fails to initiate ear branches, and minimally affects tassel development. To identify the molecular mutation responsible for the *las1* phenotype a next-generation sequencing and BSA approach was utilized to map the *las1* locus to bin 9.04. A single SNP was identified within the map window that induced a premature stop codon in a GRAS transcription factor. This SNP completely co-segregates with the *las1* phenotype across multiple generations and populations in over 2,000 individuals tested, providing evidence that this mutation is the cause of the *las1* phenotype. To further dissect the genetic pathway that the *las1* mutation affects, double mutants were analyzed between *las1* and other known axillary meristem mutants. The *teosinte branched1* (*tb1*) and *las1* double mutants had no ear branches and a reduced number of tillers indicating that *las1* functions in vegetative axillary meristem initiation or maintenance. To test if *las1* functions in reproductive axillary meristem development, double mutants between *las1* and *barren stalk* (*ba*) mutants were created. The *ba1* and *ba2* mutants fail to produce ear branches and decrease tassel branch and spikelet numbers. The *las1*/*ba2* double mutants exhibited a synergistic tassel phenotype indicating the *las1* functions in a separate but parallel pathway to *ba2* in tassel development. The *las1*/*ba1* double mutants were not significantly different from the weak single *ba1* mutant which suggests that *las1* functions in the *ba1* pathway. Taken together these results indicate that *las1* regulates both vegetative and reproductive axillary meristem development and adds a novel regulator to this developmental process.

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P185

The *tasselsheath4* gene establishes developmental fields within floral phytomers via microRNA mediated mutual repression

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The phytomer is a developmental compartment composed of stem, leaf and axillary bud. Floral phytomers differ from vegetative ones in that the leaf component is suppressed to a residual bract while the axillary bud is elaborated to initiate complex reproductive organs. In the *tasselsheath4* mutant, the floral phytomers behave like vegetative ones because the bract is de-repressed and leaf-like, growing at the expense of the axillary bud that is often reduced. *tsh4* encodes an *SBP-BOX* transcription factor that is expressed in internodes and bracts, but not meristems. In order to uncover the molecular pathway in which *tsh4* functions, a TSH4 antibody was developed and used for ChIP-seq to identify downstream target genes. From 1894 bound peaks isolated from ear chromatin, 212 corresponded to genes that are differentially expressed in *tsh4* bracts. Of these genes, several are known to play a role in bract suppression, including the *GATA* transcription factor *tasselsheath1*. Surprisingly, we discovered that several microRNA genes are directly bound by TSH4, including those that negatively regulate *tsh4* such as miR156 and miR529. Moreover, the levels of these two microRNAs are greatly increased in *tsh4* as well as in double and triple mutant combinations with related *SBP-BOX* mutants, indicating that they are negatively regulated by them. Simultaneous *in situ* localization of these microRNAs together with TSH4 protein indicates that the microRNAs occupy the axillary bud position, while TSH4 occupies the stem and bract positions within floral phytomers in a complementary pattern. This situation of adjacent developmental fields that mutually repress each other is reminiscent of what occurs during boundary formation during *Drosophila* segmentation. Finally, auxin transport and signaling genes, including several AUX/IAAs, were identified as TSH4 targets, and auxin flow was found to be enhanced in *tsh4* based on visualization of the PIN1 and DR5 reporters. We present a model in which during formation of floral phytomers, expression of *tsh4* represses auxin flow to allow establishment of meristem versus leaf domains using microRNA mediated mutual repression.

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P186

The Liguleless Narrow1 RLK and Narrow Odd Dwarf, a plasma-membrane localized Ca++ signaling protein, act in overlapping pathways to regulate immunity and maize development

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narrow odd dwarf (*nod*) is a maize mutant with pleiotropic developmental phenotypes that drastically vary in different genetic backgrounds. NOD encodes an integral plasma membrane protein that participates in Ca^{2+} signaling, which we previously proposed limits activation of pathogen defense responses in healthy plants. To define how NOD impacts plant immune signaling pathways and developmental patterning, we searched for protein interactors of NOD by affinity purification. Among candidate interactors, we identified a receptor-like kinase, Liguleless narrow (LGN), that we previously characterized in a screen for leaf developmental mutants. An antimorph allele, *Lgn-R*, is similar to *nod*, with comparable pleiotropic developmental defects, variable penetrance in different genetic backgrounds, and constitutive activation of pathogen defense responses. We confirmed that LGN and NOD interact by reciprocal co-immunoprecipitation with native antibodies, and found that LGN can phosphorylate NOD *in vitro*. To determine if LGN and NOD genetically interact, we generated a double *Lgn-R/+;nod* mutant in A619 and B73. In B73, the double mutants look like *nod*, but more severe. In A619, an inbred in which neither single mutant is severe, the double mutants are much more severe than either single mutant. Transcriptome and metabolic profiling support the idea that LGN and NOD act in overlapping but distinct pathways to regulate immune signaling and shoot development in maize. We expect that our ongoing efforts to define the molecular functions of LGN and NOD and identify their genetic modifiers in different inbred backgrounds will elucidate how these mutations impact diverse developmental processes and coordinate growth, patterning, and pathogen defenses.

Gene / Gene Models described: *LGN, NOD*; GRMZM2G134382, GRMZM2G027821
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P187

The REL2 co-repressor enables recapitulation of the maize auxin response circuit in yeast

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The hormone auxin plays a crucial role in plant growth and development through the nuclear auxin response circuit (ARC), which consists of receptors, repressors, co-repressors, and transcription factors. These components each belong to large gene families, many members of which have yet to be studied in maize. To further our understanding of how the Auxin/Indole-3-Acetic Acid (Aux/IAA) repressor proteins function during maize inflorescence development, we built a synthetic maize ARC system in Saccharomyces cerevisiae (yeast). When paired with the maize RAMOSA1 ENHANCER LOCUS2 (REL2) co-repressor, maize Aux/IAA repressor proteins showed differing repression levels against both maize and Arabidopsis Auxin Response Factors (ARFs). The maize ARCs were also responsive to auxin stimulation, and were highly-sensitive compared to Arabidopsis ARCs. These results demonstrate the utility of the yeast system for functional annotation and characterization of maize auxin repressor and co-repressor genes, and suggest the utility of this system to study the auxin repression machinery in other plant systems.

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P188

The alternative splicing in response to the increased daily temperature in maize

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Due to global warming, heat stress poses an increasingly serious threat to the growth, development, and productivity of maize. Under heat stress conditions, plants alter their gene expression and metabolic patterns to cope with high temperatures. Alternative splicing (AS) of the mRNA precursors is a critical mechanism for gene regulation and generating proteomic diversity. We analyzed alternative RNA splicing to determine if the pattern of gene expression in response to increased maximum daily temperature (MDT) is also influenced by AS. In this study, an average of 30,000 AS events were identified in maize leaves at the V4 and the V5 stage. As in other species, skipped exons (SE) and retained introns (RI) are the two major types of AS. They contribute 54-61% of the total AS events in maize leaves. Global AS events increased under high MDT. Using GO analysis, we found that the ~900 differentially AS genes at higher temperature were enriched for two major biological processes, RNA processing, and development. Twenty-five differentially AS genes were involved in mRNA processing and 18 of these in RNA splicing. Twenty-eight of the isoforms of the 18 genes involved in RNA splicing were differentially spliced at high MDT (35° or 37°C) compared with 31°C MDT. Compared with the core spliceosome components, the genes involved in splicing regulation are more highly subject to differential alternative splicing (DAS), including the genes coding for SR proteins and a hnRNP gene reportedly involved in the regulation of stress-responsive AS. These may contribute to AS, because splice-site selection is determined not only by core spliceosomal components but also to a large extent by other RNA-binding proteins, such as SR proteins and hnRNPs, which bind to cis-regulatory elements located in either introns or exons, thereby activating or repressing splicing.

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P189

The maize *unstable factor for orange1* plays an important role in endosperm development

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The maize *unstable factor for orange 1* (*ufo1*) was identified as a modifier of *pericarp color1* (*p1*). The hypomethylation of *p1* distal enhancer in *P1-wr; Ufo1-1* plants have been associated with enhanced *p1* expression and aberrant accumulation of phlobaphenes in the floral and vegetative tissues. We cloned *ufo1* and it encodes an unannotated protein of 326 amino acids. No structural and functional domains could be identified or predicted with high confidence. The *ufo1* is temporally expressed during early endosperm development; 6-12 days after pollination (DAP) and is spatially expressed in conducting zone (CZ), embryo surrounding region (ESR) and basal endosperm transfer layer (BETL). Maize endosperm is a highly differentiated primary nutrient storage tissue that undergoes intense metabolic and developmental changes within a short span of the lifecycle. We found that the gain of function *Ufo1-1,* as well as the loss of function *ufo1-Dsg* alleles show reduced seed weight, abnormal carbohydrate accumulation, altered hormone homeostasis and increased expression of stress response genes. Endosperm related and seed storage protein genes are differentially expressed in these two alleles indicating the involvement of *ufo1* in kernel development. Further, differential expression analysis of specific genes confirms the role of *ufo1* in BETL. In addition, several physiological and molecular processes including DNA repair and ribosome biogenesis are affected in *Ufo1-1*. Interestingly, UFO1-GFP shows subcellular localization in nucleoplasm and nucleolus. These current results along with pleiotropic developmental defects and constitutive stress-like phenotypes of *Ufo1-1* plants support a model for the *ufo1* function in developing kernel. Further studies are allowing us to explore *ufo1*'s involvement in specialized tissue formation during endosperm development.

Gene / Gene Models described: *p1, ufo1*; Zm00001d028854, Zm00001d000009
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P190 @ErinSparksPhD

The molecular regulation of brace root development

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Brace roots arise from aboveground nodes in maize and sorghum, and function in nutrient uptake and lodging resistance. Despite the important function of these roots, there is little know about the molecular pathways that regulate the development of brace roots in maize. We show that brace root initiation occurs adjacent to the peripheral vascular plexus, which is a specific anatomical feature of grass stems. However, the cell-type of origin for brace root initiation remains a mystery. The first identifiable stage of brace root development is after a primordium has already formed. This occurs in the first node aboveground at approximately the V4 stage. At the V6 stage, the lowest aboveground node has mature primordia, the next highest node has immature primordia, and the third highest node has no notable signs of initiation. The V6 stage is where we focus our attention to obtain a complete picture of the progression through brace root development. We have acquired RNA-sequencing from these three above-ground nodes in B73 plants at V6. The results from this RNA-sequencing experiment identify distinct signals at each stage of brace root development. We focused on the role of auxin in the first stage of brace root initiation. To determine if auxin is both necessary and sufficient, we have analyzed the expression of auxin reporter genes (DR5::erRFP and PIN1-YFP) in the context of initiation, and developed approaches to exogenously apply auxin to developing nodes. Our current results will be presented to begin defining the molecular regulation of brace root development.

P191

Transcriptomic response of maize nodal root growth under precisely controlled water deficit

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Drought is the number one limitation to agricultural productivity worldwide. Understanding the mechanisms that govern root growth responses to water deficits is critical to improve crop productivity under drought conditions. In maize, the stem-borne nodal roots perform the majority of water uptake after seedling establishment. Nodal roots are produced sequentially from the stem nodes and, under drought conditions, may have to grow through dry upper soil layers to reach water at depth. Despite early findings that nodal roots have a superior ability to continue elongation at low water potentials relative to other organs, the molecular controls that determine this ability remain poorly understood. In the present study, we utilized a model system that separates the seedling (primary and seminal) from the nodal roots within distinct compartments, allowing for independent adjustment of water availability. This system mimics the situation in the field where upper soil layers dry and the continued growth of new nodal roots depends on water supplied via the stem base from already established roots. The system is highly robust, allowing for precise and repeatable nodal root growth kinetics over a range of defined soil water potentials. We used this system to profile transcriptomic changes of the growth zone of elongating nodal roots in response to water deficit. The project focuses on inbred line FR697, which exhibits a superior ability for nodal root growth maintenance under water deficit conditions. Our results indicate distinct responses of the meristematic, rapidly elongating, and decelerating regions of the growth zone that may govern the physiological adaptations that allow for maintenance of root growth during water deficit. The results will be integrated with proteomic and metabolomic analyses to develop a mechanistic understanding of the physiological genomics of maize nodal root growth under water stress.

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P192

Understanding the role of gibberellic acid accumulation in endosymbiosis

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The association of microbes at the root-soil interface is an ancient adaptation integral for nutrient acquisition. The majority of land plants associate with mutualistic fungi called arbuscular mycorrhizae (AMS) to acquire phosphate from the surrounding environment. Besides AMS, legumes have a specialized symbiosis in the form of adapted root structures termed nodules for association with bacteria (rhizobia) that can fix nitrogen assimilated by the host plant, a process termed rhizobia-legume symbiosis (RLS). The key signaling regulators in AMS and RLS are recycled in a pathway termed the common signaling symbiosis pathway. Several hormones downstream of the common symbiosis signaling pathway have been demonstrated to have crucial roles in RLS and AMS. Among these is the hormone gibberellic acid (GA), which is of particular interest in crop species as selection in cereal breeding has resulted in several GA-deficient mutants for their dwarf phenotypes. In legumes and cereal species, GA has been shown to have a role in inhibiting infection during AMS. In contrast, there are conflicting reports for both positive and negative roles of GA in RLS depending on the legume species and treatment conditions. My research aims at understanding the role and dynamics of GA accumulation during symbiosis in the legume *Medicago truncatula* and *Hordeum vulgare* (barley). To achieve this, I am using an *in planta* visualization tool called Giberellin Perception 1 ("nGPS1") to detect the accumulation of GA during RLS and during AMS. In combination, I am using a gene inducible system (XVE/β-estradiol) to perturb GA biosynthesis during symbiosis in order to determine the functional importance of GA accumulation. This work is impactful because it provides an understanding of hormone signaling in RLS and AMS, and will provide targets for engineering nodule organogenesis in non-nodulating species.

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P193

Verifying the molecular identity of a cell fate gene in maize endosperm

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The *thk aleurone1* (thk1) mutant of maize (*Zea mays*) features reduced or aborted embryos, reduction in starch biosynthesis, and multiple (4-6) aleurone layers in the kernel. Interest in this phenotype stems from the abnormal development of aleurone, a tissue responsible for secreting enzymes that metabolize endosperm storage compounds during seed germination. Aleurone also contains high concentrations of protein and oil bodies. The original *thk1-R* mutant is caused by a 2 megabase deletion on chromosome 1, which precluded gene identification. Two EMS alleles were generated and differential expression RNA-seq reads that mapped within the 2MB deletion interval identified a candidate gene. Knockout alleles produced with CRISPR-Cas9 editing produced homozygous mutant phenotypes comparable to original *thk1-R* lines and failed to complement existing *thk1* mutant alleles, confirming the identity of the causal gene. The *thk1* gene encodes a NOT1 homolog, which is a scaffolding protein belonging to the CCR4-NOT regulatory complex. CCR40NOT regulates many cellular processes, notably, mRNA degradation via deadenylation of the poly-A tail, microRNA processing, and protein ubiquitination. Subsequent experiments will seek to elucidate the role of THK1 in controlling maize endosperm development.

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P194 @externelly

mutRank: An R Shiny web-application for mutual rank-based coexpression analysis combined with tools for gene candidate prioritization

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Gene coexpression is widely used as evidence to predict gene function and functional associations. Numerous useful databases and tools have been developed for gene coexpression analyses, but incorporation of user-provided expression and trait data is either not an option or requires coding experience. We developed mutRank, an R Shiny web-application, which offers a user-friendly and customizable interface for simplified coexpression analysis combined with tools for gene candidate prioritization. We use Mutual Rank (MR) as a measure of coexpression because it outperforms Pearson Correlation Coefficient (PCC) for gene function predictions and certain network-based pathway predictions. In addition to generating an MR coexpression table for a gene of interest, mutRank can visualize the results as a heatmap or a network graph. mutRank offers tools to annotate the list of coexpressed genes, detect coexpression clusters, predict domains and calculate GO term enrichment. Additionally, user-provided quantitative trait data can be mapped to the list of coexpressed genes to provide an additional layer of information. mutRank was successfully used to predict the function of multiple maize specialized metabolism genes and is designed to facilitate exploratory analysis of expression data, gene function prediction, and prioritization of gene candidates.

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P195 @arjunmk1

A multi-omics discriminatory analysis approach to identify drought related signatures in maize nodal roots

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Maize is one of the major food crops grown in the continental US, and as such, the major interest is directed towards understanding its adaptability to drought stress. Certain cultivars of maize have shown increased resistance to water shortages, by continuing to maintain its root growth even when under severe water stress. To better understand the molecular mechanism which leads to such adaption, we interrogate the datasets generated from the tips of nodal roots collected from B73 reference genotype plants & FR697, an inbred line that shows better adaption to drought. We use a research pipeline consisting of a discriminatory multi-omics data integration approach which combines sparse Generalized Canonical Correlation Analysis (sGCCA) and generalized Partial Least Square (PLS) analysis instead of traditional "filter funnel" approach; to incorporate all datasets into one holistic global network and form clusters spanning all omics levels. We then link significant elements from these clusters to various observations associated with drought stress in the root tip samples; reinforced by their roles in biological pathways using the dynamic algorithm IMPRES-Pro to trace their connections. The results are incorporated into a KBCommons based maize database for storage and analysis from various viewpoints. To visualize interactions between the many elements, we are also developing a new multi-layered web-based 3d visualization tool called "KBCommons Omic Studio", integrated with the KBCommons framework. This allows users to visualize, select and interrogate all the information generated in a connected graph network setting and thus allowing for elements previously unknown to be detected and become candidates for further hypothesis testing. We showcase certain possible biomarkers related to drought stress and allied observations using this method. Supported by NSF Plant Genome Program grant no. 1444448.

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P196

A webserver for *helitron* prediction and visualization

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One remarkable feature of *Helitrons* is their ability to capture gene sequences, which makes them of considerable potential evolutionary importance. It is not clear at this point how *Helitrons* acquire host sequences, but it is important to learn if *Helitrons* have the ability to trap fully functional genes and mobilize them around the genome. Helitrons are hard to detect computationally given their lack of classical transposon structural features, such as terminal inverted repeats and target site duplications. http://bo.csam.montclair.edu/helitronscanner/). Users can copy and paste or upload FASTA format DNA sequences. Four functions have been created in the back-end to ensure users are at ease with the crafted GUI. Each function can be found in the top right corner of the website. The Quick-Run tool was created for users looking to upload a file and get a rapid response from the server. The user also has the option to name the file, including the extension type, if need be. This online tool will be invaluable for biologists to data mining *Helitrons* in the genome of their interests.

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P197

Accessing the MaizeCODE data from Gramene and SciApps

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MaizeCODE is a project aimed at identifying and analyzing functional elements in the maize genome. In its initial phase, MaizeCODE assayed up to five tissues from four maize strains (B73, NC350, W22, TIL11) by RNA-Seq, Chip-Seq, RAMPAGE, and small RNA sequencing. To facilitate reproducible science and provide both human and machine access to the MaizeCODE data, we developed SciApps, a cloud-based portal, for analysis and distribution of both raw data and analysis results. Based on the SciApps workflow platform, we generated new components to support the complete cycle of MaizeCODE data management. These include publicly accessible scientific workflows for the reproducible and shareable analysis of various functional data, a RESTful API for batch processing and distribution of data and metadata, a searchable data page that lists each MaizeCODE experiment as a reproducible workflow, and integrated JBrowse genome browser tracks linked with workflows and metadata. MaizeCODE data are also integrated into the Gramene platform so that users can load the data into Gramene's genome browser, examine the associated metadata, and relaunch the reproducible workflows.
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P198

Assessing the biological ramification of alternative splicing of U12-type introns on eukaryotic gene expression

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The alternative splicing of non-coding introns from precursor mRNA augments the transcript diversity of eukaryotic gene expression. Many eukaryotes have two classes of introns known as major or U2-type as well as minor or U12-type introns. U12-type introns represent less than 1% of total introns and are spliced by a distinct minor spliceosome. Aberrant splicing of U12-type introns causes developmental and cell proliferation defects in both plants and animals. On average, the kinetics of U12-type intron splicing is slower relative to that of U2-type introns. This led to the postulation of their rate-limiting regulation of mRNA processing of minor intron containing genes (MIGs). However, U12-type intron retained versions of MIG transcript isoforms can be associated with ribosomes and potentially translated. We searched the B73_version4 reference genome for MIGs that display alternative splicing events associated with their U12-type introns in genetically normal tissues. Our data indicates that 55% of total maize MIGs display alternative splicing that results in either complete or partial retention of U12-type introns in the resultant transcript isoforms. Of these, the conceptual translation products of the retained U12 transcript isoform of 27% of MIGs encode a significant open reading frame (ORF) with a potential biological function. In 70% of cases, the alternatively spliced ORFs do not impact functional domains encoded by the wild-type transcript, whereas 22% resulted in the loss of a conserved domain. Intriguingly, 8% of U12 retained ORFs result in the addition of a new conserved domain. For selected cases, we provide compelling evidence of the potential biological ramifications of alternative splicing of U12-type introns.

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P199 @PlantsOverPants

Branchpoints in sex determination: Expression dynamics for making maize ears, tassels, and bisexual relatives

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The biological rules that govern sex characteristics are critical in cereal crops, where the agricultural product comes from a fertilized flower. Although most flowering plants produce only perfect flowers (those with both male and female structures), roughly 30% of species produce imperfect flowers, which have either male (staminate) or female (carpellate) flowers. Although other agriculturally domesticated grasses (ex. rice, wheat, millet, sorghum) produce bisexual flowers, maize forms separate, staminate and carpellate inflorescences on the same plant. Genetic mutants and chemical treatment experiments in maize suggest that this process is controlled by JA and BR-mediated pistil abortion in the male inflorescence (tassel) and by GA-mediated repression of stamen outgrowth in the female inflorescence (ear). The exact interaction and hierarchy of these sex determination signals during development remain elusive. To understand the gene expression dynamics which underlie maize sex determination events, we collected low cost, 3'UTR enriched RNAseq profiles from a time-course of 37 individual immature B73 ears spanning 21 days of development, starting with inflorescence transition until silk outgrowth. From the 37 collected time points, we imputed complete expression trajectories for 20k genes that comprise the ear transcriptome. We compared this ear molecular ontogeny with a meta analysis of RNAseq for the developing tassel plus data from sorghum and *Setaria* panicles which produce perfect, bisexual flowers. Through a combination of gene ontology and network analysis we identified hormone-related gene expression modules and tracked their activity during the production of female flowers in the ear, male flowers in the tassel, or bisexual flowers in the sorghum and *Setaria* panicle. We scanned these hormone-related transcriptional modules and our entire molecular time-course for signals of transcriptomic selective pressure and compared the relative importance of sex determination pathways during maize inflorescence morphogenesis.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P200 @turfyinjie

Building a reference transcriptome for the hexaploid hard fescue turfgrass (*Festuca Brevipila*) using a combination of iso-seq and illumina sequencing

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Hard fescue (*Festuca brevipila* Tracey, 2n=6x=42) is a cool season turfgrass that has fine leaf texture and does well in shade and low fertility soils. For these reasons, it has been used for low-input areas. Breeding and genetics studies of *F. brevipila* have been limited due to the nature of the complexity hexaploid genome. To advance our knowledge of *F. brevipila* genomics, we used PacBio isoform sequencing to develop the reference transcriptome of this species. Here, we report the *F. brevipila* reference transcriptome generated from root, crown, leaf, and seed head tissues using 4 Pacbio SMRT cells. We obtained 59,510 full-length transcripts and further refined them into 38,595 non-redundant full-length transcripts which has N50 of 2,585 bp. The longest and shortest transcripts were 11,487 and 58 bp, respectively. We used the long reads assembly for the *de novo* short reads assembly improvement and researched the phylogenetic relationship between selected species that were closely related to *F. brevipila*. Overall, the *F. brevipila* Pacbio reference transcriptome developed in this study improved the short reads assembly quality and provided evolutionary insight of this hexaploid species.

Funding acknowledgement: United States Department of Agriculture (USDA)

P201

Changes in growth, flavonoids and polyphenol contents according to sowing time of corn in Korea

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The agriculture sector is affected by climate change, namely by a crisis factor and an opportunity factor. The crisis factor is more frequent and can lead to more intense disasters. On the other hand, there are also opportunity factors such as extension of the crop cultivation period etc. Total 15 different corn hybrids, Kwangpyeongok, Gangdaok, Yanganok, Singwangok, Jangdaok, Cheonganok, Cheongdaok, Andaok, Dapyeongok, Pyeongkangok, Pyeonganok, Daanok, Sunwon P3394, Gangilok, P3394, were used to investigate the plant growth and yield depending on the sowing date. Sowing dates were April 5th and July 5th, respectively, and all experiments were repeated in three replicates. The growth of Gangdaok was the best. However, the growth of Kwangpyeongok was not the best to compare with Gangdaok's, but the stem to ear height ratio was lower than Gangdaok's, therefore, it may be better to select Kwangpyeongok for the stable cultivation in the late sowing time. Both of the growth and yield of Daanok were not good regardless of planting date, but the yield and ear shape of Pyeongkangok and Dapyeongok were good for gain the fresh corn. The growth and yield of 15 different corn hybrids were variable depending on the planting date, however, the growth degree days (GDD) were the most important factor for the corn maturity. GDD above 1500 ℃ was enough to harvest mature corn hybrids in the central region of Korea. Total polyphenol and flavonoid contents in roasted corn increased significantly with increasing roasting temperature and time. The total polyphenol content of roasted corn was 4.02±0.04 mg GAE / g sample at 200, 220, 250, 270 and 300°C, and the total polyphenol content of roasted corn was 2.8 ~ 3.1, 2.7 ~ 4.0, 3.7 ~ 5.4, 4.3 ~ 6.8, and 4.2 ~ 10.7 mg GAE / g sample. Besides the yield and growth, other characters such as sweetness and taste should be investigated for the further study of the characteristic of corn quality.

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P202

Characterizing knob structure and position in the maize pan genome

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Knobs in the maize genome have historically been defined cytologically, with 34 defined positions in maize and teosinte, and characterized by the major repetitive sequence—either TR-1 or knob180. With the assembly of the pangenome, however, which has 26 high-resolution genomic assemblies, we can better characterize structure and genomic position of knobs at the sequence level. In this study, we identify knob repeat arrays of TR-1 and knob180 and show that there are more repeat arrays present than are cytologically visible and most arrays are a mix of both TR-1 and knob180. Further, we use alignment data to compare genomic positions of repeat arrays. Together these results provide insight into the evolution and integrity of knobs.

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P203 @shanwai1234

Cold-responsive gene prediction across Panicoid grasses

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Cold stress triggers transcriptional responses in plant species. Some of these changes are adaptive and others are likely neutral molecular phenotypes mediated by transposons. While many of the genes which respond to cold in maize, sorghum, and other grasses are syntenically conserved, cold responsive expression is often not conserved between syntenic orthologs in related species. Two pairs of grass species were selected that were closely related, yet varied in their susceptibility to cold stress: foxtail millet and pearl millet, and switchgrass and proso millet. In each species, genes which responded transcriptionally to cold were identified using time course data from paired control and cold stress treated samples. A supervised classification algorithm was trained on a set of sequence, chromatin, and evolution/diversity features and showed a promising ability to predict which genes would transcriptionally respond to cold. A sequence only model that trained using only data which can be calculated directly from genomic sequence and gene model annotations performed almost as well as the complete model. The sequence only model trained in one grass species were also able to accurately predict which genes would respond to cold in another species. This effect was consistent after controlling for the confounding effects of baseline expression level and using gene family guided splitting to avoid learning the features of particular gene families that tended to respond to cold stress. The accuracy of prediction was consistent for models trained and tested in species which were both cold sensitive, both cold tolerant, or when training and testing species belonged to different phenotypic classes. In conclusion, classifiers trained on stress response expression data from well studied species may suffice to predict expression patterns in related, less studied species with sequenced genomes but limited published gene expression data.

P204 @ArunSeetharam

Consolidated approach for characterizing genomic structural variants across the maize NAM founders

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Accurate detection of large-scale structural variants (SVs) is challenging for repeat-rich genomes like maize. Yet, the characterization of these SVs remains crucial for gaining biological insights from sequenced genomes. SVs are defined as regions in the DNA sequence that have undergone changes such as deletions, insertions, translocations, and inversions when compared to the reference genome. The recently sequenced genomes of the 26 founder lines of the maize Nested Association Mapping (NAM) population encapsulate much of the diversity found in modern maize and represent an excellent resource for characterizing structural variation and its phenotypic consequences. In this study, we identify a high-confidence set of SVs for all NAM parents by comparing each line to the B73 V5 reference using a suite of SV detection algorithms. Specifically, large SVs (over 1 Kb) were detected through in silico simulation of direct labeling restriction enzyme digestion followed by pairwise comparison with BioNano Solve software; medium-sized SVs (1kb-100Kb) were identified using the pbSV program after mapping circular consensus sequences (reads) to the reference; small SVs (50bp-1Kb) were detected by mapping subreads (raw reads) to the reference and then processing these alignments with the SVIM and SNIFFLES SV detection algorithms. A filtered set of SVs were generated by consolidating SVs from all programs followed by manual curation. Preliminary results show that insertions and deletions are the most common type of SVs and are ubiquitous across the genome. We describe these and other results in our summary of SVs across the NAM founders.

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P205 @BalreetPawar

Context-specific network analysis in maize using tissue-specific transcriptomics data

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Maize (Zea mays, L) is the most greatly produced grain globally, and has significant usage for food and feed and serves as a model organism in biological research. Understanding the mRNA gene expression and abundance in maize can help clarify the mechanisms that affect nutritional value and development of various tissues. By analyzing publicly available RNA-seq transcriptomics data, we were able to identify tissue specific transcriptome behavior in maize tissues. We collected 68 samples from multiple tissues such as the root, vegetative meristem, embryo, leaf, kernel, ear primordium, pollen, and silk in maize. Then, we were able to analyze and annotate context-specific networks at the transcriptome level by using both top-down and bottom-up analysis strategies. Following the ad hoc practice in which genes with context specificity are defined as those having mRNA levels in a particular tissue at least five times its average levels, we have developed an in-house script in Python to conduct bottom-up analysis. We also used the BicMix method (Gao et al., 2016) to conduct a top-down in order to compare the results from both methods. We found that both methods effectively identified context-specific transcriptome behavior and that top-down analysis, though more computationally expensive, more effectively reduces redundancy than bottom-up analysis.

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P206

Dynamic patterns of protein-coding and noncoding elements across maize development in a maize F1 hybrid and its inbred parents

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Heterosis is widely utilized in agriculture, but its genetic and molecular basis is still largely unclear. With the development of omic technologies, we now have the unprecedented chance to collect large omic datasets to decipher heterosis. In order to evaluate the contribution of different expressed elements to heterosis throughout maize development, we have collected a comprehensive transcriptomic dataset on 26 different tissues or developmental stages of two maize inbreds, B73 and Mo17, as well as their F1 hybrid. We conducted comparative analyses on parental and progeny expression variations with different expression elements throughout maize development, which exhibited dramatic expression variation in B73, Mo17 and F1s. We found that lncRNA is the most related to heterosis, and the analysis on the relationship of all different functional elements across 26 tissues/stages for heterosis is ongoing.

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P207

Establishment of *Ustilago maydis* infection in maize anthers

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Ustilago maydis is a biotrophic fungus that causes tumors in all aerial maize organs. In anthers, visible tumors appear approximately seven days post infection; however, there is limited information on how the infection progresses in the period between host plasma membrane invagination and visible tumor formation. Transcriptomics analysis of whole anthers has identified some genes that are differentially expressed in infected anthers; however, it is unable to differentiate between more subtle differences in infected and uninfected cells or how the infection impacts different cell types in the anther. By using single cell RNA-seq, we are able to address some of these questions, as well as characterize the trajectory of genes expression changes over time using pseudo time velocity.

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P208

Gapless assembly of maize chromosomes using long read technologies

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Creating gapless telomere-to-telomere genome assemblies is important for understanding structural variations that contribute to genome diversity and phenotypic variation. However, the achievement of end-to-end maize chromosome assembly has been impeded by its enrichment of repeat arrays, which are the most challenging genomic regions to assemble. Here we developed an assembly pipeline that integrates independent PacBio and Oxford Nanopore assemblies with Bionano optical as a guide, which incorporates the advantages of current technologies and performs well in the repeat-rich regions. Using this pipeline, we produced a maize genome (B73-Ab10) assembly composed of 63 contigs with a contig N50 of 162 Mb. The final assembly includes gapless assemblies of chromosome 3 (236 Mb) and chromosome 9 (162 Mb), seven highly repetitive centromeres and multiple heterochromatic knobs. This genome assembly captured nearly all the gene and regulatory information, and provided first view of Ab10 haplotype. In addition, the complete assembly over repetitive domains revealed the internal structures of knobs and centromeric repeats, which were previously known only by cytological techniques. We found that *Cinful-Zeon* elements preferentially target repetitive domains and are abundant in other heterochromatic regions across the genome. The above results demonstrated that with long read technologies, gapless assembly of all maize chromosomes is within reach, though challenges remain in massive repetitive regions.

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P209

Genetic and bioinformatic analysis for gene identification of *emb* mutations

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The developing maize embryo passes through the proembryo, transition, coleoptilar and stage 1 (first leaf primordium) followed by the iterative formation of additional leaf primordia during stages 2 through 6 (Abbe and Stein, 1954). Using ethyl methanesulfonate (EMS), which causes single nucleotide polymorphisms (SNP), we produced lethal embryo specific (*emb*) mutants which have normal appearing endosperms. The pollen from the W22 inbred was treated with EMS and applied to silks of a W22 or B73 inbred plant. The mature kernels were planted, self-pollinated (F1), and scored for an *emb* phenotype; each *emb* mutations was further backcrossed at least 6 times to B73. The embryo phenotypes of 34 mutations were examined by dissection of the mature embryos and reported (Brunelle, Clark and Sheridan, 2017). In order to identify these genes we are using genetic tests and bioinformatic analysis. B-A translocations are used to identify the chromosome arm location of the gene of *emb* mutations. Those *emb* mutations located on the same chromosome arm will be subject to complementation tests to find allelic mutations. For each allele in a non-complementing set, individuals confirmed as heterozygous (+/*emb* mutant) are pooled into a DNA-Seq library and individuals confirmed as homozygous wildtype (+/+) are pooled into a second DNA-Seq library: both libraries are sequenced using Illumina. The sequence data and SNPs specific to the B73 genome compared to the sequence data and SNPs specific to W22 genome will be used to identify a region of the genome that is consistently W22 sequence and SNPs. The gene that shows up in all alleles of a set is most likely the gene of interest. Additionally, Nanopore sequencing will be used to identify DNA methylation, mutations and their phasing. The mutated genes that result in an *emb* phenotype are essential in developing a Systems Biology for maize embryogenesis.

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P210

Genomic, metabolic, and transcriptomic responses of the extremophile grass *Paspalum vaginatum* to nutrient deficit stress

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Many wild grass species have been shaped by natural selection to thrive under environmental conditions or resource constraints far outside the range experienced by crop species. And improved understanding of the molecular and evolutionary strategies natural selection employed to achieve these changes in stress tolerance and nutrient use efficiency in crop wild relatives has the potential to aid efforts to engineer resilient and low input crops and advance food security. Here we focus on *Paspalum vaginatum* a crop wild relative which is multiple abiotic stress tolerant and exhibits greater tolerance to both nitrogen and phosphorous deficiency. Paspalum exhibits no significant decrease in biomass accumulation under nitrogen or phosphorous deficient conditions that significantly impact the biomass accumulation of Maize and Sorghum. All three species exhibit increased root elongation and branching in response to nitrogen deficit. Metabolomic and transcriptomic analyses identified many commonalities in the molecular responses to stress in all three species. However, uniquely, Paspalum exhibits significantly increases accumulation of trehalose under nutrient deficit conditions, and genes involved in metabolic pathways leading to trehalose production are experiencing more rapid protein sequence evolution in the lineage leading to Paspalum than in other grass species. Efforts are underway to experimentally test the link between Paspalum's unique strategy of accumulating trehalose in response to stress and Paspalum's resilience to nutrient deficit.

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P211 @SchnableLab

High-throughput hyperspectral imaging as a tool to explore natural diversity in biochemical traits over time in maize association panel

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Linking natural phenotypic variation to genetic loci responsible for regulating that variation is a critical tool used to gain new insight into functions of both previously uncharacterized and well-studied genes. However, manual phenotyping is slow, labor-intensive, and normally destructive, especially for biochemical traits. Here we employed hyperspectral imaging to identify pixels originating from different plant organs and trained a set of machine learning algorithms to predict 15 leaf properties ranging from chlorophyll and water content to N, P and K abundance. These models were applied to a dataset collected from a maize association panel grown and imaged using a hyperspectral camera over a 40-day time period starting in late vegetative development and including early reproductive development for some genotypes. To validate the models, we conducted manual (destructive) measurements of plant composition for the same set of genotypes. Cross-validation accuracy on the last day of imaging prior to destructive sampling was used to estimate the accuracy with which hyperspectral imaging recapitulated conventional destructive measures of these leaf properties. We estimated narrow sense heritability to determine which traits were both accurately measured AND subject to significant variable genetic control.

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P212 @Yan_Geneticist

Identification of genetic determinants of phenotyping errors in image-based high-throughput phenotyping

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The accuracy of trait measurements greatly affects the quality of genetic analyses. In automated phenotyping pipelines, phenotyping errors are often treated as random effects that can be controlled by increasing population sizes and/or numbers of replications. In contrast, some work has indicated that error in phenotype measurements from high throughput technologies may be partially under genetic control (Liang *et al.* 2018). Consistent with this hypothesis, we observed substantial non-random, genetic contributions to phenotyping errors for five tassel traits collected using an image-based phenotyping platform. Phenotyping accuracy relative to manually collected ground truth data varied according to whether a tassel exhibits "open" or "closed" branching architecture. A GWAS conducted for this open vs. closed tassel trait detected three distinct trait-associated SNPs (TASs); two of these are adjacent to known inflorescence genes. Surprisingly, TASs identified via GWASs conducted on five tassel traits that had been phenotyped both manually (i.e., ground truth) and via an image-based method exhibit little overlap. Further, it was possible to identify TASs obtained via GWAS on the *differences* between ground truth and automated measurements (i.e., phenotyping errors), indicating that this limited overlap is under genetic control. Similar results were obtained in an analysis of data collected from another image-based phenotyping pipeline. Therefore, this study suggests that phenotyping errors cannot always be controlled simply by increasing population size and/or replication number. Further, our ability to identify candidate genes associated with tassel architecture via GWAS on phenotyping errors that were not detected via GWAS of either automated or manually collected tassel trait data, highlights a complementary method of gene discovery.

Funding acknowledgement: National Science Foundation (NSF), Plant Science Institute

P213 @katerinaholan

Image-based quantification of *Puccinia sorghi* pustules in maize

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Foliar diseases of maize are a constant threat, and changing climates are leading to expanding distribution of the pathogens that lead to these diseases. Disease phenotyping is an extremely important part of their study, as effects of pathogen growth need to be accurately assessed due to their variable nature. However, many foliar diseases can be difficult to phenotype, including the rusts caused by fungi of the order *Pucciniales*. These pathogens form small, numerous pustules on their hosts that are typically assessed visually with the aid of a scoring system. One such pathogen, *Puccinia sorghi*, is the causal agent of common rust of maize, and it can form hundreds of pustules per leaf. As such, counting each pustule is extremely time-consuming and traditional scoring systems are unreliable and subjective. To have a faster, more reliable, reproducible, and quantitative method, we have developed an image-based phenotyping platform for *P. sorghi* that isolates pustules based on color. Using only a flatbed scanner and a laptop, individual leaves are scanned, and characteristics such as total leaf coverage and pustule number, size, and distribution are extracted from the images of inoculated leaves. The images are analyzed in an entirely autonomous manner, and the same parameters are applied to each leaf, resulting in unbiased phenotyping between images. Computer vision allows us to identify small but significant changes between experimental conditions, leading to more accurate common rust phenotyping needed for functional genomics studies of maize-*P. sorghi* interactions.

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P214

Investigation of NKD1, NKD2 and OPAQUE2 interaction on gene network associated with maize endosperm development

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NKD1, NKD2 and OPAQUE2 (O2) are three important transcription factors (TFs) that control gene networks of maize endosperm development. To explore their interactions, a set of *nkd1, nkd2* and *o2* homozygous lines, including all single, double and triple mutant combinations and wildtype control, was generated. RNA sequencing-based differential gene expression analysis indicated a dynamic influence of the three TFs on downstream genes at 8, 12 and 16 DAP. Weighted gene co-expression network analysis (WGCNA) and multifactorial analysis were performed to investigate the effects of NKD1, NKD2 and O2 interactions on downstream genes and sub-networks (modules). GO term enrichment analysis was used to annotate main functions or processes for each module. The results suggest that at 8 DAP, NKD1 and NKD2 interactively regulate auxin-activated signaling pathway and transmembrane transport, and NKD2 and O2 regulate transcription factor activity; at 12 DAP, NKD1, NKD2 and O2 together are involved in response to environment stresses; and at 16 DAP, NKD1 and O2 regulate nutrient reservoir activity, and NKD1, NKD2 and O2 together affect DNA replication and packaging, which may be associated with endoreduplication. Several hub TF genes were identified that are putative direct targets, protein-protein binding partners or significantly correlate with at least one of the three primary TFs suggesting they may be central regulators of their modules. For example, ARFTF30 and bHLH51 are both putative NKD1 targets, and are putative hub TFs of modules associated with auxin-activated signaling pathway and transmembrane transport activity, respectively. In all, NKD1, NKD2 and O2 interactively and dynamically regulate multiple modules of endosperm development through putative module hub TFs, which may further regulate other genes in the modules, and form a hierarchical network architecture.

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P215

Long-read sequencing reveals genomic structural variations that underlie creation of Quality Protein Maize

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Mutation of *o2* doubles maize endosperm lysine content, but it causes an inferior kernel phenotype. Developing quality protein maize (QPM) by introgressing *o2 modifier*s (*Mo2*s) into the *o2* mutant benefits millions of people in developing countries where maize is a primary protein source. Here, we report genome sequence and annotation of a South African QPM line K0326Y, which is assembled from single-molecule, real-time shotgun sequencing reads collinear with an optical map. We achieve a N50 contig length of 7.7 million bases (Mb) directly from long-read assembly, compared to those of 1.04 Mb for B73 and 1.48 Mb for Mo17. To characterize *Mo2*s, we map QTLs to chromosomes 1, 6, 7, and 9 using an F_2 population derived from crossing K0326Y and W64A*o2*. RNA-seq analysis of QPM and *o2* endosperms reveals a group of differentially expressed genes that coincide with *Mo2* QTLs, suggesting a potential role in vitreous endosperm formation.

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P216

MaizeGDB's MaizeMine facilitates genomic data mining

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The availability of high-throughput genomic technologies has accelerated the generation of massive quantities of genomic datasets. Maize researchers often wish to perform comparative analysis between their datasets and published or publicly available data. MaizeMine (http://maizemine.maizegdb.org), MaizeGDB's data mining warehouse, enables researchers without scripting skills to integrate their data with publicly available data and perform meta-analysis. The MaizeMine List tool allows users to upload identifiers to create custom lists, perform sets of operations such as unions and intersections, and execute template queries with lists. Users can easily compare their results with published results by uploading genomic coordinates or identifiers. MaizeMine uses the InterMine data warehousing system to integrate genomic sequences from the B73-RefGen_v3 and RefGen_v4 genome assemblies, three sets of gene annotations (AGPv3, AGPv4, RefSeq), Gene Ontology (GO), protein annotations (UniProt), protein families and domains (InterPro), homologs (Ensembl Compara), and pathways (CornCyc, KEGG, Plant Reactome). Additional MaizeMine datasets include three insertional mutagenesis collections, all dbSNP variants, shoot and root transcriptional start sites, maize-GAMER annotations, and more. MaizeMine also contains pre-computed variant effects and expression levels based on RNA-seq data from the Zea mays Gene Expression Atlas (NCBI BioProject PRJNA171684). MaizeMine provides sophisticated and straightforward search tools, including a keyword search, built-in template queries with intuitive search menus, and a QueryBuilder tool for creating custom queries. The release of the B73-RefGen_v5 assembly and associated annotations provides an opportunity to add an additional assembly to MaizeMine. Over the next year, multiple planned updates for MaizeMine will incorporate not only previously included datasets now updated to B73-RefGen_v5, but additional new datasets describing quantitative traits (GWAS and QTLs), cis regulatory elements, and the epigenetic landscape.

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P217 @MaizeGDB

MaizeGDB: A history of our maize genetics research community

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Members of the Maize Genetics Research Community have historically been known as "Maize Cooperators", people who share an interest in maize genetics, genomics, and breeding research, and who embrace collaboration and cooperation through sharing materials, data, and ideas. This community has a strong history of cooperation dating back to the 1920s when researchers would hold "cornfabs" to share ideas. In 1929 Rollins A. Emerson, his student George Beadle, and Allan C. Fraser, sent a letter sharing maize linkage data. This example of sharing led to a worldwide community of thousands of maize cooperators and free public resources to help organize the collaborative efforts and promote maize research. These resources include: Maize Genetics Newsletter, Maize Genetics Cooperation Stock Center, Maize Genetics and Genomics Database (MaizeGDB), and the Maize Genetics Meeting. As the maize community continues to grow and evolve, we reflect on the history of cooperation in this great community.

Funding acknowledgement: United States Department of Agriculture (USDA)

P218 @MaizeGDB

MaizeGDB: Hosting a plethora of maize genomes at MaizeGDB including B73 RefGen_v5 and the NAM founder genomes

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MaizeGDB currently hosts 44 genome assemblies including the recent release of B73 RefGen_v5 and the 25 NAM founder genomes. While these genome assemblies have great potential to advance maize research, hosting so many genomes in a manner that is beneficial for maize researchers and breeders is not straight-forward. Many assemblies also present the option for constructing pan-genomes and identifying pan-gene sets, as well as presenting challenges for handling gene model annotations from many different assemblies. Here we describe our approaches to handling multiple genome assemblies, including the release of the NAM founder genomes. In coordination with the NAM sequencing consortium, this release has 26 new genome pages, over 1 million new gene model pages, 206 new files in the FTP, and 134 additional BLAST targets. In addition, MaizeGDB created JBrowse genome browsers for each genome with over 1,000 total tracks of data across the 26 browsers. MaizeGDB continues to updates tools and resources to support multi-genomic and pan-genomic data sets.

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P219

Mapping and functional characterization of cis-regulatory variation in plants

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Transcription factors (TFs) bind to short DNA sequence motifs in regulatory regions of target genes and control the gene expression changes responsible for plant developmental programs and environmental responses. Yet, in plant genomes, the vast majority of TF-DNA binding events and the gene expression changes they elicit remain largely uncharacterized. We aim to understand how TF-DNA binding and its variability in different genetic backgrounds affects phenotypic outcomes in maize, the highest producing grain crop in the world, and in Arabidopsis, the most-studied model plant. We will generate detailed regulatory information maps and new methodologies to identify relationships between TF binding and variability in gene expression, providing new tools for the rational design of crops with improved traits. Our specific objectives and approaches are: i) to develop a modified DAP-seq assay (pooledDAP-seq) and computational methods to quantitatively test TF binding among different maize inbred backgrounds and Arabidopsis accessions, and to correlate TF binding with expression differences; ii) to develop an approach called doubleDAP-seq to analyze binding of obligate and facultative heterodimers as well as TFs complexed with repressor proteins; and iii) to use genome editing to functionally characterize newly defined regulatory regions. Guided by high-resolution maps of proximal and distal cis-regulatory modules, we will disrupt specific elements within these modules and measure phenotypic outcomes, focusing on plant architecture and biotic stresses (maize), and floral development (Arabidopsis).

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P220

Mining maize with Gramene

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Need to find orthologs for your favorite maize genes? How about expression? Would obtaining a sorghum mutant of your gene of interest accelerate your research? Or perhaps you need to compare entire pathways, and visualize the maize interactome? Gramene (http://www.gramene.org) is an integrated resource for comparative functional analysis in plants. We provide access to 67 reference genomes, and pathways for 93 plants species. Built upon Ensembl, Reactome, and Expression Atlas infrastructures, Gramene is committed to open access and reproducible science based on the FAIR data principles. Gramene provides integrated search capabilities and interactive views to visualize gene features, gene neighborhoods, phylogenetic trees, expression profiles, pathways, and cross-references. Maize reference genomic data include the B73 RefGen_V4 assembly with i) gene functional descriptions, ii) sub-genome designation and ohnologs, iii) annotated transposable elements, iv) methylation data, iv) V3-V4 gene ID mappings and an assembly converter to lift-over genomic coordinates across V2-V4. Gramene hosts genetic variation for 12 genomes including the Panzea 2.7 GBS and HapMap2 datasets in maize, and the USDA-ARS sorghum EMS collection. The Plant Reactome hosts 306 reference pathways curated in rice and projected to maize and other species by orthology. Visualizations of EBI Expression Atlas data, from over 800 experiments, are integrated into the search results panel, and both the genome and pathway browsers. Finally, in collaboration with the genome assembly of 26 NAM founders project (NSF IOS-1445025, PI: K. Dawe, Co-PIs: M. Hufford & D. Ware), we are developing new resources to interrogate the maize pangenome, including the updated RefGen_V5 assembly. The Gramene Maize Pangenome subsite (http://maize-pangenome-ensembl.gramene.org) features uniformly annotated and comprehensively mapped NAM founder genomes for studying structural variation impacting CNV and PAV. Gramene is supported by an NSF grant IOS-1127112, and partially from USDA-ARS (001-8062-505 002).

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P221

New from the UniformMu National Public Resource for maize genetics

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First --- New insertion assignments have emerged among the 70,515 unique germinal insertions of the UniformMu resource due to their re-mapping in v4 of the B73 genome. The collective insertions, including multiple alleles, account for a total of 97,852 seed-stock assignments. Of these, 7,573 (7.7%) changed from their original destinations after re-mapping to v4. The migration from B73v2 to B73v4 added 4,460 new insertion sites to the resource while removing 6,111. The latter were either duplicates or not well-supported by alignments in B73v4. However, the missing insertions did include over 200 sites clearly detected by alignments in v2, but absent in v4. Moreover, a majority of missing insertions had mapped to loci with well-documented syntelogs in the sorghum genome. We anticipate that the improved accuracy of stock assignments will further enhance the overall quality and reliability of the UniformMu resource for users. In addition to accessibillity via the genome browser at MaizeGDB, a "UniformMu Downloadable," which lists B73v4 coordinates of all insertions, is available at https://download.maizegdb.org/UniformMu/UniformMu_Release 9_B73v4_7_1_2019.xlsx. **Second ---** We have replenished high-demand lines depleted by user requests. New seed have been deposited for 223 UniformMu stocks, and an additional 112 were grown and sib-pollinated in Spring 2019. **Lastly ---** Coming this spring at MaizeGDB: 4,000 new mutants were revealed by mapping the UniformMu insertion coordinates in the native W22 inbred genome. The unique insertion sites were not detected in B73, consistent with genetic differences between the inbred genomes.

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P222 @adam_thrash

PAST: The Pathway Association Study Tool for interpreting GWAS results in light of metabolic pathway data

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We present a recently developed bioinformatics method for interpreting genome-wide association study (GWAS) results using metabolic pathway analysis. PAST (Pathway Association Study Tool) has been used in several studies to find significant pathways and mechanisms explaining phenotypic traits of interest in plants. PAST has been implemented as a package for the R language. Two user-interfaces are provided; PAST can be run by loading the package in R and calling its methods, or by using an R Shiny guided user interface. An online, maize specific version of PAST has been added to MaizeGDB. In testing, PAST completed analyses in up to one hour by processing data in parallel. PAST has many user-specified options for maximum customization. It has been used to analyze data from multiple independent maize GWAS panels on grain color, oil and fatty acid grain concentrations, corn earworm resistance, and aflatoxin accumulation resistance, with results that make clear and biological sense. PAST has also been used with GWAS data from other plant species. The user-friendliness and unequivocal results make PAST accessible and useful to researchers interested in associating metabolic pathways with GWAS datasets to better understand the genetic architecture and mechanisms affecting phenotypes. Links to Bioconductor, Github, and MaizeGDB, where PAST and the user manual can be accessed, are presented in this poster.

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P223 @corncolors

Pangenome of the maize NAM founder inbreds

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De novo genome assemblies of the 25 maize NAM founder inbreds and a new B73 (version 5) genome have been publicly released. The genomes were assembled as a part of a single large project using a combined PacBio/Bionano approach, with the intent of making all data directly comparable. mRNA was sequenced from ten tissues for each inbred, and the annotations are available with browser support at MaizeGDB. The NAM assemblies have contig N50s ranging from ~17-78 Mb and scaffold N50s from ~95-201 Mb. Our interpretations of the data, including pangenome content, fractionation across inbreds, and the prevalence and impact of structural variation will be presented.

Funding acknowledgement: National Science Foundation (NSF)

P224

Persistence and evolution of polyploid complexes within a species

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Whole genome duplication events are common across the green tree of life and often create mating barriers within a species. Many grass species have the potential to overcome sexual inviability by clonal propagation through a rhizome. Andropogon gerardii is an ecologically dominant prairie grass composed of two cytotypes (6x and 9x) that represents more than 80% of the biomass in endangered tallgrass prairies. The 9x individuals are sexually sterile and reproduce only through clonal propagation. Despite inviability, 9x individuals are found across the full range of A. gerardii and dominate populations in the southwestern United States. Although asexuality is initially advantageous for overcoming reproductive barriers, it creates a bottleneck that reduces standing genetic variation. Additionally, loss of recombination can lead to the accumulation of deleterious mutations. These processes may consequently decrease fitness and adaptive potential. We have completed initial collection and whole genome sequencing of 140 individuals from 14 populations across the natural range of A. gerardii. Individuals have been aligned to a new A. gerardii reference genome and genotypes called using a novel maximum likelihood method. Here we will present the method used to call genotypes in a hexaploid and preliminary data on the population genetics of A. gerardii.

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P225 @zhzheng92

Prediction of phenotypic plasticity using root system architecture

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Phenotypic plasticity characterizes the phenotypic variation of a trait when a genotype is exposed to different environments. Root system architecture (RSA) plays critical roles in plant productivity and stress tolerance. As such, we hypothesized that it might be possible to extract from RSA explanatory variables for phenotypic plasticity prediction complementary to SNPs and variation in gene expression. We applied a deep learning framework to more than 8,000 2-D images of RSA from separate diversity panels of maize inbred lines and G2F hybrids. In total, 1,032 latent features, novel traits that distinguish individuals based on treatments inferred automatically from image data using deep learning, were extracted. Based on the accuracy of classifying the RSA of plants that had or had not been irrigated, these latent features outperformed traditional RSA traits defined by biologists and engineers. Moreover, these RSA latent features were used to successfully predict the phenotypic plasticity of above-ground traits of both inbred lines and G2F hybrids, including flowering time, plant height and yield components across multiple environments. Finally, we investigated the biological and functional significance of these latent features by conducting an association study that simultaneously used SNPs and gene expression data as explanatory variables for RSA latent features.

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P226 @Valiancy_miao

Semantic segmentation of sorghum and maize using hyperspectral data identifies genetic associations with variation in plant size

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This study describes the evaluation of a range of approaches to semantic segmentation of hyperspectral images of sorghum plants, classifying each pixel as either non-plant, or belonging to one of three organ types (leaf, stalk, panicle). While many current methods for segmentation mainly focus on separating plant pixels from background, organ-specific semantic segmentation makes it feasible to measure a wider range of plant properties. Manually scored training data for a set of hyperspectral images collected from a sorghum association population was used to train and evaluate a set of supervised classification models. Many algorithms show acceptable accuracy for this classification task such as LDA (Linear Discriminant Analysis), MLR (Multinomial Logistic Regression), and ANN (Artificial Neural Networks). Algorithms trained on sorghum data are able to accurately classify maize leaves and stalks, but fail to accurately classify maize reproductive organs which are not directly equivalent to sorghum panicles. Trait measurements extracted from semantic segmentation of sorghum organs can be used to identify both genes known to be controlling variation in a previously measured phenotypes (e.g panicle size and plant height) as well as identify signals for genes controlling traits not previously quantified in this population (e.g stalk/leaf ratio). Organ level semantic segmentation provides opportunities to identify genes controlling variation in a wide range of morphological phenotypes in sorghum, maize, and other related grain crops.

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P227

Sequence, assembly and annotation of maize inbred B104: A maize transformation resource

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Emergence of new gene editing tools like CRISPR-Cas systems and TALENs have heightened the need to develop new and efficient transformation technologies in plants including maize. However, maize transformation is not straightforward. Not all lines are easily transformed and therefore genome edited. The maize genome reference sequence, for example, is currently based on the B73 inbred line, which is not readily transformed. B104 is a maize inbred line which is highly similar to B73 and is known to be transformable. Thus, to capture the genome-wide variation of these two lines belonging to the same heterotic group (Iowa stiff stalk synthetic lines) and enhance our knowledge of maize transformation, we have generated a high quality genome assembly of the maize inbred line B104. The new assembly was generated using high-depth PacBio data and a Bionano optical map and is significantly more contiguous and complete (32 scaffolds and 98Mb N50) than the previous draft assembly based on short-read data. RNA-seq from multiple tissues was used to annotate the assembly using an evidence-driven pipeline that includes gene models generated using Mikado software with PASA updates combined with ab-initio gene models from the BRAKER pipeline. We present a summary of genomic similarities and differences between B73 and B104 and assess the potential role of structural, epigenetic (chromatin accessibility and DNA methylation), and gene expression variation in the determination of agronomic and transformation traits.

Funding acknowledgement: United States Department of Agriculture (USDA)

P228 @stevepiccolo

ShinyLearner: Enabling biologists to perform benchmark machine-learning classification algorithms

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Machine-learning classification is growing in use among biologists. In one type of application, researchers use classification algorithms to predict whether biological entities belong to a given category (e.g., disease or no disease). Alternatively, researchers may use such algorithms to identify biological features (e.g., genes) associated with a particular biological outcome. Although the research community has developed many classification algorithms and corresponding software libraries, considerable barriers exist for non-computational biologists to take advantage of these tools. Different algorithms are written in different programming languages and require different input formats. Software libraries may require dependencies that are difficult to install, and the software may fail if incompatible versions are installed. Thus, identifying which algorithm is best for a given problem is difficult. We developed ShinyLearner (https://github.com/srp33/ShinyLearner), an open-source software tool that reduces these barriers. ShinyLearner integrates several popular machine-learning libraries (e.g., scikit-learn, mlr, weka) within a Docker container that includes all software dependencies. Accordingly, ShinyLearner can be installed with ease. ShinyLearner supports Monte Carlo and k-fold cross validation and provides an option for feature selection. When multiple parameter combinations are used for a given algorithm, ShinyLearner dynamically selects the best combination via nested evaluation. A simple Web interface facilitates the process of configuring and executing the software. Output files are in "tidy-data" format to enable easier processing with external analysis tools.

P229

Strategies for imputing genotypes from low coverage DNA sequence using the practical haplotype graph

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The Practical Haplotype Graph (PHG) consists of a relational database and associated software that organizes genomic sequence for many different samples and provides methods for imputing genotypes from low coverage sequence. Imputation inputs include a PHG database of reference haplotypes and fastq files of DNA sequence generated by any of a number of sequencing protocols. The PHG software implements two imputation methods. The first uses a first-order hidden Markov model (HMM) to impute genotypes. The second uses a positional Burrows-Wheeler transform (pBWT), which organizes haplotypes in memory to provide fast searching and matching. The HMM method is quite fast for a limited number of reference haplotypes, but its computational time scales linearly with the number of haplotype states. To handle the potentially large number of haplotypes that would be available from a breeding program, a pBWT method (fastLS) was added. The pBWT algorithm executes in constant time with respect to the number of haplotype states, so can handle imputation based on large numbers of reference haplotypes. This poster describes the use of both methods for imputing homozygous and heterozygous diploid samples.

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P230 @hwb333

The NUPRIME project: Innovations in identifying candidate cis-regulatory elements from small particle MNase footprints

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The goal of this project (referred to as NUPRIME) is to develop micrococcal nuclease (MNase) profiling as a foundational resource for integration of maize epigenomic data. The web page at maizenucleosome.org describes the project, supported by the Plant Genome Research Program (NSF IOS 1444532). Chromatin structure is dynamic and intimately linked to gene regulation and other genetic functions. We applied nuclease sensitivity profiling (DNS-seq) to reveal sites of open chromatin in four reference tissues (Turpin et al., 2018, Data in Brief 20:358-363, DOI: 10.1016/j.dib.2018.08.015). Comparison of DNS profiles identifies tissue-specific variation in chromatin structure. We also report on an innovation in fine mapping of chromatin particles. This improved MNase-based open and accessible (MOA-seq) chromatin profiling technique maps small fragments from light nuclease digests of chromatin from formaldehyde-fixed nuclei at ultra-high resolution. MOA-seq earshoot coverage peaks identified by iSeg (Girimurugan et al., 2018) generally match those from hypersensitive or TF-occupied footprints identified by other methods such as DNS-seq, ATAC-seq, or ChIP-seq. Motif analysis of the top 1% of iSeg MOA-seq peaks identified hundreds of known and unknown DNA sequence motif families, collectively defining the occupied earshoot cistrome. Together, DNS-seq and MOA-seq define open chromatin regions as well as small-particle footprints within these broader accessible chromatin regions. These approaches enable comparative analyses across tissues or treatments to identify regulatory chromatin regions and nominate candidates for functional cis-elements.

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P231 @SchnableLab

The effects of nitrogen stress on leaf angle in sorghum and maize

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Past research focusing on leaf angle has shown an association with increased yields in high planting densities in maize, a trait that was inadvertently selected during breeding for maize improvement. Studies show sorghum varieties with more erect leaf angles are linked to increased yields with greater nitrogen accumulation in vegetative tissue. However, these studies focus on crop response under optimal conditions for diverse sorghum varieties. As part of a study of differential responses to nitrogen stress in sorghum, under greenhouse conditions. It was observed that eight of the ten diverse sorghum accessions exhibit a decrease in adaxial leaf angle when grown under nitrogen deficit conditions. A parallel analysis in maize examined leaf angles of seventeen maize lines drawn from the Buckler_Goodman 282 Association panel, were grown in a field environment under nitrogen deficit and nitrogen replete conditions. Which did not identify any equivalent plasticity of leaf angle in response to nitrogen deficit stress. The difference in leaf angle among sorghum varieties was observed under nitrogen stress. More field research is needed to better understand this leaf angle response under less than optimal conditions. The influence of leaf angle on canopy architecture could be affected by nutrient stress as well but it is unclear if this response is adaptive or maladaptive. This research will help better understand plasticity within both sorghum and maize. Based on the close lineage of maize and sorghum this could also be used in improvements for future maize applications.

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P232

The maize B-chromosome content

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The maize B-chromosome was discovered a century ago and has been actively studied since then. This peculiar chromosome, which is dispensable, remains in maize lines by an accumulation mechanism that consists of nondisjunction at the second pollen mitosis followed by preferential fertilization of the egg, as opposed to the polar nuclei, by the B chromosome containing sperm. The nondisjunction occurs at the centromeric region and requires other portions of the B chromosome that act in trans, given that they do not need to be present on the same chromosome to produce nondisjunction. We report here the completion of the first plant B-chromosome pseudomolecule sequence assembly. Using the MAKER pipeline we have discovered 758 genes with 686 located on the 107Mbp long pseudomolecule. Using available RNA-seq data extracted from leaves of B containing plants, we found that B genes were expressed in this tissue. Combining gene location and ontology information, candidates for the distal trans-acting factor required for nondisjunction has been narrowed. By analyzing homologous genes from related species, we estimated the timing of gene insertion into the B-chromosome and found that this process is continuous over evolutionary time.

P233

The sequence of the mysterious B chromosome reveals some of its secrets

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B chromosomes are dispensable, supernumerary and "selfish" genetic elements found in representatives of plants, animals and fungi. In a population, they are present only in some individuals and in several species they are selectively eliminated from specific tissues and organs. Interestingly, they do not follow rules of mendelian inheritance and can accumulate through a process called non-disjunction. The B chromosome in maize is one of the first discovered (a hundred years ago) and most studied. We combined available genomics tools to sequence and assemble the maize B chromosome. With a pseudomolecule of 107 Mb, it is the first plant B chromosome ever assembled to chromosome-scale. A high-quality sequence enabled a detailed analysis of repetitive elements as well as its gene content. Transposable elements contribute more than 90% to the pseudomolecule size and clustering of LTR retrotransposons resulted in twelve families with more than 100 copies on the B chromosome, all of them being found also on A chromosomes. While it had been believed for decades that B chromosomes lack gene sequences, annotation of the maize B chromosome with the Maker pipeline identified more than seven hundred genes. The analysis of the closest orthologs/paralogs of B-localized genes in the genomes of maize and Sorghum bicolor reveal a history of gene transpositions followed by their degradation. Any synteny of genes from a potential progenitor chromosome cannot be recognized. Gene Ontology analysis indicated a prevalence of GO-terms linked to B-chromosome behavior among those over-represented on the B chromosome compared to the A-chromosome complement. Finally, sequencing of B-A translocations allowed a narrowing down number of candidates for a "distal element" involved in non-disjunction to 34 genes.

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P234

Tissue-specific transcriptional activity determined by iRNA-seq analysis

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Transcript abundance is commonly used to measure gene expression. However, this type of analysis does not discriminate between differential expression caused by mRNA degradation or acute transcriptional activity. We have adapted a computational method called intron RNA-sequencing (iRNA-seq) analysis to determine genome-wide transcriptional activity in plants using total RNA-seq datasets. iRNA-seq analysis assesses intron coverage and mature transcript levels to identify changes in gene expression that are predicted to result from changes in transcriptional activity, thus taking differences between mRNA degradation and acute transcriptional activity into account. Utilizing this technique, we have identified differential transcription in husk and inner stem tissue in maize. Genes undergoing transcription were found to correspond with predicted enhancer activity and transcription factor activity was found to highly regulate the observed transcriptional activity. We demonstrate that iRNA-seq analysis is an effective way to predict the relative contribution of transcriptional changes to important plant developmental and regulatory events, thus improving our understanding of transcriptional regulation in plants.

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P235

Utilizing adaptive diversity in Tripsacum for improving freezing tolerance in maize

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Tripsacum is the sister genus to Zea and is widely distributed across temperate and tropical latitudes in the North and South America. It is largely untouched by domestication and encompasses a tremendous diversity in cold tolerance traits. The natural adaptive variation for freezing tolerance in Tripsacum could be utilized to breed for frost tolerant maize in two ways: to create overwintering maize in southern regions, and support earlier planting in northern regions. However, there are very few genomic resources available to dissect the variation. A mapping population was created from multiple crosses between clones belonging to species dactyloides and floridanum across latitudinal gradients, and clonally propagated F1s were open-pollinated in the field. The segregating F2 progenies were screened for freezing tolerance by exposing them to hard frost, and individuals with extreme phenotypes were pooled and sequenced for bulk segregant analysis (BSA). Short WGS reads from bulks were aligned against de novo genome assemblies generated for each species using long nanopore sequencing reads. The assemblies were at 30x coverage with N50 of 396K and 370K for floridanum and dactyloides respectively. The BSA of this unique genetic mapping system allows for a larger number of recombination to be sampled than in a traditional mapping population, resulting higher power and resolution of QTL identification.

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Zimmerman, David **P13**
Zobrist, Jacob **P153**
Zwiener, Mackenzie J **P231**

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History of the Maize Genetics Conference

Year	Annual	Location	Dates	Chair
2020	62	Kailua-Kona, Hawai‘i	March 12-15	Clinton Whipple
2019	61	St. Louis, Missouri	March 14-17	Michael Muszynski
2018	60	Saint-Malo, France	March 22-25	Alain Charcosset
2017	59	St. Louis, Missouri	March 9-12	Erich Grotewold
2016	58	Jacksonville, Florida	March 17-20	David Braun
2015	57	St. Charles, Illinois	March 12-15	Mark Settles
2014	56	Beijing, China	March 13-16	Ann Stapleton
2013	55	St. Charles, IL	March 14-17	Phil Becraft
2012	54	Portland, OR	March 15-18	John Fowler
2011	53	St. Charles, IL	March 17-20	Erik Vollbrecht
2010	52	Riva del Garda, Italy	March 18-21	Jane Dorweiler
2009	51	St. Charles, IL	March 12-15	Steve Moose
2008	50	Washington, DC	February 27 - March 2	Thomas Brutnell
2007	49	St. Charles, IL	March 22-25	Anne Sylvester
2006	48	Asilomar, Pacific Grove, CA	March 9-12	Jay Hollick
2005	47	Lake Geneva, WI	March 10-13	Martha James
2004	46	Mexico City, Mexico	March 11-14	Mike Scanlon
2003	45	Lake Geneva, WI	March 13-16	David Jackson
2002	44	Kissimmee, FL	March 14-17	Sarah Hake and Sue Wessler
2001	43	Lake Geneva, WI	March 15-18	Torbert Rocheford and Sue Wessler
2000	42	Coeur d'Alene, ID	March 16-19	Rebecca Boston and Sue Wessler
1999	41	Lake Geneva, WI	March 16-19	Julie Vogel and Cliff Weil
1998	40	Lake Geneva, WI	March 19-22	Mike McMullen
1997	39	Clearwater Beach, FL	March 13-16	Paul Sisco
1996	38	St. Charles, IL	March 14-17	Paul Chomet
1995	37	Asilomar, Pacific Grove, CA	March 16-19	Karen Cone
1994	36	St. Charles, IL	March 24-27	Kathy Newton
1993	35	St. Charles, IL	March 18-21	Tim Nelson
1992	34	Asilomar, Pacific Grove, CA	March 19-22	Sarah Hake
1991	33	Lake Delavan, WI	March 21-24	Jim Birchler
1990	32	Lake Delavan, WI	March 8-11	
1989	31	Lake Delavan, WI	March 2-5	
1988	30	Madison, WI	March 25-27	
1987	29	Lake Delavan, WI	March 20-22	
1986	28	Lake Delavan, WI	March 21-23	Curt Hannah
1985	27	Lake Delavan, WI	March 29-31	Hugo Dooner
1984	26	Champaign, IL	March 10-11	Earl Patterson
1983	25	Allerton Park, IL	March 12-13	Earl Patterson
1982	24	Allerton Park, IL	March 13-14	Earl Patterson
1981	23	Allerton Park, IL	March 14-15	Earl Patterson
1980	22	Allerton Park, IL	March 8-9	Earl Patterson
1979	21	Allerton Park, IL	March 10-11	Earl Patterson
1978	20	Allerton Park, IL	March 11-12	Earl Patterson
1977	19	Allerton Park, IL	March 12-13	Earl Patterson

Year	Annual	Location	Dates	Chair
1976	18	Allerton Park, IL	March 13-14	Earl Patterson
1975	17	Allerton Park, IL	March 8-9	Earl Patterson
1974	16	Allerton Park, IL	March 9-10	Earl Patterson
1973	15	Allerton Park, IL	March 10-11	Earl Patterson
1972	14	Allerton Park, IL	March 11-12	Earl Patterson
1971	13	Allerton Park, IL	March 13-14	Earl Patterson
1970	12	Allerton Park, IL	March 14-15	Earl Patterson
1969	11	Allerton Park, IL	March 15-16	Earl Patterson
1968	10	Allerton Park, IL	March 16-17	Earl Patterson
1967	9	Allerton Park, IL	March 11-12	Earl Patterson
1966	8	Allerton Park, IL	March 12-13	Earl Patterson
1965	7	Allerton Park, IL	March 13-14	Earl Patterson
1964	6	Allerton Park, IL	March 14-15	Earl Patterson
1963	5	Allerton Park, IL	March 9-10	Earl Patterson
1962	4	Allerton Park, IL	March 17-18	Earl Patterson
1961	3	Allerton Park, IL	March 18-19	Earl Patterson
1960	2	Allerton Park, IL	March 12-13	Earl Patterson
1959	1	Allerton Park, IL	January 8-9	John Laughnan, Ed Coe, Gerry Neuffer, and Earl Patterson

Notes

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