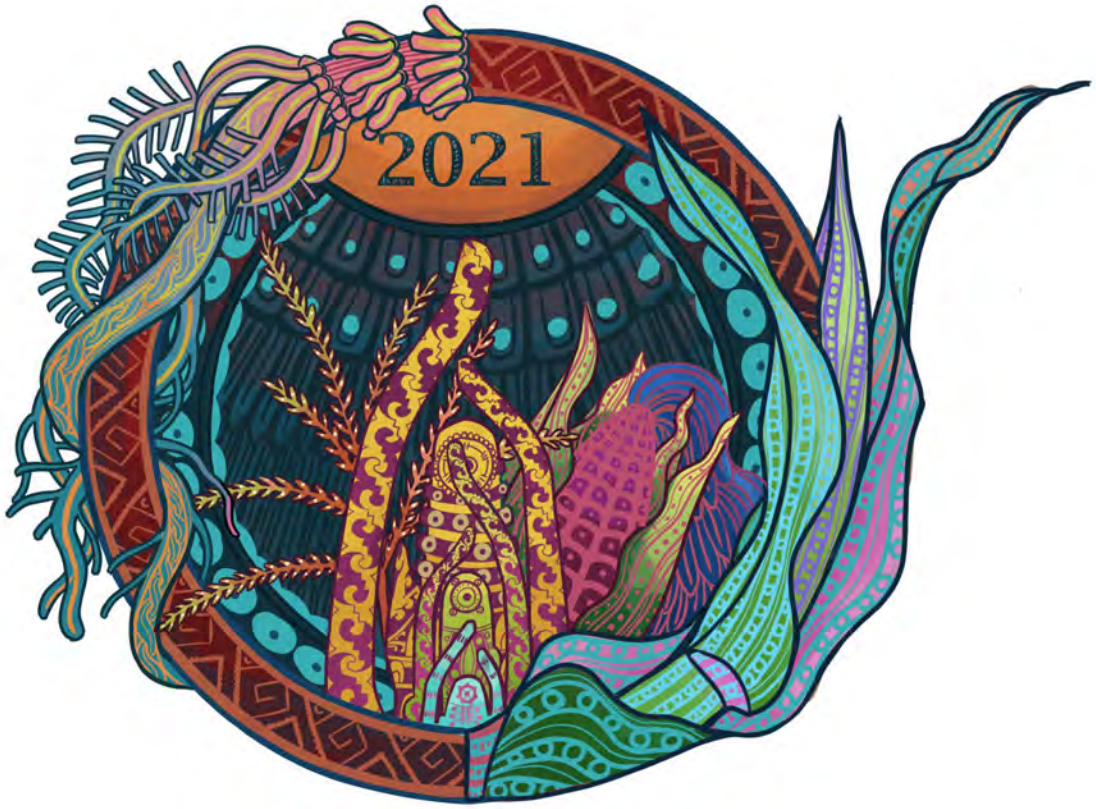
63rd Annual Maize Genetics Meeting

Program and Abstracts

March 8 – March 12, 2021



MGC

Maize Genetics Cooperation, Inc.

2nd Virtual Maize Meeting
Facilitated in partnership with

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This conference received financial support from:

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We thank these sponsors for their generosity!

A special thank you for the in-kind support from the USDA-ARS.

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Cover image description

Hand-drawn logo consisting of different major maize organs, using motifs of the art of the indigenous peoples of Mesoamerica, including the Maya, Mixtec Zapotec, Mexica and Nahuas, by whom maize was domesticated and dispersed.

Cover art by

Dr. Thai Dao
Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon USA

General Information

Meeting Etiquette: Sharing of unpublished data

The Maize Meeting is a respected forum for presentation and discussion of unpublished material. For this virtual format there are no privacy guarantees beyond that of an in-person meeting. **Photographing, screenshots, downloading, or recording of talks and posters is not permitted**. All poster images, poster videos and talk recordings will be accessible only by registered meeting attendees who have access to the password-protected site. Please honor the Maize Genetics community's long-standing tradition of sharing unpublished data without concern of inappropriate distribution.

Poster and Talk Viewing

All posters are available for viewing as clickable images from the list of posters on the virtual meeting SwapCard platform.

Viewing posters:

- Go to the Posters tab on the virtual meeting site.
- Click a poster title to access a page for that poster. Many poster authors have provided a lightning video, which will automatically begin playing upon selecting a poster title.
- To view a static image of the poster, click "See Poster in Detail" and a new window will open to display the poster.
- Questions or comments can be posted at any time using the Chat function in the "Live Discussion" box on the righthand side of the poster page in the virtual platform. Because attendees are joining the conference from many time zones, responses may not be immediate.

Daily poster sessions (Monday to Thursday) are scheduled for poster viewing, for posting questions and comments in the "Live Discussion" box and for connecting with poster presenters via 10-minute individual meetings.

Arranging 10-minute meetings during Poster Sessions:

- To arrange a meeting with a poster presenter, click on the poster of interest.
- Scroll to the "Speakers" heading and click on the name of the poster presenter, which will redirect you to the person's profile page.
- Scroll to the "Meet [Attendee's Name]" section, and select "See more slots". Identify the time slots that fall within the poster session at which you wish to connect and select a 10-minute slot.
- By selecting a time slot, it will automatically be added to My Event for both you and the for the poster presenter.
- Poster presenters should be sure to modify their availability to reflect the times they are available for meetings, including during poster sessions (e.g. if you are unavailable due to time zone differences). This can be done by selecting the "My Event" tab, and selecting "My Meetings" on the left-hand navigation. All attendees are default selected as available for anytime talks are not in session.

All poster images and videos are available for viewing by registered attendees at any time during the March 8-12 meeting, as well as for one month following the conference.

Asynchronous talk viewing: All live talks will be recorded and will be posted within 24 hours after the presentation, allowing for asynchronous viewing to accommodate our global community. Questions can be posted in the chat box accompanying the video and will be monitored by the presenter. Alternatively, you may select to directly message the presenter or arrange for an individual meeting. All recordings will be available at the virtual meeting platform for at least one month following the conference.

Virtual Maize Genetics Meeting platform

Our 2021 virtual meeting is being hosted on the SwapCard platform. Each meeting registrant will receive a personalized invitation email sent by SwapCard (noreply@swapcard.com). If this invitation has not arrived or if you are having issues accessing the site, please go to https://app.swapcard.com/login and enter the email address that you used when registering for the Maize Genetics Meeting. This will generate a sign-in email. If you have issues upon entering the meeting site on Swapcard, please access the Support Zoom Room on Monday, 3/8 during either of the two conference blocks that day. If you are still experiencing issues, please email virtual@conferencedirect.com.

Virtual Networking and Socializing

Socializing and networking can occur via the Gather.Town platform, that can be accessed by clicking the "Social Networking in GatherTown" button on the home page of the virtual conference platform. Daily Networking and Socializing times are noted in the meeting schedule. This platform allows for virtual mingling via video chat. A quick guide for navigating the Gather.Town platform and socializing with friends and colleagues can be found upon entering Gather.Town. The Gather.Town platform will be available for networking from 12pm-4pm CST and 8-12pm CST (unless otherwise noted on the schedule) on Monday through Thursday, and 12pm-1pm CST on Friday.

Steering Committee

A post-meeting survey will be sent out for you to share your feedback, or you may directly share your suggestions and comments about the meeting with the 2021 Steering Committee.

Marna Yandeau-Nelson, Chair.....(myn@iastate.edu)
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Madelaine Bartlett……..(mbartlett@bio.umass.edu)
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Yongrui Wu .....(yrwu@sibs.ac.cn)

Ex officio:
Carson Andorf - MaizeGDB
David Braun – Treasurer
Darwin Campbell – Meeting Planning
John Portwood - Abstract Coordinator
Marty Sachs - Maize Meeting Guru

Acknowledgements

Many thanks go to John Portwood, Carson Andorf, and the MaizeGDB staff from the USDA-ARS, as well as Darwin Campbell (Iowa State University) for their tremendous efforts in organizing, assembling, and advertising the conference program. We also greatly thank Carrie Good and her team at ConferenceDirect, and Ian Popewitz and Lynne Navis from the Alliance of Crop, Soil, and Environmental Science Societies (ACSESS) for helping to organize and implement the virtual conference platform, handling registration and dealing with a multitude of other issues. Thanks go to Mei Guo and Todd Jones for their efforts in securing funding to offset meetings costs. Finally, many, many thanks go to the Steering Committee for organizing the 2nd virtual Maize Genetics Meeting.

From the Maize Genetics Cooperation Board of Directors:

Maize Genetics Awards:

The 2021 M. Rhoades Early Career Awardee:

Andrea Eveland at the Donald Danforth Plant Science Center.

The 2021 L. Stadler Mid-Career Awardee:

Nathan Springer at the University of Minnesota.

The 2021 R.A. Emerson Awardee:

Don Auger at the South Dakota State University (posthumous).

The 2021 Cooperator Awardee:

Marty Sachs at USDA-ARS and the Maize Genetics Cooperation Stock Center.

The 2021 Leadership Awardee:

Natalia de Leon 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. This year we will honor both the 2020 and 2021 awardees.

The **2020** Barbara McClintock Prize for Plant Genetics and Genome Studies has been awarded to Dr. James Birchler who will present a McClintock Prize Address on Thursday, March 11, 5:15pm CST (See Abstract #M2).

The **2021** Barbara McClintock Prize for Plant Genetics and Genome Studies has been awarded to Dr. John Doebley, who will present a McClintock Prize Address on Wednesday, March 10, 9:15am CST (See Abstract #M1).

(See https://maizegdb.org/mgc/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 Advocacy Committee. The grant funds activities at the Maize Genetics Meeting including the MaGNET program and travel awards to increase disciplinary breadth and underrepresented participation. In addition, the funding allows the Maize Genetics Meeting 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. The third mid-year virtual conference focused on public-private partnerships and career development. White papers summarizing conclusions of the mid-year conferences are available on MaizeGDB (https://www.maizegdb.org/mgc/advocacy/docs.php). 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.

Introducing the Committee on Outreach, Diversity, Inclusion, and Education:

The Committee on Outreach, Diversity, Inclusion, and Education (CODIE) of the Maize Genetics Community was established in 2020, and is dedicated to fostering a diverse and inclusive community of researchers. To achieve this goal, the committee will specifically work on the following three objectives:

1. Improving the recruitment, retention, and recognition of scientists from underrepresented groups at different steps of their career.
2. Broadening participation in the Maize Genetics Research Community, funded research and training opportunities, and in the Maize Genetics Meeting by international collaborators and US scientists from research and educational institutions.
3. Encouraging Maize Research Community members to learn about the expression and negative effects of systemic racism and promote inclusivity and equity in science and education.

More information, including funding and training opportunities, can be found at the CODIE website (https://www.maizegdb.org/mgc/outreach/). CODIE members are volunteers from the community and all are welcome to join the committee. If you are interested in becoming a member of CODIE, please contact Dr. Marilyn Warburton (marilyn.warburton@ars.usda.gov) and Dr. John Fowler (fowlerjo@oregonstate.edu).

The MaGNET Program and 2021 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 Meeting (MGM). 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, and to meet with plenary speakers. Each MaGNET Award helps defray the cost of attending the Maize Genetics Meeting, including registration, and for in person-meetings- food, lodging and airfare. In addition, each awardee is paired with an experienced 'Maize Mentor', who will help the awardee navigate the conference. Awardees are identifiable by a special notation on their virtual name tags, and many of them are attending the MGM 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.

2021 MaGNET Awardees

Undergraduate:
Isabella Higgins, University of Massachusetts Amherst — Poster #184
Abelina Jackson, University of California, Davis

Graduate:
Katharhy Grossman, CUNY Lehman College

Faculty mentor
Madelaine Bartlett, University of Massachusetts Amherst

Primarily Undergraduate Institution & Disciplinary Breadth Awards

Primarily Undergraduate Institutions (PUI) and Disciplinary Breadth (DB) are two 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 Meeting (MGM). The PUI program seeks to welcome students and faculty from Primarily Undergraduate Institutions into the maize community, by encouraging their attendance at the MGM. The Disciplinary Breadth (DB) program seeks to recruit and retain scientists (advanced graduate students, post-docs, and early-career faculty) from plant-related disciplines into the maize research community. The DB program has recently been expanded to support attendance at the meeting for graduate students and postdocs from historically underrepresented groups, regardless of discipline. Both programs provide an opportunity for researchers from diverse disciplines that have potential to enrich the maize community to learn about maize genetics by connecting with scientists in the maize genetics community, exploring potential collaborations, and developing career contacts.

Each award helps defray the cost of attending the Maize Genetics Meeting, including registration, and for in person-meetings- food, lodging and airfare. Awardees are identifiable by a special notation in their conference profiles, and many of them are attending the MGM 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 Meeting for student awardees, faculty who accompany one or more eligible student applicants are also eligible to apply for a PUI or DB award.

2021 PUI Awardees

Faculty
Irina Makarevitch, Hamline University — Poster #37

2021 DB Awardees

Postdoc
Subbaiah Chalivendra, on job market — Poster #172

Graduate Student
Melissa Lehrer, West Virginia University — Poster #191

Faculty
Melinda Yerka, University of Nevada-Reno — Poster #40

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

Broadening International Participation Awards

The 2021 Broadening International Participation Award program seeks to promote international attendance at the virtual meeting of researchers from countries that are historically under-represented at the Maize Meeting. This 2021 award program seeks to enrich the maize community and broaden the opportunities to learn about maize genetics by connecting with scientists in the maize genetics community, exploring potential collaborations, and developing career contacts.

Faculty

Paula Casati, Centro de Estudios Fotosinteticos y Bioquimicos, Argentina — Poster #128

Lohithaswa Chandappa, University of Agricultural Sciences, Bangalore, India

Zahoor Ahmed Dar, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, India — Poster #62

Research Scientists

Jazmin Abraham-Jaurez, Instituto Potosino de Investigación Científica y Tecnológica (IPICYT), Mexico — Poster #192

Sneha Adhikari, ICAR-Indian Institute of Wheat and Barley Research (IIWBR) Flowerdale, Shimla, H.P., India — Poster #85

Allen Oppong, Council for Scientific and Industrial Research – Crops Research Institute, Kumasi, Ghana — Poster #54

Graduate Students

Mary Emeraghi, University of Abomey-Calavi, Benin

Stefani Ramos, Universidad de la República, Uruguay

Data Management Made Simple

…A Reminder from the MaizeGDB team

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 and annotations should go to the long term repositories NCBI (USA - https://www.ncbi.nlm.nih.gov), EBI or DDBJ, where they are given stable identifiers (accessions). These three resources share data 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 NCBI Genomes or EBI. Contact us for help..
- 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 the data in your article.** When reviewing papers, please insure reported data is actually present and FAIR.

2. Use established unique identifiers for genes and gene models. 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. If reporting on a gene sequence, please use the exact gene model number (which will also ID the genome from which it came). If there is no gene model, please deposit your sequence at NCBI, and report the NCBI identifier. If reporting on a protein, please use the correct ID from NCBI or https://www.uniprot.org. If it's not there, please submit your protein sequence to NCBI or Uniprot.

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. Ensure data sets are "machine readable". 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.

2021 63rd Annual Maize Genetics Meeting Schedule

All times listed are in CST.

Wednesday March 3rd Pre-conference events

3:00 PM - 4:00 PM	**Maize Genetics Meeting MaGNET Networking Event**

Monday March 8th 9:00 AM - 8:00 PM CST

9:00 AM - 9:30 AM	Welcome and announcements
9:30 AM - 10:50 AM	**Session I: Genome Biology** **Chair: Erin Sparks**
9:30 AM - 9:50 AM	**(T1) Antoine Allier, Université Paris-Saclay** *Genetic diversity management and introduction in maize breeding programs using genomic selection*
9:50 AM - 10:10 AM	**(T2) Alex Bousios, University of Sussex** *Gene capture by transposable elements leads to epigenetic conflict in maize*
10:10 AM - 10:30 AM	**(T3) Jing Li, Iowa State University** *Annotating and characterizing the orphan genes of Zea mays B73 and NAM lines*
10:30 AM - 10:50 AM	**(T4) Kelly Dawe, University of Georgia** *De novo assembly, annotation, and comparative analysis of 26 diverse maize genomes*
10:50 AM - 11:00 AM	Break
11:00 AM - 12:00 PM	**Session II: Plenary Speaker** **Chair: Marna Yandeau-Nelson**
11:00 AM - 12:00 PM	**Frank Hochholdinger, University of Bonn** *Genetic dissection of maize root development*
12:00 PM – 12:30 PM	Student conversation with Dr. Hochholdinger (attendance is limited, sign up in meeting platform required)
12:00 PM – 5:00 PM	Socializing, networking, and open poster viewing

Monday March 8th (continued)

5:00 PM - 8:00 PM	**Session III: Maize & the Environment I** **Chair: Madelaine Bartlett**
5:00 PM - 5:20 PM	**(T5) Laura Tibbs Cortes, Iowa State University** *Identifying Environmental and Genetic Factors Underlying Phenotype in the Maize NAM Population*
5:20 PM - 5:40 PM	**(T6) Johannes Scharwies, Stanford University** *Diversity of moisture regulated root branching uncovered in maize*
5:40 PM - 6:00 PM	**(T7) Marcin Grzybowski, University of Nebraska-Lincoln** *Genetic architecture of nonphotochemical quenching (NPQ) kinetics in maize*
6:00 PM - 6:40 PM	**Fostering Diversity in the Maize Research Community** **(T8) Beronda Montgomery, Michigan State University** *Planting Equity – Lessons from plants on cultivating equitable ecosystems*
6:40 PM - 7:00 PM	**(T9) Rubén Rellán-Álvarez, North Carolina State University** *Teosinte introgression modulates phosphatidylcholine levels and induces early maize flowering time*
7:00 PM – 8:00 PM	**Poster session**
8:00 PM	Socializing, networking, and open poster viewing

Tuesday March 9th 9:00 AM-8:00 PM CST

9:00 AM - 9:10 AM	Welcome and announcements
9:10 AM - 10:50 AM	**Session IV: Regulating Genes and Genomes** **Chair: Maud Tenaillon**
9:10 AM – 9:30 AM	**(T10) Thomas Hartwig, Heinrich Heine University** *FIND-CIS: High-resolution mapping of functional cis-elements in the maize drought response*
9:30 AM - 9:50 AM	**(T11) Maria-Angelica Sanclemente, University of Florida-Gainesville** *Sugar Modulation of Anaerobic-Response Networks in Maize Root Tips*
9:50 AM – 10:10 AM	**(T12) Zhaobin Dong, University of California-Berkeley** *The tasselsheath4 gene establishes developmental fields within floral phytomers via microRNA mediated mutual repression*
10:10 AM - 10:30 AM	**(T13) Maud Fagny, Université Paris-Saclay** *Identifying key tissue-specific, biological processes by integrating enhancer information in maize gene regulatory networks*
10:30 AM - 10:50 AM	**(T14) Zongliang Chen, Waksman Institute** *Structural variation at the maize wuschel1 locus alters stem cell organization in inflorescences*
10:50 AM - 11:00 AM	Break
11:00 AM - 12:00 PM	**Poster session**
12:00 PM - 1:00 PM	Opportunity to remember Drs. Don Auger and Nick Lauter with family in Gather.Town networking space (link available in meeting platform)
12:00 PM – 5:00 PM	Socializing, networking, and open poster viewing

Tuesday March 9th (continued)

5:00 PM - 7:00 PM	**Session V: Emerging Tools & Applied Research I** **Chair: Todd Jones**
5:00 PM - 5:20 PM	**(T15) Yan Zhou, Iowa State University** *Identification of genetic determinants of trait measurement errors in image-based, high-throughput phenotyping*
5:20 PM - 5:40 PM	**(T16) Sagnik Banerjee, Iowa State University** *Constructing Zea mays genes from RNA-Seq expression data using FINDER - a fully automated gene annotator*
5:40 PM - 6:00 PM	**(T17) Daniel Runcie, University of California-Davis** *A statistical model for genomic predictions of high-dimensional traits*
6:00 PM - 6:20 PM	**(T18) Anju Giri, Cornell University** *Haplotype Associated RNA Expression (HARE) improves prediction of complex traits in maize*
6:20 PM - 6:40 PM	**(T19) Apurba Anirban, University of Queensland** *Investigating anthocyanin and sugar development in purple pericarp sweetcorn*
6:40 PM - 7:00 PM	Break
7:00 PM - 8:00 PM	**Session VI: Plenary Speaker** **Chair: Erin Sparks**
7:00 PM - 8:00 PM	**Bin Han, Chinese Academy of Sciences** *A quantitative genomics study on rice heterosis*
8:00 PM – 8:30 PM	Student conversation with Dr. Han (attendance is limited, sign up in meeting platform required)
8:00 PM	Socializing, networking, and open poster viewing

Wednesday March 10th 9:00 AM-8:00 PM CST

9:00 AM - 10:00 AM	**Session VII: McClintock Prize Presentation** **Chair: Jeff Ross-Ibarra**
9:00 AM – 9:10 AM	**Jeff Ross-Ibarra, UC Davis** *McClintock Prize introduction*
9:10 AM – 10:00 AM	**John Doebley, University of Wisconsin-Madison** *Barbara McClintock and the Genetic Foundation for Understanding Maize Domestication*
10:00 AM – 10:15 AM	Break
10:15 AM - 12:15 PM	**Session VIII: Emerging Tools & Applied Research II** **Chair: Marna Yandeau-Nelson**
10:15 AM - 10:35 AM	**(T20) Thomas Widiez, University of Lyon (INRAE)** *Haploid induction requires targeting of NOT-LIKE-DAD to pollen endo-plasma membrane by lipid anchoring and electrostatic interactions*
10:35 AM - 10:55 AM	**(T21) Simon Rio, Université Paris-Saclay** *Can admixed individuals help to study the differences in allele effects between maize heterotic groups?*
10:55 AM - 11:15 AM	**(T22) Nathaniel Korth, University of Nebraska-Lincoln** *Do the major changes in seed proteins in quality protein popcorn influence growth of beneficial, health-promoting bacteria in the human gut?*
11:15 AM - 12:15 PM	**Poster session**
12:15 PM - 1:15 PM	Career Networking Session
12:15 PM – 5:00 PM	Socializing, networking, and open poster viewing

Wednesday March 10th (continued)

5:00 PM - 7:00 PM	**Session IX: Cellular Processes** **Chair: Clint Whipple**
5:00 PM - 5:20 PM	**(T23) CJ Rachel Wang, Academia Sinica, Taipei Taiwan** *Super-resolution expansion microscopy (ExM) reveals the nature of critical recombination intermediates during meiosis*
5:20 PM - 5:40 PM	**(T24) Xinxin Ding, University of Wisconsin-Madison** *Microautophagy of storage proteins in maize aleurone cells*
5:40 PM - 6:00 PM	**(T25) Tyler McCubbin, University of Missouri-Columbia** *The COBRA-like gene BK2L3 affects cellulose deposition and sucrose export from source leaves*
6:00 PM - 6:20 PM	**(T26) Joseph Gallagher, University of Massachusetts-Amherst** *Duplicate transcription factor genes GT1 and VRS1 repeatedly evolve roles in growth repression*
6:20 PM - 6:50 PM	Award Presentations
6:50 PM - 7:00 PM	Break
7:00 PM - 8:00 PM	**Session X: Plenary Speaker** **Chair: Madelaine Bartlett**
7:00 PM - 8:00 PM	**Sarah Hake, UC Berkeley** *Organogenesis in maize - lessons from mutants*
8:00 PM – 8:30 PM	Student conversation with Dr. Hake (attendance is limited, sign up in meeting platform required)
8:00 PM	Socializing, networking, and open poster viewing

Thursday March 11th 9:00 AM-7:00 PM CST

9:00 AM - 10:00 AM	**Session XI: Plenary Speaker** **Chair: Matt Hufford**
9:00 AM – 10:00 AM	**Giles Oldroyd, University of Cambridge** *Transforming agricultural sustainability through beneficial microbial associations*
10:00 AM -10:30 AM	Student conversation with Dr. Oldroyd (attendance is limited, sign up in meeting platform required)
10:00 AM – 10:30 AM	Break
10:30 AM - 12:00 PM	**Maize Community Meeting** (all attendees encouraged to attend)
12:00 PM – 5:00 PM	Socializing, networking, and open poster viewing
5:00 PM – 7:00 PM	**Session XII: McClintock Prize Presentation** **Chair: David Braun**
5:00 PM - 5:10 PM	**David Braun, University of Missouri** *McClintock Prize introduction*
5:10 PM - 6:00 PM	**Jim Birchler, University of Missouri** *The Gene Balance Hypothesis: How gene stoichiometries affect expression, the phenotype and evolutionary processes*
6:00 PM - 7:00 PM	**Poster session**
7:00 PM	Socializing, networking, and open poster viewing

Friday March 12th 9:00 AM-1:00 PM CST

9:00 AM – 9:10 AM	Welcome and announcements
9:10 AM - 10:30 AM	**Session XIII: Maize & the Environment II** **Chair: Hilde Nelissen**
9:10 AM – 9:30 AM	**(T27) Peng Yu, University of Bonn** *Genetic Control and Environmental Regulation of Root-Microbiome Interaction in Maize*
9:30 AM – 9:50 AM	**(T28) Mihai Miclaus, National Institute for Biological Sciences, Cluj-Napoca, Romania** *Cytolines as models to study the impact of different cytoplasms on gene expression under heat stress conditions*
9:50 AM – 10:10 AM	**(T29) Giulia Castorina, University of Milan, Italy** *The drought-responsive ZmFDL1/MYB94 transcription factor regulates cuticle biosynthesis*
10:10 AM – 10:30 AM	**(T30) Elly Poretsky, University of California-San Diego** *Uncovering the Genetic Basis of Maize Sensitivity to Herbivore-Associated Molecular Patterns*
10:30 AM – 10:50 AM	Break
10:50 AM - 12:00 PM	**Session XIV: Genome Biology II** **Chair: Mei Guo**
10:50 AM - 11:10 AM	**(T31) Heather Manching, University of Delaware** *Using a parallel selection experiment to test modalities of haplotype-by-latitude effects underlying phenotypic variation in flowering time.*
11:10 AM - 11:30 AM	**(T32) Juliette Aubert, University of Montpellier, France** *Paramutation: a global silencing mechanism in maize?*
11:30 AM - 11:50 AM	**(T33) Guillaume Ramstein, Arhus University, Denmark** *Prediction of evolutionary constraint by genomic annotations improves prioritization of causal variants in maize*
11:50 AM - 12:00 PM	Closing remarks
12:00 PM - 1:00 PM	Socializing, networking, and open poster viewing
1:00 PM	**Meeting Adjourns**

Posters

Computational and Large-Scale Biology

P1	**Lisa Harper** <lisaharper@me.com>	*A history of maize genome sequence assemblies*
P2	**Liya Wang** <wangli@cshl.edu>	*Accessing the MaizeCODE data from SciApps*
P3	**Raksha Singh** <Raksha.Singh@usda.gov>	*Analysis of leaf-associated bacterial and fungal microbiomes of corn inbred lines related with Tar Spot disease*
P4	**Alain Charcosset** <alain.charcosset@inrae.fr>	*De novo assembling 19 maize inbred lines of importance for European breeding*
P5	**Travis Hattery** <Thattery@iastate.edu>	*Evaluating the impacts of growth environment on maize silk cuticular lipid composition*
P6	**Ksenia Krasileva** <kseniak@berkeley.edu>	*Evolutionary trajectory of innate immune receptors in maize*
P7	**Jutta A. Baldauf** <baldauf@uni-bonn.de>	*Gene expression complementation in the field from an above and below ground perspective in maize hybrids*
P8	**Guifang Lin** <guifanglin@ksu.edu>	*Genome assembly of A188 and genetic mapping of regeneration*
P9	**Julien Roziere** <julien.roziere@inrae.fr>	*Genome-wide de novo analysis of preferentially located motifs in 5′ and 3′-proximal regions from arabidopsis and maize*
P10	**Minghui Wang** <mw729@cornell.edu>	*Genome-wide identification of crossover sites using deep leaning*
P11	**Jianing Liu** <jl03308@uga.edu>	*Genomic history of maize as interpreted from whole-genome alignment of 26 lines*
P12	**Janlo Robil** <jmrp76@mail.missouri.edu>	*GrasVIQ: An image analysis framework for automatic quantification of veins in grass leaves*
P13	**Heidi Salihovic** <hfs17@my.fsu.edu>	*Identification of transposable element families that exhibit tissue-specific variation in chromatin structure as revealed by global MNase sensitivity profiling*
P14	**Tingting Guo** <tguo@iastate.edu>	*Identifying environmental index (EI) for genome-wide association studies (GWAS) and genomic selection (GS) for current and future environments*
P15	**Yinjie Qiu** <qiuxx221@umn.edu>	*Maize pan-genome construction using 26 NAM genome assemblies*
P16	**Jack Gardiner** <jack.m.gardiner@gmail.com>	*MaizeMine facilitates meta-analysis of diverse data sets through genomic data integration*
P17	**Allen Hubbard** <ahubbard@danforthcenter.org>	*Methods to effectively leverage LC-MS for population level biochemistry phenomics*
P18	**Marcela Tello-Ruiz** <telloruiz@cshl.edu>	*Mining maize with Gramene*
P19	**Travis Wrightsman** <tw493@cornell.edu>	*Modeling chromatin accessibility across angiosperms*
P20	**Fabio Gomez-Cano** <gomezcan@msu.edu>	*Multi-networks integration to prioritize regulatory genes of maize phenolic metabolism in maize*
P21	**Keting Chen** <kchen@iastate.edu>	*Multi-omics dissection of the maize seedling cuticle*

P22 **Kapeel Chougule** <kchougul@cshl.edu>
One-stop pangenome browser for exploring the rich genetic diversity in maize

P23 **Jose Valdes Franco** <jav246@cornell.edu>
Pangenome-guided super-contiging and scaffolding of long-read assemblies of diverse CIMMYT maize lines

P24 **Hank Bass** <bass@bio.fsu.edu>
Project update: Nuclease profiling in maize - MNase sensitivity and frenter profiles for B73v5.

P25 **Andrew Olson** <olson@cshl.edu>
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P26 **Nancy Manchanda** <nancym@iastate.edu>
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P27 **Ethalinda Cannon** <Ethy.Cannon@usda.gov>
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P28 **Peter Bradbury** <pjb39@cornell.edu>
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P29 **Brandon Monier** <bm646@cornell.edu>
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P30 **Arnaud Ronceret** <ronceret@ibt.unam.mx>
Analysis of the interaction between proteins of the meiotic chromosome axis and AFD1 in Zea mays

P31 **Jing Zhang** <zhangjing@genetics.ac.cn>
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P32 **Hua Yang** <yanghu@missouri.edu>
Genetic mapping and mutagenesis analysis of the trans factor 1 required for the B chromosome non-disjunction in maize

P33 **Yishuang Sun** <sunys16@genetics.ac.cn>
Identification and characterization of ZWINT-1 as an important protein in the maize cell division process

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P34 **Chuck Dietrich** <charles.dietrich@bayer.com>
Bayer's SmartStax® PRO technology to launch in US in 2022 with industry first three modes of action for corn rootworm control, including a novel RNAi.

P35 **Britney Moss** <mossbl@whitman.edu>
Building an educational network focused on community curation of plant genomes

P36 **John Gray** <jgray5@uoft02.utoledo.edu>
Employing the maize TFome to Foster the Integration of Research with Education (F.I.R.E.)

P37 **Carson Andorf** <carson.andorf@usda.gov>
Maize genetics Committee on Outreach, Diversity, Inclusion, and Education (CODIE) 2020-2021 update

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P38 **Michael Tross** <mtross2@huskers.unl.edu>
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P39 **Eric Rodene** <eric.rodene@huskers.unl.edu>
A drone-based high-throughput phenotyping pipeline to estimate time-series nitrogen responses in maize

P40 **Melinda Yerka** <myerka@unr.edu>
A new sorghum MAGIC population for genome-to-phenome investigations of grain end use qualities

P41 **Nina Chumak** <Nina.chumak@botinst.uzh.ch>
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P42	**Paul-Louis Lopez-Marnet** <Paul-Louis.Lopez-Marnet@inrae.fr>	*An efficient method to segment pictures from FASGA stained stem cross section to highlight variation of histological profiles between maize genotypes.*
P43	**Chase Krug** <chkrug@iastate.edu>	*Analysis of plant height in Zea mays*
P44	**Baoxing Song** <bs674@cornell.edu>	*Constrained non-coding sequence provides insights into regulatory sequence and loss of gene expression in maize*
P45	**Aimee Schulz** <ajs692@cornell.edu>	*Cooperate or Compete: modeling competitive traits in maize*
P46	**Valerie Craig** <craigv@uoguelph.ca>	*Development of an index for remotely sensing physiological maturity in maize (Zea mays L.)*
P47	**Pragya Adhikari** <adpragya@illinois.edu>	*Differential regulation of maize and sorghum orthologs in response to the fungal pathogen Setosphaeria turcica*
P48	**Alper Adak** <alperadak@tamu.edu>	*Discovery of temporal loci controlling segregation of vegetation Indices through maize hybrid growth*
P49	**Sarah Odell** <sgodell@ucdavis.edu>	*Dissecting quantitative trait variation in a multi-parent maize population*
P50	**Norbert Bokros** <norbert.bokros@uky.edu>	*Diurnal fluctuations in turgor pressure impart negligible effects on sweet sorghum stalk strength*
P51	**Jessica Wedow** <jwedow@danforthcenter.org>	*Elemental profiling in maize to understand genotype-by-environment interactions*
P52	**Merritt Khaipho-Burch** <mbb262@cornell.edu>	*Elucidating the extent of pleiotropy in maize and its functional relevance towards trait prediction*
P53	**Silvio Salvi** <silvio.salvi@unibo.it>	*Gaspé Flint 1.1.1, a small-size early-flowering maize inbred line*
P54	**Zhongjie Ji** <jizhongj@msu.edu>	*Genetic analysis of field measured data from Genomes to Fields in Michigan*
P55	**Allen Oppong** <alnopp@yahoo.co.uk>	*Genetic analysis of new maize hybrids for yield and resistance to aflatoxin accumulation*
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P57	**Blake Trygestad** <trygesta@msu.edu>	*Genetic resistance to tar spot (Phyllachora maydis) in maize*
P58	**Maxime Laurent** <maxime.laurent@uclouvain.be>	*Genetic variability of the expression of plasma membrane aquaporins in maize leaves: from eQTLs to characterization of cis- and trans-acting regulatory factors*
P59	**Jonas Rodriguez** <jrodriguez36@wisc.edu>	*Genome-wide association reveals candidate genes for saccharification efficiency and stalk anatomical features in maize*
P60	**Jinlong Li** <jli89@unl.edu>	*Genomic selection to optimize doubled haploid-based hybrid breeding in maize*
P61	**Qiuyue Chen** <qchen295@wisc.edu>	*Harnessing knowledge from maize and rice domestication for new crop breeding*
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P65	**Jialu Wei** <jlwei@iastate.edu>	*How does maize respond to heat stress: An old question with an expanded study scope*
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P68	**Namrata Maharjan** <namrata.maharjan@sdstate.edu>	*Identification of loci influencing Teosinte crossing barrier1 (Tcb1) efficacy in maize by Quantitative Trail Loci (QTL) mapping and Genome-Wide Association Study (GWAS)*
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P70	**Robert Shrote** <shrotero@msu.edu>	*Improving genomic selection through the addition of a competing diversity objective*
P71	**Ruijuan Tan** <tanruij1@msu.edu>	*Integrating GWAS results to increase predictive ability for ear leaf area in Maize*
P72	**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*
P73	**Rafael Della Coletta** <della028@umn.edu>	*Leveraging structural variant information in GxE genomic prediction models*
P74	**Ashmita Khanal** <akhanal2@illinois.edu>	*Mapping resistance to an Illinois isolate of anthracnose leaf blight in sorghum*
P75	**Alexander Mullens** <mullens3@illinois.edu>	*Mechanisms of resistance to bacterial pathogens in maize*
P76	**Ravi Mural** <rmural2@unl.edu>	*Meta-analysis identifies pleiotropic loci controlling phenotypic trade-offs in sorghum and maize*
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P79	**Sarah Lipps** <slipps@illinois.edu>	*Novel loci for leaf blight resistance in sorghum aids in understanding of E. turcicum pathosystem*
P80	**Robert Twohey III** <twohey2@illinois.edu>	*Optimizing Leaf Morphology in Zea mays Using a QTL Mapping Approach to Identify the Genetic Regulators of Specific Leaf Area*
P81	**Travis Rooney** <ter56@cornell.edu>	*Perennial relatives of maize may contain the key to effective nutrient recycling in maize production*
P82	**Sanzhen Liu** <liu3zhen@ksu.edu>	*Phenotypic association and prediction through integrative K-mer analysis*
P83	**Hongyu Jin** <hjin5@huskers.unl.edu>	*Pollen-sequencing: A rapid and cost-effective method to construct genetic map using sequencing data from the hybrid pollens*
P84	**Yacine Djabali** <yacine.djabali@inrae.fr>	*Presence/absence variations and SNPs equally contribute to the variations of protein and metabolite abundance.*
P85	**Sneha Adhikari** <sneha.adhikari@icar.gov.in>	*Revealing the genetic diversity in teosinte introgressed maize population by morphometric traits and microsatellite markers*
P86	**Mackenzie Zwiener** <mzwiener3@huskers.unl.edu>	*The effects of nitrogen stress on leaf angle in sorghum and maize*
P87	**Guangchao Sun** <s.guangchao@gmail.com>	*Unravel the roles of genetic regulation of transcriptional variation in temperate adaptation in maize*

P88 **Dorothy Sweet** <kirsc168@umn.edu> — *Variability in maize growth rates and utility in predicting end of season performance*

P89 **Samantha Snodgrass** <snodgras@iastate.edu> — *Variance component analysis of MOA-seq identified transcription factor binding sites for 143 maize traits*

P90 **Xuan Zhang** <zhangxuancau@cau.edu.cn> — *WD40 repeat-protein under parallel domestication enhances grain yield in maize and rice*

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P92 **Mowei Li** <li.10425@buckeyemail.osu.edu> — *Cytosine methylation profiles distinguish different regulatory states of a paramutable purple plant 1 allele*

P93 **Michelle Stitzer** <mcstitzer@cornell.edu> — *Elevated transposable element copy number is associated with reduced fitness in maize*

P94 **Qian Liu** <liuqian376@genetics.ac.cn> — *Epigenetic adaptation of maize centromere in oat-maize addition lines*

P95 **Yan Win** <yann@uni-bonn.de> — *Expanding BonnMu - The transposon-induced mutant resource in the European flint maize line F7*

P96 **Kaitlin Higgins** <km.higgins26@gmail.com> — *Exploring genetic and epigenetic contributions to imprinting in maize*

P97 **Yang Liu** <yangliu@genetics.ac.cn> — *Functional characterization of R-loops in maize centromere*

P98 **Jinge Tian** <jtian69@wisc.edu> — *Genetic regulation of sexual conversion of the terminal lateral inflorescence in maize during domestication*

P99 **William Clore IV** <whclore@iastate.edu> — *Identifying putative LTR retrotransposons in maize insertions using LTR Predictor*

P100 **Yibing Zeng** <yz77862@uga.edu> — *Insights into gene methylation through the NAM founder methylomes*

P101 **Julie Dazeniere** <jd493@sussex.ac.uk> — *Patterns of natural selection in a plant transposable element*

P102 **Natalie Deans** <deans.11@osu.edu> — *Pl1-Rhoades paramutation is associated with molecular changes at downstream tandem repeats*

P103 **Wei Guo** <guo342@purdue.edu> — *Rapid, heat-induced transgenerational reactivation of a silenced transposable element in maize*

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P105 **Elias Primetis** <eliasprimetis93@gmail.com> — *The diversity and evolution of the cis-regulatory region of LTR retrotransposons in maize*

P106 **Shujun Ou** <oushujun@iastate.edu> — *The evolution of 26 diverse maize genomes driven by transposable elements*

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P108 **Benjamin Oakes** <oakes.105@osu.edu> — *The role of RNA polymerase IV in effecting heritable regulatory changes*

P109 **Sharu Paul Sharma** <sharu@iastate.edu> — *Transposon-induced rearrangements activate gene expression in maize*

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P123 **Swaraj Thaman** <thamans@whitman.edu> — *Confirmation of alternative splicing for the auxin repressor gene ZmIAA1 in Zea mays*

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P129 **Lina Castano-Duque** <Lina.Castano.Duque@usda.gov> — *Flavonoids play a role in resistance to accumulation of aflatoxin in corn*

P130 **Ran Tian** <rtian@ttu.edu> — *Genome-wide dissection of the regulation of epicuticular wax in sorghum: the first defense line of plants against environmental threats*

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P135 **Sarah Hamade** <shamade@oakland.edu>
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P136 **virginia protto** <virginiaprot@gmail.com>
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P137 **Jacob Olson** <olson169@purdue.edu>
Major determinants of above ground architecture and flowering time affect brace root node number in sorghum

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P139 **Usha Bhatta** <ukb45206@uga.edu>
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P140 **Sophia Bigio** <bigiosd@whitman.edu>
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P144 **Jae-Hyung Lee** <jalee@cshl.edu>
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P152 **Shailesh Karre Satyanarayana Guptha** <skarres@ncsu.edu>
Two maize E3 ubiquitin ligases control hyper-sensitive response in maize

P153 **Manwinder Singh Brar** <mbrar@g.clemson.edu>
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P154 **Ryan Benke** <rbenke@purdue.edu>
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P155 **Jennifer Arp** <jarp@danforthcenter.org>
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P157 **Liangwei Yin** <yinl8@miamioh.edu>
Chromatin types and epigenomic features shape the differentiation of the maize subgenomes

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Knob diversity and conservation in the maize pangenome

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Maize domestication is a stable process during evolution

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Megabase-scale structure variation with Tripsacum origin contributes to maize adapation

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P165 **Maud Tenaillon** <maud.tenaillon@inrae.fr>
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Two teosintes made modern maize

Cell and Developmental Biology

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A genetic and molecular approach to identify transcription factors control maize root adaptive response to water deficit

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A maize male gametophyte-specific gene encodes ZmLARP6c1, a potential RNA-binding protein required for competitive pollen tube growth

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Analysis of aberrant TANGLED1 localization in the preprophase band defective maize mutant discordia1

P171 **Allison Phillips** <allison.phillips@wlc.edu>
Analysis of stunter2 and stunter3, maize maternal effect mutants with reduced kernel size

P172 **Subbaiah Chalivendra** <schalivendra@hotmail.com>
Aspergillus flavus SrbA is required for conidial germination and survival under hypoxia, and colonization of intact maize seeds

P173 **Jie Tang** <jietang@iastate.edu>
Autophagy is upregulated in response to high temperatures in maize

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*Characterization of ramosa suppressor locus*12.2995, a novel allele of opaque1 that regulates plant architecture in maize*

P175 **Emily Wear** <emily_wear@ncsu.edu>
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P176 **Zhaoxia Li** <zhaoxial@iastate.edu>
Daily temperature cycles promote alternative splicing of RNAs encoding SR45a, a splicing regulator in maize

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P193	**N/A**	*This abstract has been pulled from the program at the author's request.*
P194	**Xiaosa Xu** <xxu@cshl.edu>	*New insights into CLAVATA-WUSCHEL signaling in the maize inflorescence meristem using single-cell RNA sequencing (scRNA-seq)*
P195	**Lei Liu** <lliu@cshl.edu>	*Patterns of transcriptional response to overproliferating inflorescence meristems in fasciated ear mutants*
P196	**Hilde Nelissen** <hilde.nelissen@psb.vib-ugent.be>	*Spatial transcriptomics of the maize SAM*
P197	**Arif Ashraf** <mohammadarif@umass.edu>	*Tale of the nucleus: A protein in the nuclear membrane is required for correct division plane orientation*
P198	**Janlo Robil** <jmrp76@mail.missouri.edu>	*Temporal regulation of cell division and expansion by auxin and gibberellins underlies medio-lateral growth and vein proliferation in maize leaf*
P199	**Amber de Neve** <adeneve@umass.edu>	*The development and evolution of unisexual floral specification in Poaceae*
P200	**Yan Zhou** <yzhou86@iastate.edu>	*The genetics of leaf orientation and its impact on the interception of solar radiation*

P201 **Diana Ruggiero** <ruggiedi@oregonstate.edu>
The secret lives of veins: single cell genomics and quantitative genetics of maize leaf vascular development

P202 **Lise Pingault** <lise.pingault@unl.edu>
Transcriptomic signatures associated with maize defense against corn leaf aphid.

P203 **Ashley Hostetler** <ahende@udel.edu>
Utilizing high-throughput brace root phenotyping data to predict lodging susceptibility across 53 maize genotypes

P204 **Qiong Nan** <qnan@umass.edu>
WPRs acting as a downstream of PAN2 receptor is involved in the polarity establishment in maize stomata

P205 **Samuel Leiboff** <leiboffs@oregonstate.edu>
Wavy Auricle in Blade2 (Wab2) is a semidominant ectopic auricle mutant suppressed by one copy of wavy auricle in blade1 (wab1) loss-of-function

Late Poster Submissions

P206 **Songtao Gui** <songtaogui@sina.com>
ZEAMAP, a comprehensive database adapted to the maize multi-omics era

P207 **Rachel Baschieri** <baschier@oregonstate.edu>
Comparing cytokinin oxidase/dehydrogenase gene expression in sorghum inflorescence and maize tassel development

P208 **Xinxin Ding** <xding4@wisc.edu>
Microautophagy of storage proteins in maize aleurone cells

P209 **Carolyn Rasmussen** <crasmu@ucr.edu>
Cell cortex microtubules contribute to division plane positioning during telophase in maize

P210 **Yan Zhou** <yzhou86@iastate.edu>
Identification of genetic determinants of trait measurement errors in image-based, high-throughput phenotyping

Plenary Speaker Abstracts

Plenary Speaker 1 (@HochholdingerF) **Monday, March 8 11:00 AM CST**

Genetic dissection of maize root development

(presented by Frank Hochholdinger <hochholdinger@uni-bonn.de>)
Full Author List: Hochholdinger, Frank[1]
[1] Crop Functional Genomics, Institute for Crop Science and Resource Conservation, University of Bonn, 53113 Bonn, Germany

To exploit limited soil resources and to respond to alterations of the biotic and abiotic environment, plant root systems continuously adjust their architecture and their physiological, biochemical and metabolic performance. This enormous developmental plasticity is facilitated by the fact that plant root systems are not fully predetermined during embryo development. In maize, the root system consists of several structurally and functionally distinct root types. Most of these roots are formed during postembryonic development, i.e. after germination.

We have identified a number of monogenic mutants that are affected in the formation of different root types and root hairs. Molecular cloning of these genes demonstrated that key elements of auxin signal transduction such as LBD and Aux/IAA proteins are instrumental for shoot-borne, seminal and lateral root initiation. Moreover, genes related to exocytotic vesicle docking, cell wall loosening as well as cellulose synthesis and organization contribute to root hair elongation. The identification of interaction partners and downstream targets of these proteins together with cell-type specific transcriptome analyses provided novel insights into the regulatory networks controlling maize root development and architecture. More recently, we have extended our genetic analyses of root system development to other cereal species. In this context we cloned a gene that controls the root angle in barley and wheat. The corresponding mutant exhibits a steeper root growth of seminal and lateral roots.

Other aspects of our genetic analysis of maize root development include the transcriptomic analysis of heterosis manifestation in seedling roots. In this work, we demonstrated that gene expression complementation is frequently observed in maize hybrids and that hybrids express more genes than their parental inbred lines. Finally, we have established a novel reverse genetics sequence-indexed resource for maize genetics called BonnMu which currently contains mutations in ca. 60% of maize genes.

Plenary Speaker 2 **Tuesday, March 9 7:00 PM CST**

A quantitative genomics study on rice heterosis

(presented by Bin Han <bhan@ncgr.ac.cn>)
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[1] Chinese Academy of Sciences

Exploitation of heterosis in crop has achieved remarkable yield advantages over traditional inbred line breeding. So far, hybrid breeding that combines elite alleles from both parental lines to generate a better F1 variety is still one of the fastest and most efficient breeding approaches in rice and many of other crops. However, the genetic mechanisms of heterosis are only partly understood, and a global view of heterosis from a representative number of hybrid combinations is lacking. We have developed an integrated genomic and forward genetic approach to construct a genome map for elite hybrid rice varieties and their inbred parental lines. We identified that the accumulation of numerous rare superior alleles with positive dominance is an important contributor to the heterotic phenomena. The large data of genomics and phenomics from the well-designed populations enabled us to identify the genetic contributors and find out the exact causes of heterosis using “a composite interval-mapping method”. For the individual yield components, the heterozygous state of the heterosis-related genes generally acted through the way of dominance complementation. These results inform on the genomic architecture of heterosis for yield traits in rice, which will be useful information for crop improvement program.

Since cultivated rice is a self-pollinating plant or an inbreeding crop, the rice male sterility varieties, which are unable to produce functional pollen grains and are used as female parents to avoid self-pollination, are prerequisite for efficiently generating hybrid rice varieties to produce large quantities of hybrid seeds. However, we know little about maternally inherited cytoplasmic genome variations for rice hybrids. In our study, we also analyzed the cytoplasmic genomes of a collection of 1495 elite hybrid rice varieties, and revealed major types of hybrid cytoplasm that were adopted in different hybrid production strategies. We found that the appliance of cytoplasmic male sterility associated gene WA352c, a new type of mitochondrial genome from wild rice (O. rufipogon) population, was introduced to cultivated rice (O. sativa) population and broaden the organelle resource pool for rice hybrids. We therefore figured out the association between nucleus and cytoplasm for male sterile lines. This work demonstrated diversity of hybrid cytoplasm and association between organelle and nucleus helped us get better understanding of hybrid rice breeding.

Unlocking genetic diversity of Oryza species will provide insights into genomics of rice complex traits and rice breeding. We have implemented an integrated approach of genome-wide association study (GWAS) and phenomics with functional analysis to catch up agronomic trait genes or quantitative trait loci (QTLs) in a diverse cultivated rice population. This approach informs that the associated loci with the agronomic traits such as panicle length, grain sizes, grain weight and grain filling rate can be further characterized through expressional profiling, in-depth genome analysis, transgenic study, genome editing, and population genetic analysis. Allelic genetic variations responsible

for complex traits can be effectively explored. We have also constructed a pan-genome dataset of the O. sativa–O. rufipogon species complex through deep sequencing and de novo assembly of about 70 divergent accessions. Inter-genomic comparisons identified 23 million sequence variants in the rice genome. This catalog of sequence variations includes many known quantitative trait nucleotides and will be helpful in pinpointing new causal variants that underlie complex traits. In particular, we systemically investigated the whole set of coding genes using this pan-genome data, which revealed extensive presence and absence of variation among rice accessions. This pan-genome resource will further promote evolutionary and functional studies in rice. We also believe that the rich genetic diversity in Oryza sativa and Oryza rufipogon serves as the main sources in rice breeding.

Organogenesis in maize - lessons from mutants

(presented by Sarah Hake <hake@berkeley.edu>)
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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.

Plenary Speaker 4 (@gilesdoldroyd) **Thursday, March 11 9:00 AM CST**

Transforming agricultural sustainability through beneficial microbial associations

(presented by Giles Oldroyd <giles.oldroyd@slcu.cam.ac.uk>)
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[1] Russell R Geiger Professor of Crop Science, Crop Science Centre, Department of Plant Sciences, University of Cambridge, UK.

Plants associate with microorganisms in the rhizosphere, with potential for benefit or detriment. Facilitation of nutrient acquisition is the primary potential benefit for plants engaging with soil-based microorganisms: arbuscular mycorrhizal fungi expand the surface area for the capture of nitrates, phosphates and water, while nitrogen-fixing bacteria provide a novel source of nitrogen through conversion of atmospheric dinitrogen into ammonia. Arbuscular mycorrhizal associations have a very ancient evolutionary origin in the plant kingdom and as such are widespread across plant phylogeny, while the nitrogen-fixing association is a more recent adaptation, that is restricted to some angiosperms. These microbial associations have relatively limited application currently in agriculture: legume crops are inoculated with nitrogen-fixing bacteria, but mycorrhizal fungi have limited use in arable farming. Instead much of agriculture uses inorganic fertilizers as the primary means for crop nutrition. We are looking at the mechanisms required in crops to facilitate the broader use of beneficial microorganisms, as a means to drive greater sustainability and equity in agriculture, by augmenting crop nutrition without the need for extensive fertilization.

McClintock Prize Abstract

McClintock Prize (M1) **Wednesday, March 10 9:10 AM CST**

Barbara McClintock and the Genetic Foundation for Understanding Maize Domestication

(presented by: John Doebley <jdoebley@wisc.edu>)
Author list: Doebley, John[1]
[1] Genetics Department, University of Wisconsin-Madison

Barbara McClintock published the first genetic map of maize and inferred the presence of mobile elements in the maize genome from a combination of cytological and genetic observations. Her work laid the foundation for future genetic dissection of maize domestication with a prescience for one of the underlying genetic mechanisms. Here, I will review research from my laboratory group on maize domestication, highlighting its intersections with McClintock's discoveries. The domestication of maize from a wild Mexican grass called teosinte occurred about 9,000 years ago and resulted in dramatic changes in plant morphology. The genetic changes that underlie maize domestication have been investigated using quantitative trait locus (QTL) mapping, QTL cloning, and genome-wide scans for altered gene expression. QTL analyses suggest that some morphological traits are governed by relatively large numbers of genes (30 or more), but that other traits have relatively simple inheritance involving a few QTL. We have identified and characterized QTL with large effects on some domestication traits. First, *teosinte branched* (*tb1*) affects the difference between the long branches of teosinte versus the short branches of maize. Second, *teosinte glume architecture* (*tga1*) affects the formation of a casing that surrounds teosinte seeds that is lacking in maize. Third, *grassy tillers* (*gt1*) contributes to differences between having many small ears like teosinte or a few large ears like maize. While QTL studies enabled the identification and characterization of a few domestication genes for morphological traits, a genomic scan identified hundreds of genes that show evidence for differential expression between maize and teosinte, suggesting there is a broad array of genes controlling both known and unknown domestication traits. Much remains to be learned.

The Gene Balance Hypothesis: How 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

The inverse dosage effect was described in maize decades ago. A dosage series of the long arm of chromosome 1 produced 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 this effect on target genes. RNA-Seq experiments in maize and Drosophila indicate that global trans effects caused by additional chromosomes or segments 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 are also found that extend positively and negatively. RNA-Seq studies of monosomics show a trend toward global upregulation. Whole genome dosage changes (polyploidy) show many fewer modulations suggesting a stoichiometric or balance component to gene regulation. RNA-Seq on the five trisomies and three ploidies of Arabidopsis has been analyzed in collaboration with the Matzke lab. These experiments were conducted such that absolute amounts of gene expression were sought to produce the ratio distributions given the knowledge that trans-acting reductions are common. These studies in maize, Drosophila, and Arabidopsis indicate a connection between dosage compensation of the varied genes in aneuploids 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 from whole genome duplication or segmental duplication. The stoichiometric effects are postulated to explain this trend. The recognition of similar aneuploidy effects in monocots, dicots, fungi, insects, and mammals 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 I – GENOME BIOLOGY
Chair: Erin Sparks Monday, March 8. 9:30 AM – 10:50 AM CST

T1 @AllierAntoine

Genetic diversity management and introduction in maize breeding programs using genomic selection

(submitted by Antoine Allier <allierantoine@gmail.com>)
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There is an increasing awareness that crop breeding programs should balance short- and long-term objectives by maintaining genetic diversity to cope with future challenges. The advent of high density genotyping opened new avenues to further enhance the efficiency and sustainability of breeding programs. This involves the evaluation of genetic diversity in elite breeding pools, its efficient conversion into short- and long-term genetic gain and the efficient identification, improvement and introduction of extrinsic variability into breeding pools.
Selection of parental crosses that generate superior progeny while maintaining sufficient diversity is a key success factor at short- and long-term. We derived analytical solutions to predict the joint distribution of a quantitative trait and genetic diversity in the progeny of multiparental crosses (referred to as Usefulness Criterion Parental Contributions UCPC). We used UCPC to extend the Optimal Cross Selection (OCS) that aims at maximizing the performance in progeny while maintaining diversity. In a simulated maize inbred line breeding program, UCPC-OCS proved to be more efficient than OCS to convert the genetic diversity into short- and long-term genetic gains.
The narrow genetic base of an elite population might however compromise its long-term improvement. An efficient strategy to broaden its genetic base is therefore required. Many genetic resources are accessible to breeders but cannot all be considered. We compared different predictive criteria for selecting genetic resources that best complement elite recipients, based on genomewide marker effects estimated on a collaborative maize diversity panel. We also evaluated the interest of UCPC-OCS to improve genetic resources (pre-breeding), to bridge these with elite breeding (bridging), and to manage recurrent introductions into the breeding population. In a simulated maize inbred line breeding program, we demonstrated that recurrent introductions of genetic resources through a bridging population maximize long-term genetic gain while maintaining genetic diversity constant, with only limited short-term penalty.

Funding acknowledgement: ANRT-CIFRE, RAGT2n

T2 @abousios

Gene capture by transposable elements leads to epigenetic conflict in maize

(submitted by Alex Bousios <alexandros.bousios@gmail.com>)
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[4] School of Life Sciences, University of Sussex, Brighton, UK

Transposable elements (TEs) regularly capture fragments of genes. When the host silences these TEs, siRNAs homologous to the captured regions may also target the genes. This epigenetic crosstalk establishes an intragenomic conflict: silencing the TEs has the cost of silencing the genes. If genes are important, however, natural selection may maintain function by moderating the silencing response, which may also advantage the TEs. In this study, we examined this model by focusing on Helitrons, Pack-MULEs, and Sirevirus LTR retrotransposons in the maize genome. We documented 1263 TEs containing exon fragments from 1629 donor genes. Consistent with epigenetic conflict, donor genes mapped more siRNAs and were more methylated than genes with no evidence of capture. However, these patterns differed between syntelog versus translocated donor genes. Syntelogs appeared to maintain function, as measured by gene expression, consistent with moderation of silencing for functionally important genes. Epigenetic marks did not spread beyond their captured regions and 24nt crosstalk siRNAs were linked with CHH methylation. Translocated genes, in contrast, bore the signature of silencing. They were highly methylated and less expressed, but also overrepresented among donor genes and located away from chromosomal arms, which suggests a link between capture and gene movement. Splitting genes into potential functional categories based on evolutionary constraint supported the synteny-based findings. TE families captured genes in different ways, but the evidence for their advantage was generally less obvious; nevertheless, TEs with captured fragments were older, mapped fewer siRNAs, and were slightly less methylated than TEs without captured fragments. Collectively, our results argue that TE capture triggers an intragenomic conflict that may not affect the function of important genes but may lead to the pseudogenization of less-constrained genes.

Funding acknowledgement: National Science Foundation (NSF), The Royal Society, EMBO, HFSPO

T3

Annotating and characterizing the orphan genes of Zea mays B73 and NAM lines

(submitted by Jing Li <jingli@iastate.edu>)
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Thousands of unannotated transcripts in eukaryotic genomes have significant transcriptomic, Ribo-Seq, and proteomic evidence. Many of the pervasively-expressed transcripts have the potential to encode proteins, without a homolog in any other species- "orphan genes". Because orphan genes lack sequence similarity to genes of other species and canonical sequence motifs have not yet developed, identifying orphan genes and predicting their function is a challenge[1]. Of the orphan genes functionally characterized to date, most enable organisms to survive abiotic and biotic stresses. Here, we identify orphan genes and other young genes in the maize B73 and 25 NAM founder lines. Gene models are predicted by a novel pipeline, BIND[1], which combines traditional *ab initio* prediction by BRAKER with direct inference from RNA-Seq alignments. We collected from NCBI-SRA 1,460 samples of high-quality RNA-Seq representing multiple tissues and diverse conditions for B73, and 20 tissue samples from each of the NAM founder lines. The BIND pipeline predicted ~34,136 maize-specific genes (including ~867 line-specific genes) per genome. 1092 *Zea mays*-specific genes (including 82 B73-specific genes) had a translation signal, based on 75 samples of Ribo-Seq data. We classify the relationship among orphan genes in each NAM line and outgroup species by a synteny-based approach, fagin[2] to determine the evolutionary origin of line-specific genes. Finally, we define key structural and expression characteristic of the orphan genes from each maize lines. The BIND annotation pipeline can be applied to data from other maize lines, or other eukaryotic species; we have made it available in a flexible, modifiable, and reproducible manner via the python program, pyrpipe[3]. This configuration was embedded in the workflow manager, Snakemake, for ease of high throughput data management.**1** Li *et al. bioRxiv,* 2021 | **2** Arendsee *et al. BMC bioinformatics*, 2019 | **3** Singh *et al. bioRxiv*, 2020

Funding acknowledgement: National Science Foundation (NSF)

T4

De novo assembly, annotation, and comparative analysis of 26 diverse maize genomes

(submitted by Kelly Dawe <kdawe@uga.edu>)
Full Author List: Dawe, R Kelly[1]; Consortium, NAM sequencing[2]
[1] University of Georgia
[2] Multiple

We recently reported de novo genome assemblies, transcriptomes, annotations, and methylomes for the 26 inbreds that serve as the founders for the maize nested association mapping population. The data indicate that the number of pan-genes exceeds 103,000 and that the ancient tetraploid character of maize continues to degrade by fractionation to the present day. Excellent contiguity over repeat arrays and complete annotation of centromeres further revealed the locations and internal structures of major cytological landmarks. We demonstrated that combining structural variation with SNPs can improve the power of quantitative mapping studies. Finally, we documented variation at the level of DNA methylation, and demonstrated that unmethylated regions are enriched for cis-regulatory elements that overlap QTL and contribute to changes in gene expression. I will highlight the main outcomes and provide perspectives on how the data can be used.

Funding acknowledgement: National Science Foundation (NSF)

T5

Identifying environmental and genetic factors underlying phenotype in the maize NAM population

(submitted by Laura Tibbs Cortes <ltibbs@iastate.edu>)
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[1] Department of Agronomy, Iowa State University, Ames, IA USA 50011

Maize phenotypes are determined by the complex interplay of genetics and environmental factors including day length, temperature, and precipitation. Identifying an environmental index consisting of an environmental parameter and critical window that strongly influence a given phenotype can help to dissect this complexity, enabling accurate within-season predictions in new environments. When combined with genomic prediction, these accurate predictions are possible even for previously unobserved genotypes. In this project, the CERIS-JGRA package was used to identify environmental indices for 19 traits measured in the Maize NAM population in 11 environments. These indices, along with genomic prediction, were used to accurately predict phenotypes even in unobserved genotypes in new environments. In most cases, environmental indices identified from each training set were remarkably consistent, reinforcing the conclusion that these environmental indices are biologically relevant. Rather than considering only environmental indices depending on temperature and day length, such as photothermal time or growing degree days, this analysis also included indices measuring precipitation and evapotranspiration. These additional indices were found to be better predictors of important traits including plant height and anthesis-silking interval, emphasizing the important effect of drought stress on these traits. Finally, the unique structure of the NAM population permits both GWAS and QTL mapping to identify loci responsible for the plastic response to these environmental indices. This approach enables biologically-informed dissection of phenotypic plasticity from both genetic and environmental perspectives.

Funding acknowledgement: National Science Foundation (NSF)

T6

Diversity of moisture regulated root branching uncovered in maize

(submitted by Johannes Scharwies <joscha@stanford.edu>)
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Moisture regulated root branching, called hydropatterning, has been observed in maize, rice, and Arabidopsis. Plants that exhibit hydropatterning are able to detect spatial differences in water distribution around their root growth zone, which leads to pre-patterning of lateral root primordia towards regions of higher water availability. Hydropatterning may explain some of the developmental plasticity that allows plants to adapt to changing environments. While SUMOylation of the transcription factor ARF7 was found to influence hydropatterning in Arabidopsis, genetic factors involved in hydropatterning in maize remain unknown. Furthermore, variation in hydropatterning amongst populations of plants has not been studied. Here, we characterize the hydropatterning responses of a diverse population of 250 maize inbred lines using a novel high-throughput phenotyping platform. Differences in lateral root branching were quantified for roots grown along moist germination paper, while the other side of the root was exposed to air. Genotypes that showed preferential branching of lateral roots towards the moist germination paper were classified as strong hydropatterning. We observed that root branching in this population was skewed towards strong hydropatterning. The highest variance in hydropatterning was observed in inbred lines of the non-stiff stalk subpopulation. Interestingly, the propensity for root branching on the air-exposed side showed the best correlations to in-field root architecture and above-ground traits compared to branching density on the moisture exposed side. SNP-based GWAS identified eight hydropatterning-associated SNPs and transcriptome-based GWAS identified 11 hydropatterning-associated genes, some of which had previously been identified as being differentially regulated across the root growth zone during hydropatterning. Future investigation of these loci will illuminate the molecular mechanisms controlling moisture-regulated root branching and the role of these loci as potential targets for breeding more water-use efficient crops.

Funding acknowledgement: Advanced Research Projects Agency-Energy (ARPA-E)

T7

Genetic architecture of nonphotochemical quenching (NPQ) kintetics in maize

(submitted by Marcin Grzybowski <mgrzybowski2@unl.edu>)
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Light is primary source of energy for plants, however excess light energy is dangerous to plants, as it can produce reactive oxygen species, and damage the photosynthetic apparatus. To prevent this damage, plants evolved mechanisms to harmlessly dissipate damaging excess light energy as heat. These mechanisms are collectively referred to as called nonphotochemical quenching (NPQ). The activity of NPQ changes dynamically throughout the day in response to changes in light levels. Despite this, it has been estimated that lag in the adjustment of NPQ to changing light levels reduces potential photosynthetic productivity by ~20% under filed conditions. Genetic engineering to accelerate NPQ increased dry matter production by ~ 15% in tobacco. Here we sought to evaluate the extent of naturally occurring variation in the kinetics of NPQ within maize and the potential of this trait as a target for crop improvement. The kinetics of NPQ were measured across maize association panel (n=752). NPQ curves are complex traits and we employed both data- and expert-driven approaches to generate sets of traits describing how the curves could vary with respect to each other. Both approaches produced multiple heritable traits with the mean proportion of variation explainable by genetic factors being 0.63. Genome wide association studies conducted with these traits identified 15 candidate genes, including one gene, *photosystem II subunit S* (*PSBS*), previously employed to engineer accelerated NPQ in tobacco. The NPQ peak associated with *PsbS* in maize is also a cis-eQTL for expression of the same gene. The favorable allele is associated with statistically significant increase in grain yield in the same population. Substantial naturally occurring variation in NPQ exists within current maize germplasm which gives an opportunity for sustainably achieving increase in yield of maize and other C4 crops.

Gene / Gene Models described: *PsbS*; Zm00001d042697
Funding acknowledgement: National Science Foundation (NSF), Foundation for Food & Agriculture Research (FFAR)

T8 @BerondaM

Planting Equity – Lessons From Plants on Cultivating Equitable Ecosystems

(submitted by Beronda Montgomery <montg133@msu.edu>)
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Humans engage with plants with an expectation that these organisms have the capacity to grow and thrive. Plants are extremely sensitive to external environmental conditions and adapt their growth and behavior to dynamic cues, which results in optimized survival and productivity. The specific ways in which humans engage with the plants growing in their environment offer many lessons about mentoring, professional development interventions and impactful leadership, as well as a need for promoting ecosystems-based awareness, tending, and cultivation to promote the success of individuals therein. Plant-based lessons that inform effective mentoring and leadership practices and promote sense-driven success of students and colleagues in academic environments are many. Montgomery will discuss specific plant biology-inspired practices for supporting the comprehensive development of a diverse range of students, academic staff, and faculty members as researchers, scholarly thinkers, and independent practitioners. Growth-based perspectives derived from the relationships that humans regularly exhibit with plants indicate vast potential for our capacity for progressive support of diverse individuals in the academy—powerful lessons for planting and cultivating equity.

T9 @rellanalvarez

Teosinte introgression modulates phosphatidylcholine levels and induces early maize flowering time

(submitted by Rubén Rellán-Álvarez <rrellan@ncsu.edu>)
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After domestication from lowland teosinte parviglumis (Zea mays ssp.parviglumis) in the warm Mexican southwest, maize (Zea mays ssp. mays) colonized the highlands of México and South America. In the highlands, maize was exposed to lower temperatures that imposed strong selection on flowering time. Previous work in maize and other has linked variation in phospholipid metabolism to low temperature stress as well as changes in flowering time. Here, we combined linkage mapping analysis with genome scans to identify High PhosphatidylCholine 1 (HPC1), a gene which encodes a phospholipase A1 enzyme, as a major driver of phospholipid variation in highland maize. Common garden experiments demonstrated strong genotype-by-environment interactions associated with variation at HPC1, with the highland HPC1 allele leading to higher fitness in highlands, possibly by hastening flowering. The highland maize HPC1 variant results in impaired function of the encoded protein due to a polymorphism in a highly conserved sequence. A meta-analysis indicated a strong association between the identity of the amino acid at this position in a prokaryotic protein harboring this conserved sequence and optimal growth temperature of the organism. Mutagenesis of HPC1 via genome editing validated its role in regulating phospholipid metabolism. Finally, we showed that the highland HPC1 allele entered cultivated maize by introgression from the wild highland teosinte Zea mays ssp. mexicana and has been maintained in maize breeding lines from Northern US, Canada and Europe.
Thus, HPC1 introgressed from teosinte mexicana underlies a large metabolic QTL that modulates phosphatidylcholine levels and has an adaptive effect at least in part via induction of early flowering time

Gene / Gene Models described: ; Zm00001d039542, Zm00001d017584
Funding acknowledgement: National Science Foundation (NSF), Conacyt, UC-Mexus

SESSION IV – REGULATING GENES AND GENOMES
Chair: Maud Tenaillon Tuesday, March 9. 9:10 AM – 10:50 AM CST

T10

FIND-CIS: High-resolution mapping of functional cis-elements in the maize drought response

(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. Here, we identified, quantified and validated allele-specific (AS) functional variants by F1-mediated identification of non-coding *cis*-element-impacting SNPs (FIND-CIS). FIND-CIS combines an 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 150-250k ~60 bp interaction footprints (FPs) per hybrid. FPs correlated with TF targets identified via ChIP-Seq, putative enhancer sites, and were often centered on a known TF motif. Within these FPs, we identified ~20-30k SNPs per F1 which showed a significant allelic bias (AFPs). AFPs and AS mRNA abundance 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

T11 @MASanclemente

Sugar modulation of anaerobic-response networks in maize root tips

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Sugar supply is a key component of hypoxia tolerance and acclimation in plants. However, a striking gap remains in our understanding of mechanisms governing sugar impacts on low-oxygen responses. Here, we used a maize (*Zea mays*) root-tip system for precise control of sugar and oxygen levels. We compared responses to oxygen (21 and 0.2%) in the presence of abundant versus limited glucose supplies (2.0 and 0.2%). Low-oxygen reconfigured the transcriptome with glucose deprivation enhancing the speed and magnitude of gene induction for core anaerobic proteins (ANPs). Sugar supply also altered profiles of hypoxia-responsive genes carrying G4 motifs (sources of regulatory quadruplex structures), revealing a fast, sugar-independent class followed more slowly by feast-or-famine-regulated G4 genes. Metabolite analysis showed that endogenous sugar levels were maintained by exogenous glucose under aerobic conditions and demonstrated a prominent capacity for sucrose re-synthesis that was undetectable under hypoxia. Glucose abundance had distinctive impacts on co-expression networks associated with ANPs, altering network partners and aiding persistence of interacting networks under prolonged hypoxia. Among the ANP networks, two highly interconnected clusters of genes formed around *Pyruvate decarboxylase 3* and *Glyceraldehyde-3-phosphate dehydrogenase 4*. Genes in these clusters shared a small set of *cis*-regulatory elements, two of which typified glucose induction. Collective results demonstrate specific, previously unrecognized roles of sugars in low-oxygen responses, extending from accelerated onset of initial adaptive phases by starvation stress to maintenance and modulation of co-expression relationships by carbohydrate availability.

Gene / Gene Models described: *Pdc3, Gpc4*; Zm00001d028759, Zm00001d017121
Funding acknowledgement: National Science Foundation (NSF)

T12

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

(submitted by Zhaobin Dong <zbdong@cau.edu.cn>)
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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 pathways in which *tsh4* functions, an antibody was developed and used for ChIP-seq to identify downstream target genes. This data was compared to DAP-seq data for the duplicated SBP-box factors UNBRANCHED2/3 that function redundantly with *tsh4*. From 1894 bound peaks isolated from ear chromatin, 212 corresponded to genes that are differentially expressed in *tsh4* bracts. Several of these genes are known to play roles in bract suppression, including the GATA transcription factor *tasselsheath1*. Surprisingly, we discovered that several microRNA genes are directly bound by TSH4 and UB2/3, including those that negatively regulate their transcripts such as *miR156* and *miR529*. Moreover, the levels of these two microRNAs are greatly increased in *tsh4* and higher order combinations with *ub2/3*, indicating that they are also transcriptionally repressed by *SBP-box* genes. Simultaneous *in situ* localization of these microRNAs together with TSH4 protein indicates that the microRNAs occupy axillary bud positions, while TSH4 occupies 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 in *Drosophila*. Finally, auxin transport and signaling genes were identified as TSH4 targets, and auxin flow was found to be enhanced in *tsh4* and *tsh4/ub2/ub3* based on simultaneous visualization of the PIN1 and DR5 reporters. We present a model of floral phytomer formation in which *tsh4* represses auxin gradients to allow the establishment of meristem versus leaf domains via microRNA mediated mutual repression.

Funding acknowledgement: National Science Foundation (NSF)

T13 @mfagny

Identifying key tissue-specific, biological processes by integrating enhancer information in maize gene regulatory networks

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Enhancers are key regulators of the spatio-temporal expression of genes in eukaryotes, in particular during development. Their regulatory effect is mediated by the binding of transcription factors, which interact with target gene promoters through 3D-loops over distances reaching several dozens of megabases in some species. Groups of enhancers characterized by similar transcription factor binding sites (TFBSs) have been shown to shape complex regulatory networks, which control tissue-specific expression of genes involved in particular biological functions. While enhancers have been identified as key players in the wiring of developmental gene regulatory networks in mammals, this question remains largely unexplored in plants. Transposable Elements (TEs) of various superfamilies have been proposed as a source of new regulatory elements in plants and animals, but whether TEs contribute to the emergence of tissue-specific gene regulatory networks in plants remains to be fully elucidated.
Here, we investigate the enhancer-driven regulatory network of two maize tissues at different stages: leaves at seedling stage (V2-IST) and husks (bracts) at flowering. By combining TFBS annotation of enhancers previously detected in these two tissues (Oka et al., 2017) with mRNA-seq data using a systems biology approach, we model the regulatory relationships between TFs and their potential target genes, and identify regulatory modules specific to husk and V2-IST. We show that V2-IST leaves are characterized by the response to hormones and macromolecule biogenesis and assembly, while husks are characterized by cell wall modification and response to abiotic stresses. Analysis of enhancers sequence reveals that two different TE families have shaped part of the regulatory network in the two tissues, TIR transposon Mutator in V2-IST, and MITE Pif/Harbinger in husk, and that MITEs have provided potential new TFBSs involved in husk tissue-specificity.

Funding acknowledgement: French National Agency for Research - Agence Nationale pour la Recherche (ANR)

T14

Structural variation at the maize *Wuschel1* locus alters stem cell organization in inflorescences

(submitted by Zongliang Chen <zc176@waksman.rutgers.edu>)
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Structural variation in plant genomes is a significant driver of phenotypic variability in traits important for the domestication and productivity of crop species. Among these are traits that depend on functional meristems, stable populations of stem cells that are maintained by the CLAVATA-WUSCHEL (CLV-WUS) pathway, a negative feedback-loop that controls the expression of the *WUS* homeobox transcription factor gene, an essential regulator of meristem size and a key morphogenetic factor. While the CLV-WUS pathway is ultimately responsible for the post-embryonic development and productivity of crop species, WUS function and impact on maize development and yield remain largely unexplored. Here we show that the maize dominant *Barren inflorescence3* (*Bif3*) mutant harbors a tandem duplicated copy of the *ZmWUS1* gene, *ZmWUS1-B*, whose novel promoter enhances transcription in a ring-like pattern. Overexpression of *ZmWUS1-B* is due to multimerized binding sites for type-B RESPONSE REGULATORs (RRs), key transcription factors in cytokinin signaling. Hypersensitivity to cytokinin causes stem cell overproliferation and major rearrangements of *Bif3* inflorescence meristems, accompanied by mis-regulation of key components of the CLV-WUS as well as of different hormonal pathways, lead to the formation of ball-shaped ears and severely affect maize productivity. These findings establish *ZmWUS1* as an essential meristem size regulator in maize and highlight the striking effect of cis-regulatory variation on a key developmental program.

Funding acknowledgement: National Science Foundation (NSF)

T15 @Yan_Geneticist

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

(submitted by Yan Zhou <yzhou86@iastate.edu>)
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The accuracy of trait measurements greatly affects the quality of genetic analyses. In automated phenotyping pipelines, phenotyping errors, the differences between automated measurements and ground truth measurements, 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 these errors 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. Further, identification of TASs from GWASs conducted on the differences between the two values, indicates that a fraction of measurement error is under genetic control. Therefore, our study suggests that phenotyping errors cannot always be controlled simply by increasing population size and/or replication number.

Funding acknowledgement: National Science Foundation (NSF)

T16 @sagnik1504

Constructing *Zea mays* genes from RNA-Seq expression data using FINDER - a fully automated gene annotator

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Gene annotation in eukaryotes is a non-trivial task that requires meticulous analysis of expression data. The presence of transposable elements and sequence repeats in eukaryotic genomes adds to this complexity, as does overlapping genes and genes that produce numerous transcripts. Currently available software annotate genomes by relying on full-length cDNA or on a database of splice junctions which makes them susceptible to the errors in the input. We present FINDER, which automates downloading of expression data from NCBI, optimizes read alignment, assembles transcript and performs gene prediction. FINDER is optimized to map reads with different settings to capture all biologically relevant alignments with special attention to micro-exons (exon length less than 51 nucleotides). We configured FINDER to apply statistical changepoint detection to read coverage data which led to the discovery of overlapping genes on the same strand and accurately redefine the boundaries of some overlapping genes on opposite strands. FINDER further reports transcripts and recognizes genes that are expressed under specific conditions. FINDER integrates prediction results from BRAKER2 with assemblies constructed from expression data to approach the goal of exhaustive genome annotation. FINDER accurately reconstructed 22,198 and 25,156 transcripts in *Arabidopsis thaliana* and *Zea mays* respectively – about 4000 more transcripts than BRAKER2, MAKER2 and PASA. Even in different groups like transcripts with micro-exons, overlapping transcripts etc., FINDER reported a superior performance. The pipeline scores genes as high confidence or low confidence based on the available evidence. It is capable of processing eukaryotic genomes of all sizes and requires no manual supervision – ideal for bench researchers with limited experience in handling computational tools.

Funding acknowledgement: United States Department of Agriculture (USDA), Oak Ridge Institute for Science and Education (ORISE), National Science Foundation (NSF)

T17

A statistical model for genomic predictions of high-dimensional traits

(submitted by Daniel Runcie <deruncie@ucdavis.edu>)
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Measuring and modeling multiple traits at once can accelerate the rate of genetic gain in breeding programs, whether their goal is to improve a single target trait, or simultaneously improving many traits. Multi-trait data is widely available, from high-throughput phenotyping technologies, repeated measures, or multi-environment trials. However, the vast majority of statistical models used in plant and animal breeding today handle only a single trait (or at most a few traits) at a time. We have developed a new statistical model for genomic prediction that efficiently and robustly scales to thousands of traits, allowing simultaneous predictions of high-dimensional phenotypes using data from complex experimental designs. Our approach overcomes the computational bottlenecks and over-parameterization challenges of traditional multi-trait linear mixed models by combining recent innovations in computational algorithms and statistical theory. These include: i) efficient and tunable Bayesian priors that prioritize only the strongest, most informative signals in Big Data, ii) a latent factor structure for trait covariances, and iii) efficient approximation and implementation schemes for reducing computational costs in mixed models. We will demonstrate the utility of our approach in the context of multi-trait multi-environment genomic predictions using data from maize.

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

T18 @AnjuAnjugiri1

Haplotype Associated RNA Expression (HARE) improves prediction of complex traits in maize

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Genomic prediction using haplotype information from functionally-important genomic regions like genes can be an important approach to integrate functional and structural genomic information. Here we present a novel and cost-effective method of imputing *cis* Haplotype-Associated RNA Expression (HARE, RNA expression of genes by haplotype) and studied their transferability across tissues and evaluated genomic prediction models within and across populations. HARE can include tightly-linked *cis*-acting causal variants in direct physical contact with the gene, excluding *trans* effects from diffusion and metabolism, so they would be more transferrable across different tissues and populations. We showed that HARE estimates were more transferable across diverse tissues than the measured transcript expression, which was not surprising given that *cis* haplotype explained only a third of the variation in the expression. Genomic prediction models were evaluated within and across two diverse maize panels - Goodman Association panel and Nested Association Mapping panel for predicting 26 complex traits. HARE resulted up to 15% higher prediction accuracy than control approaches that preserve haplotype structure suggesting that HARE carries functional information in addition to information about haplotype structure. The largest increase was observed when the model was trained in NAM and tested in the Goodman Association panel. Additionally, HARE yielded higher prediction accuracy compared to measured expression values within the population. The accuracy achieved by measured expression was variable across tissues whereas accuracy using HARE was more stable across tissues. Therefore, imputing RNA expression of genes by haplotype is more stable, cheap, cost-effective, and transferable across different populations.

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

T19

Investigating anthocyanin and sugar development in purple pericarp sweetcorn

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Anthocyanins are important plant secondary metabolites that give maize kernels purple/red pigmentation, but also have putative health benefits, including preventing cancers and hypertension. Anthocyanin in maize can be produced in either the kernel aleurone or pericarp. The pericarp is maternal tissue, meaning that all kernels on a cob are therefore similarly coloured, regardless of pollen source. Anthocyanin concentration in pericarp-pigmented kernels is most commonly higher than aleurone-pigmented kernels, as pericarp comprises approximately four layers of cells, as opposed to a single layer for aleurone tissue. Most anthocyanin-pigmented maize is starchy. By contrast, super-sweet sweetcorn, which lacks anthocyanin, has a high sugar content due to the sh2 (shrunken2) mutation, which prevents starch formation. Because of an extremely close genetic linkage (0.1 cM) between a non-functional anthocyanin biosynthesis gene, *anthocyaninless-1*(*a1*), and the supersweet mutation, *sh2*, the development of purple supersweet sweetcorn is challenging. To overcome this, we crossed a white supersweet sweetcorn (a1sh2.a1sh2) with a purple-pericarp Peruvian maize (A1Sh2.A1Sh2). We initially developed F3 heterozygous purple sweetcorn (A1sh2.a1sh2) in a large field experiment by breaking the tight genetic linkage, which was followed by two consecutive field experiments to successfully develop a homozygous purple-pericarp supersweet sweetcorn (A1sh2.A1sh2) accession. Biochemical analysis revealed that the mature purple-pericarp maize parent produced sevenfold more anthocyanin compared to aleurone-pigmented kernels. The developed supersweet lines also produced similar levels of anthocyanin. The principal pigments consisted of, in decreasing order, of cyanidin-, peonidin-, and pelardonidin-based anthocyanins. The sugar content in the developed lines at eating stage was similar to that of the sweetcorn parent, with the main sugar components identified as, in increasing order, of fructose, glucose and sucrose in both the white supersweet parent and the derived purple supersweet accession. We believe this is the first report of a purple-pericarp supersweet sweetcorn, based on the sh2 mutation.

Funding acknowledgement: This research was funded in part by Hort Innovation, as part of 'Naturally Nutritious' project (HN15001).

T20 @ThomasWidiez

Haploid induction requires targeting of NOT-LIKE-DAD to pollen endo-plasma membrane by lipid anchoring and electrostatic interactions

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Although discovered more than 60 years ago by Ed Coe, maize haploid inducer lines still make headlines due to novel mechanistic insights and additional applications. Haploid inducer lines are powerful tools to create in planta maize seeds containing haploid embryos and/or to deliver genome editing machinery. The resulting haploid plantlets are then used in the doubled haploid technology, enabling either fast evaluation of phenotypic traits on homogenous progeny, or creating directly transgene free homozygous edited plants. During the past four years, molecular actors behind maize haploid induction have started to be identified, including the key *MATRILINEAL (MATL) / NOT-LIKE-DAD (NLD) / PHOSPHOLIPASE-A (PLA)* gene. Nevertheless, the MATL/NLD/PLA mode of action still remains puzzling. In this cell biology study, we surprisingly found that *MATL/NLD/PLA* is not transcribed in sperm cells, as expected, but in the neighboring vegetative cell of the pollen. Consequently, we revised the subcellular localization of NLD protein. Using isolated sperm cells and immuno-electron microscopy we demonstrated that MATL/NLD/PLA protein localizes to a specific membrane derived from the plasma-membrane (PM) of the vegetative cell. This pollen endo-PM is an internal PM that encircles the two sperm cells. So far, only one protein has been described to localize to this pollen endo-PM. By carrying out pharmacological approaches coupled with targeted mutagenesis, we were able to reveal that lipid anchoring together with electrostatic interactions between membrane and MATL/NLD/PLA positively charged amino acid are involved in targeting MATL/NLD/PLA to this atypical endo-PM. Using a set of fluorescent phospho-lipid sensors, we were able to characterize the lipid signature of the endo-PM, revealing phosphatidylinositol-4,5-bisphosphate (PIP(4,5)P2) specifically in this membrane. Our results uncover a unique example of how the polarity of a particular membrane (i.e. endo-PM) could be critical for plant reproduction and proper gamete formation.

Gene / Gene Models described: *matl/nld/pla1*; GRMZM2G471240
Funding acknowledgement: Région Auvergne-Rhone-Alpes (DH-INNOV), Agence Nationale de la Recherche (ANR-19-CE20-0012)

T21 @Sim_on_r

Can admixed individuals help to study the differences in allele effects between maize heterotic groups?

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Maize breeding populations are highly structured, notably because of the separation of the germplasm into heterotic groups. This stratification generally impacts quantitative traits and must be accounted for in the methods used to study their genetic architecture (e.g. association mapping) to avoid the detection false positives. A less documented, but just as important, consequence of population structure is the existence of group-specific allele effects at loci associated to traits of interest. These can be observed for several reasons: (i) a different linkage disequilibrium (LD) between markers and quantitative trait loci (QTL) across groups, (ii) group-specific genetic mutations in QTL regions, and/or (iii) epistatic interactions between QTLs and other loci that have differentiated allele frequencies between groups. While factors (i) and (ii) are difficult to separate, they can be distinguished from factor (iii) by applying a dedicated association mapping methodology to a structured population evaluated along with an admixed progeny generated by crossing individuals from parental groups. This approach was applied to a maize panel of flint, dent and admixed inbred lines that was evaluated for flowering time. Several associations were detected revealing a wide range of configurations of allele effects, both at known flowering QTLs (*Vgt1*, *Vgt2* and *Vgt3*) and new loci. We found several QTLs whose effect depended on the group ancestry of alleles while others interacted with the genetic background. We finally investigated how the specificity of QTL allele effects can be accounted for in genomic prediction modelling.

Funding acknowledgement: French national agency for research (ANR)

T22 @NateKorth

Do the major changes in seed proteins in quality protein popcorn influence growth of beneficial, health-promoting bacteria in the human gut?

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Americans consume an average of 40 liters of popcorn a year; improvement of popcorn's protein quality could prompt human nutrition outcomes without depending on consumers changing their diets. Like dent corn, over half the protein content in maize popcorn seed comprises zein storage proteins, which are deficient in the essential amino acids: tryptophan and lysine. The *opaque-2* mutation has been shown to increase the lysine and tryptophan content of dent corn inbreds and hybrids. Introgressing *opaque-2* and a set of quality protein modifier loci into popcorn inbreds combined with selection for the quality protein phenotype, produced popcorn lines with substantially altered protein composition, including elevated levels of lysine and tryptophan. We employed an *in vitro* fermentation system to study the effect of the *opaque-2* modified popcorn on the human gut microbiomes of four human subjects. Comparisons of the effect of multiple pairs of popcorn hybrids with and without the *opaque-2* mutation and quality protein phenotype on *in vitro* human gut microbiomes identified a statistically significant increase in microbial diversity in response to *opaque-2*/quality protein popcorn relative to wildtype controls (Adj.P = 4e-04). The abundance of multiple genera of gut bacteria including *Dorea*, *Coprococcus*, and *Butyricicoccus* increased in microbiomes fed *opaque-2*/quality protein popcorn relative to wildtype controls. These organisms are known producers of butyrate, a short chain fatty acid associated with improved host immunity and appetite regulation. Direct measurement of butyrate produced results consistent with the predicted increase in butyrate production among samples fed *opaque-2*/quality protein popcorn in two of the subject's microbiomes. Collectively these data suggest the opportunity to employ maize genetics as a tool to achieve targeted changes in the human gut microbiome, both to study interactions between the composition of human gut microbiome and health and, perhaps, to ultimately achieve desirable health outcomes.

Funding acknowledgement: Bill and Melinda Gates Foundation, Raikes Foundation, ConAgra Foods

T23 @ChungJuRachelW1

Super-resolution expansion microscopy (ExM) reveals the nature of critical recombination intermediates during meiosis

(submitted by CJ Rachel Wang <rwang@gate.sinica.edu.tw>)
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Meiosis is the essential cell division that generates haploid gametes for sexual reproduction. A central process enabling correct segregation of homologous chromosomes is the DNA double-strand break (DSB)-dependent recombination. The recombinase RAD51 and its meiosis-specific paralog DMC1 associated with DSB sites promote strand invasion and exchange, giving rise to crossovers or non-crossovers. While both proteins are required for recombination, their spatial organization during DSB repair is not fully understood. In this study, we adapted a recently described technology termed expansion microscopy (ExM) that uniformly increases the volume of a biological sample by at least 60 folds to dissect the recombination process. We show that DMC1 and RAD51 have distinct spatial localization on presynaptic filaments. First, DMC1 forms elongated signals with RAD51 foci located near chromosome axes. As prophase progressed, fewer DMC1 filaments were detected, whereas RAD51 forms elongated signals. Interestingly, we identified several RAD51/DMC1 configurations that may represent distinct recombination intermediates. In addition, maize mutants of DMC1 and RAD51A/RAD51B genes were used to elucidate the functions of these recombinases. This in-depth analysis of single-cell landscapes of RAD51 and DMC1 accumulation patterns at DSB repair sites at super-resolution revealed the variability of foci composition, and defined functional consensus configurations that change over time during maize meiosis.

Gene / Gene Models described: *RAD51A, RAD51B, DMC1, ZYP1, SPO11-1*; GRMZM2G121543, GRMZM2G084762, GRMZM2G109618, GRMZM2G143590, GRMZM2G129913
Funding acknowledgement: Academia Sinica, Ministry of Science and Technology

T24

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 the prolamin accretions in the ER of aleurone cells come in close contact with the vacuolar membrane and 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 largely unknown but a few studies show that it can be either ATG8-dependent or ATG8-independent. To identify proteins mediating the microautophagy of prolamin accretions in maize aleurone cells, we conducted RNA-sequencing studies of aleurone and starchy endosperm tissues at 18 and 22 days-after-pollination and performed mass spectrometric analyses on vacuolar membrane-enriched fractions of aleurone cells. After analyzing the RNA-sequencing and proteomic data, we identified ten candidate proteins with potential function in autophagy and/or membrane modification. By transiently expressing mCherry-tagged candidate proteins in developing aleurone cells, we identified a hydroxyproline-rich glycoprotein family protein and a phospholipase that localize to the vacuolar membrane domain surrounding the prolamin accretions during microautophagy, supporting their potential roles in the microautophagy process. Our study has provided not only valuable data and insights for the plant microautophagy process but also research methods that can be adapted to other research studying the molecular mechanisms of plant biological processes.

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

T25

The COBRA-like gene *BK2L3* affects cellulose deposition and sucrose export from source leaves

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In higher plants, phloem facilitates long-distance transport of sucrose (Suc) from photosynthetic source tissues to heterotrophic sink tissues. The loading of Suc and other compounds into the phloem generates a very high osmolarity, which attracts water from nearby xylem to create high hydrostatic pressure and drives the transport of assimilates. Maintaining the high intracellular pressure requires robust cell walls. Cellulose, a major building block of the cell wall, is synthesized at the plasma membrane by cellulose synthase complexes, but additional proteins are required for proper cellulose deposition, such as the COBRA proteins. COBRA family proteins are involved in primary cell wall development across angiosperms and affect plant height, brittleness, and the development of root hairs, seed coat, and pollen, but no *cobra* mutant has exhibited perturbed sucrose transport to date. We identified two maize mutants from EMS-mutagenized populations, based on phenotypes associated with increased carbohydrate accumulation. Radioisotope studies of mutants showed severely decreased export from mature leaves, resulting in significantly increased soluble sugar and starch levels. We identified the causative mutation for both alleles in a member of the COBRA family, the *Brittle Stalk2-Like3* (*BK2L3*) gene. Hence, we investigated the composition and ultrastructure of the cell wall in the mutants and found that they contained reduced cellulose content in mature leaves, along with altered phloem cell wall ultrastructure in mature and immature phloem in comparison to wild type. The data suggest that BK2L3 is critical for normal cellulose deposition and cell wall formation during phloem development, which is necessary to establish and maintain the high phloem turgor pressure required for sucrose export from source leaves. This work is the first to our knowledge to mechanistically link the structure of the phloem cell wall to its role in resisting the high hydrostatic pressure required for long-distance transport.

Gene / Gene Models described: *BK2L3*; Zm00001d034049
Funding acknowledgement: National Science Foundation (NSF)

T26

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 and derepression of inflorescence structures in both Brachypodium and maize. Gene expression localization analyses further illuminate how these two gene lineages have diverged following whole genome duplication. Additionally, dissection of these genes' upstream regulatory regions using CRISPR/Cas9 genome editing reveals a likely path for evolutionary divergence between these two gene lineages. 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)

T27

Genetic control and environmental regulation of root-microbiome interaction in maize

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Plant roots are a largely untapped resource and a key for crop improvement because virtually all mineral nutrients in the human diet are extracted from soil by root system. Our research focuses on the genetic control and environmental regulation of the formation and functioning of the root system and its biotic interaction with soil microbes such as arbuscular mycorrhizal fungi (AMF) and plant growth-promoting bacteria (PGPB) in maize. We aim to develop a theoretical framework to incorporate beneficial root-microbe interactions into crop breeding and alleviation of environmental costs originated from excessive agricultural inputs. Multi-omics study using RNA sequencing and metagenomic sequencing identified that root growth and development associates with external AMF and PGPB in maize root and its rhizosphere, which is designated as the narrow area of soil affected by the root activities. Co-variance and network analyses demonstrate that transcriptomic gradients along the longitudinal root axis drive specific shifts in rhizosphere microbial diversity. Moreover, we established that root-derived flavonoids specifically promote the enrichment of bacteria of the taxa Oxalobacteraceae in the rhizosphere, which in turn promote maize growth and nitrogen acquisition. Additional inoculation experiments found that both Oxalobacteraceae and AMF restore the defective root growth in lateral rootless 1 mutant under nutrient poor conditions. Further laser capture microdissection (LCM) together with RNA sequencing and CRISPR/Cas9 identified key ERF genes interacting with AMF underlying lateral root formation and nutrients uptake in maize. In summary, these studies reveal the genetic basis of the reciprocal interactions between root architecture and the composition and diversity of specific microbial taxa in the rhizosphere resulting in improved plant performance. These findings might open new avenues towards the breeding of high-yielding and nutrient-efficient crops by exploiting their interaction with beneficial soil microbes and will safeguard food security and sustainability of agriculture.

Funding acknowledgement: Deutsche Forschungsgemeinschaft (DFG)

T28 @mihai_miclaus

Cytolines as models to study the impact of different cytoplasms on gene expression under heat stress conditions

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Crops are under a constant pressure due to global warming. Nowadays' technologies offer a unique opportunity to probe how maize responds to this process from an omics perspective and speed up the breeding programs accordingly. Recently, an atlas of gene expression responses to heat stress in various tissues of maize was developed (He et al., 2019). Complementary to testing the response to heat stress of a single nucleus hosted in a single cytoplasm, here we use three maize cytolines (i.e., same nucleus but different cytoplasms) (i) to probe how one nucleus performs in different cytoplasmic environments and (ii) to link the different gene expression patterns to existing genetic variability in the organellar genomes. There is a consistent common response as differentially expressed genes (DEG) and enriched GO terms in all three cytolines, but the cytoplasm-donor lines trigger the expression of new genes that are not DEG in the nucleus-donor line. Cytoline-specific DEG may be linked to the existing variability at mitochondrial level. A SNP in the mitochondrial *Atp4* gene seem to impact the proper functioning of its protein, explaining the tremendous difference in gene expression of one cytoline vs. the other two. By corroborating gene expression data with the existing polymorphism at organellar genomes and measurements of photosynthesis rates in the three cytolines, our results offer a unique perspective on the existing interplay between one nucleus and three different cytoplasms. Our results contribute molecular data to the paradigm used to explain the impact of transferring elite nuclei on cytoplasms that confer heat resistance as an alternative to traditional breeding in the context of global warming.

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T29 @CastorinaGiulia

The drought-responsive ZmFDL1/MYB94 transcription factor regulates cuticle biosynthesis

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In higher plants, the outer surface of aerial parts, including vegetative organs, flowers, fruits, seeds and pollen grains, is constituted by a continuous hydrophobic layer termed the cuticle, which consists of two major components, the polymer cutin and cuticular waxes. The cuticle provides a primary barrier that protects from environmental stresses, reduces leaf water loss and plays an important function in plant development. To gain insight into the genetic and hormone regulation of cuticle deposition in maize, we analyzed the role of the MYB transcription factor ZmMYB94/FUSED LEAVES 1 (ZmFDL1). Transcriptome analysis allowed to characterize the ZmFDL1-dependent gene regulatory network underlying cuticle deposition and identify novel candidate genes involved in lipid metabolism in maize. Candidate genes were selected whose transcriptional variations nicely correlated with biochemical defects observed in fdl1-1mutant seedlings. A decrease in cuticle-dependent leaf permeability in maize seedlings exposed to drought as well as abscisic acid treatment, was also observed, which implies coordinated changes in the transcript levels of ZmFDL1/MYB94 and associated genes. These results suggest that the ZmFDL1 regulatory pathway integrates external cues to increase cuticle-mediated response to water scarcity.

Gene / Gene Models described: *fdl1*; GRMZM2G056407, Zm00001d022227

T30

Uncovering the genetic basis of maize sensitivity to herbivore-associated molecular patterns

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Herbivorous pests are major contributors to crop loss, posing a significant threat to food stability. Bacillus thuringensis (Bt) toxins and related molecules have helped protect maize from herbivore attack, but evolving pest resistance threatens long-term utility. A better understanding of plant perception of and defense against herbivore attack will facilitate development of additional strategies to enhance crop plant resistance. Maize has been a model system for the study of plant responses against herbivore-associated molecular patterns (HAMPs), molecules from insects that induce plant anti-herbivore defenses. Fatty acid-amino acid conjugates (FACs) present in the oral secretions of Lepidopteran herbivores are well-studied HAMPs, but the molecular basis for their perception by plants remains unknown. To understand the genetic basis for maize sensitivity to FACs, maize inbred lines were screened for sensitivity to N-linolenoyl L-glutamine (Gln-18:3), a representative FAC as compared to the endogenous plant defense signal ZmPep3. We identified two maize inbred lines with differential sensitivity to Gln-18:3, but similar responsiveness ZmPep3. Screening of the intermated mapping population for Gln-18:3 sensitivity through measurement of induced volatile emission revealed a single quantitative trait locus (QTL) significantly associated with Gln-18:3 sensitivity. Among the ~20 genes within the mapping interval we have identified a candidate receptor gene. Multiple approaches indicate a role for the candidate receptor in mediating maize sensitivity to FACs, and heterologous expression of the receptor gene confers FAC sensitivity.

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

T31

Using a parallel selection experiment to test modalities of haplotype-by-latitude effects underlying phenotypic variation in flowering time.

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Genetic diversity is key to adaptation and breeding for crop improvement. In maize, there is wide variation in tropical germplasm that could be used to create improved varieties. Capitalizing on this diversity is challenged by polygenic maladaptive phenotypes linked to late flowering time, requiring pre-adaptive selection before an individual's merit in a target population of environments can be realized. To study the genomic basis for pre-adaptation, we created a Tropical Synthetic (TropicS) population of maize and performed parallel selection for early flowering time across a latitudinal transect at eight locations for two generations. During each generation of selection and at each location, extreme mapping was used to detect significant associations with flowering time. These results are being examined to test for local versus global patterns of haplotype-by-latitude associations. Moreover, using ancestral haplotype maps, we are resolving the founder alleles that underlie these associations. Uncovering environmental trends in response to selection will provide insights important for the development of more efficient allele mining and breeding strategies for pre-adaptation.

Funding acknowledgement: United States Department of Agriculture (USDA)

T32 @Juliett_Aubert

Paramutation: a global silencing mechanism in maize?

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Paramutation is a rare exception where reprogramming is both mitotically and meiotically stable over many generations. Since its discovery in 1956, only 4 locus of paramutation have been described in Zea mays, all associated with a red pigmentation phenotype: red1 (r1), booster1 (b1), plant color1 (pl1), and pericarp color1 (p1). To our knowledge, no research was conducted to identify paramutable genes that are not involved in a visible phenotype in maize. To determine the importance of paramutation in gene silencing, we crossed independent inbred lines (B73, M37W and M162W) and backcrossed their F1s. We sequenced and analysed leaf messenger RNA from all 4 successive generations, and identified 80 differentially expressed genes in the inbred lines that were stably silenced in the F1s and in all the backcrosses. These alleles meet all criteria of paramutation as their silencing is stable through meiosis, and the newly silenced genes in turn silence active genes in backcrosses. They cover diverse functions in maize, but carry no significant differences in TEs or small-RNA density. With these 80 new candidates to paramutation, we argue that this phenomenon is more common than initially expected in maize, and should not be overlooked when crossing independent lines.

Funding acknowledgement: Agence Nationale de la Recherche (ANR)

T33

Prediction of evolutionary constraint by genomic annotations improves prioritization of causal variants in maize

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Traditional quantitative genetics analyses have successfully identified genomic regions responsible for genetic variability, but they have generally lacked the resolution to detect the exact genomic sites causing differences among individuals. To detect causal polymorphisms at single-site resolution, plant geneticists can rely on evolutionary constraint across many species. However, its usefulness can be limited due to missing data and low evolutionary depth. In this study, we take an approach based on computational annotations to accurately predict evolutionary constraint of any single-nucleotide polymorphism. Using only sequence analysis, we annotated non-synonymous mutations in about 27,000 maize gene models, with information about genomic structure (GC content, transposon insertion, k-mer frequency) and protein function (predicted effects of polymorphisms on peptide sequence or protein representation). We learned the relationship between these genomic annotations and evolutionary constraint, used as a proxy for the effect size of polymorphisms on fitness. Predictions by these annotations were validated by experimental information at polymorphisms: within-species conservation (minor allele frequency), chromatin accessibility (MNase hypersensitivity), gene expression (protein abundance across 32 tissues) and Gene Ontology enrichment (metabolic processes). Importantly, these predictions also captured effects of polymorphisms on fitness-related traits in hybrid maize (grain yield), which suggests their functional relevance for practical applications in plant breeding. Together, our results suggest that our proposed approach may effectively prioritize the single-site polymorphisms most likely to impact important agronomic traits in maize. Such prioritizations could be useful to select markers for accurate genomic prediction and candidate mutations for efficient base editing.

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

Posters

P1

A history of maize genome sequence assemblies

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It has been 12 years since the first maize genome sequence was announced at the 2008 Maize Meeting in Washington DC, but the quest for a contiguous genome sequence of maize started long before. In 1998 a huge effort was launched to generate the tools to allow a whole genome sequence assembly of maize. On the molecular side, technology was developed to create large insert size BACs, public BAC libraries were generated, and BACs were fingerprinted with new strategies that allowed BACs to be aligned to the genetic map of maize, and placed in large contigs. On the genetic side, a finer resolution genetic map was created by the critical step of inter-mating F2s for three generations to build the populations that lead to the IBM (Intermated B73 x Mo17) genetic maps. The markers used to fingerprint the BACs were also genetically mapped, allowing the merging of the physical and genetic maps. When these tools were ready, a minimum tiling path through the BACs was selected, those BACs were sequenced and assembled into contigs and scaffolds, and the first BAC-based genome assembly was made available in 2008. A year later in 2009, a pseudomolecule version for each of the ten chromosomes of the B73 genome (RefGen_v1) was published and released. The community has come light years since these remarkable achievements and currently has 46 complete genome assemblies. In January 2021, the NAM sequencing consortium released the official gene models for a set of 26 complete genomes (the NAM founders and B73) that were identically sequenced, assembled and annotated so that all 26 genomes are directly comparable. In this poster, we document the history of maize genome assemblies, review the major advances that sequenced genomes have brought to maize research, and honor the hundreds of scientists that made the complete genome sequence of maize possible.

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

P2

Accessing the MaizeCODE data from 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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P3

Analysis of leaf-associated bacterial and fungal microbiomes of corn inbred lines related with Tar Spot disease

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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-shaped lesions scattered on the surface of leaves, stalks, and husks of corn. Almost nothing is known about the molecular basis of the *Phyllachora maydis*-Zea mays pathosystem. Plant-associated microorganisms known as the microbiome play important roles in plant health. To evaluate whether leaf-associated bacterial and fungal microbes have a role in disease resistance, we analyzed the phylloplane microbiomes of susceptible (S) and non-susceptible/resistant (R) corn NAM (Nested Associated Mapping) parental lines under *P. maydis* infestation in natural field conditions. Comparative metagenomics analysis of phylloplane microbiomes from susceptible (TX303 and B97) and resistant (CML52 and CML103) NAM lines revealed the microbiome patterns associated with Tar Spot. Further study on the difference on the microbiome composition between susceptible and resistant cultivars could lead to the development of novel and more effective biological control agents against Tar Spot and therefore is a high priority topic for future study.

Funding acknowledgement: United States Department of Agriculture (USDA)

P4

De novo assembling 19 maize inbred lines of importance for European breeding

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Characterizing the genomic diversity of maize is critical to understand the molecular origin of structural variation, and is a prerequisite to underpin the functional variation underlying phenotypic variation. Whole genome sequence assemblies at the chromosome scale with low amount of missing data are critical resources for answering such questions. Whole genome sequence assemblies are now available for several maize inbred lines, but a number of key founders used in European maize breeding programs have not yet been sequenced de novo. This concerns the dent genetic group and the Europe specific flint genetic group. The consortium has established a list of lines priority lines for de novo sequencing. The SeqOccIn project (Sequencing Occitanie Innovation, https://get.genotoul.fr/seqoccin/) aims at acquiring expertise on the optimal combination of long fragment sequencing technologies and associated applications to better characterize complex genomes for species of agronomical interest (maize, sheep, cow). In the framework of this project, we are *de novo* assembling whole genome sequences of these 19 maize inbred lines. The poster will present the list of these maize lines, together with first assembly results using PacBio long read sequencing.

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P5

Evaluating the impacts of growth environment on maize silk cuticular lipid composition

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As a conduit for pollination and fertilization of the ovule, silks are integral for reproductive success in maize. The emergence of silks from the encasing husk leaves is necessary for pollen reception, but also subjects silks to a host of biotic and abiotic stressors. The cuticle is the first layer of protection between the silk and the external environment and is comprised of a cutin matrix infused and laid atop with hydrophobic metabolites, including hydrocarbons, fatty acids, and fatty alcohols. To define the genetic architecture of these silk cuticular lipid traits, a Genome Wide Association Study (GWAS) was performed using cuticular lipid profiles from a phenologically restricted set of 448 inbreds from the Wisconsin Diversity (WiDiv) Panel grown in three environments. Previous work in this panel has identified genotype-by-environment (GxE) interactions that impact silk cuticular hydrocarbon composition; in some instances, we observe up to 10-fold variation across inbreds. To further assess GxE effects, silk cuticular lipid composition was profiled for ten strategically selected WiDiv inbreds grown in 12 environments (6 planting dates x 2 locations). Companion weather data collected for each environment will be evaluated via multivariate approaches to predict specific weather parameters that influence cuticular lipid composition. Taken together, these approaches will provide insights into the role of environment in cuticular lipid composition, increasing our understanding of the genetic architecture underlying the accumulation of protective surface lipids.

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P6 @kseniakrasileva

Evolutionary trajectory of innate immune receptors in maize

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Plants rely on germline encoded immune receptors to recognize rapidly evolving pathogens. The major class of plant immune receptors belong to the Nucleotide Binding Leucine Rich Repeat (NLR) protein family. NLRs show extensive copy number variation, allelic variation as well as domain shuffling across plants. The genomes of domesticated elite maize varieties show a distinct and unusual evolutionary history of NLRs. In comparison with Sorghum and other grasses, maize contains a highly reduced set of NLRs, retaining only a few members of each sub-family. Specifically, a clade responsible for domain shuffling is lost across 26 sequenced genomes of NAM lines. Despite major losses in copy number variation, several maize genes still exhibit extensive allelic variation that is characteristic of highly variable NLRs (hvNLRs) suggesting ongoing generation of allelic diversity. Arabidopsis hvNLRs overlap with hybrid incompatibility loci, suggesting their importance in compatibility of wide crosses. Understanding NLR evolution in maize can inform breeding strategies and uncover mechanisms of NLR diversification.

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P7

Gene expression complementation in the field from an above and below ground perspective in maize hybrids

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Highly heterozygous F_1-hybrids display a superior phenotypic performance in comparison to their genetically diverse parental inbred lines. The classical dominance model of heterosis explains the superiority of hybrid plants by the complementation of deleterious parental alleles by superior alleles of the second parent at many loci [1]. Genes active in one inbred line but inactive in another represent an extreme instance of allelic diversity, which has been defined as single-parent expression (SPE) [2]. The present study systematically investigates the transcriptomic plasticity of a diverse panel of maize inbred lines and their F_1-hybrids under field conditions in vegetative and generative above ground organs. We demonstrate that extreme gene expression complementation in F_1-hybrids is a general mechanism throughout plant development. In all genotype-by-organ combinations ~1,500 genes show SPE patterns even under uncontrolled environmental conditions. Among the analyzed above ground organs and previously published primary root data, SPE genes reveal a high organ-specificity. A gene co-expression network analysis identified some clusters of highly connected genes which are especially enriched by specific SPE patterns and show significant correlations to phenotypic traits. Functional analyses of key genes in these SPE-enriched clusters will provide further understanding how genes displaying expression complementation contribute to the superior plasticity of maize hybrids in above ground organs. [1] Jones, D.F. (1917). Dominance of linked factors as a means of accounting for heterosis. Genetics 2, 466–479. [2] Paschold, A., et al. (2012). Complementation contributes to transcriptome complexity in maize (*Zea mays* L.) hybrids relative to their inbred parents. Genome Res. 22, 2445–2454.

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P8

Genome assembly of A188 and genetic mapping of regeneration

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The inbred line A188 is an attractive model for elucidation of maize gene function due to its highly embryogenic character and transformation amenability. However, the lack of a genome sequence of A188 limits the use of A188. Here, we first constructed a chromosome-level genome assembly of A188 using Oxford Nanopore long readsand obtained an assembly (A188Ref1) of 2.25 Gb, including 10 chromosomal pseudomolecules, a mitochondrial genome, a chloroplast genome, and 986 scaffolds or contigs.Annotation of A188Ref1 resulted in 40,747 high-confidence gene models with 62,142 transcripts (A188Ref1a1).Genome comparison of A188 with B73 based on both whole genome alignments and read depths from sequencing reads identified approximately 1.1 Gb syntenic sequences as well as extensive structural variation. To understand the genetics of embryogenic and transformable trait of A188, we performed genotype-by-sequencing (GBS) and bulk segregant RNA-seq (BSR-seq) with an F2 population of A188 and B73, the transformation-recalcitrant line, and identified at least three genomic loci associated with regeneration. The project provides both genetic and genomic resources for genetically understanding maize regeneration and other important agronomic traits.

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

P9 @julien_roziere

Genome-wide *de novo* analysis of preferentially located motifs in 5' and 3'-proximal regions from arabidopsis and maize

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Identifying *cis*-regulatory motifs controlling gene expression is an arduous challenge that is actively explored to discover key genetic factors responsible for traits of agronomic interest. The PLMdetect (Preferentially Located Motif detection) method was developed to identify over-represented motifs (PLMs) in promoters at a preferred distance from the transcription start site in the model plant Arabidopsis (Bernard et al., 2010, doi: 10.1139/g10-042). Taking into account this biological information, this method has the advantage of limiting the false-positive rate compared to other *in silico* methods. Here, we expanded the PLMdetect method to comprehensively analyze *de novo* the promoters as well as the untranslated transcribed regions of Arabidopsis and the important crop maize. We sought to determine how their differences in genome content and architecture would be reflected in features of their PLMs in 5'- and 3'-proximal regions of each gene locus. We have currently identified three groups of PLMs for each species in each targeted region. An assessment of these PLMs using known plant transcription factor (TF) binding site (TFBS) data (Fornes et al., 2020, doi:10.1093/nar/gkz1001) revealed that a subset of these PLMs (9.4% and 7.3% in Arabidopsis and maize, respectively) are previously characterized TFBSs (tPLMs), while the others (90.6% and 92.7% in Arabidopsis and maize, respectively) represent novel and uncharacterized motifs (uPLMs), not captured by the current collection of plant TFBSs. Enrichment analyses of the tPLMs revealed positional preferences of TFBSs from several TF families as previously reported in Arabidopsis (Yu et al., 2016, doi:10.1038/srep25164). Finally, GO term enrichment analyses showed that 15.3% of the uPLMs are able to infer functional predictions which are not provided by tPLMs. In the near future, we will add comparisons between the data sets obtained from each species.

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P10

Genome-wide identification of crossover sites using deep leaning

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Studying genome-wide crossover distribution is challenging due to the high cost of genotyping that is required to construct the complete crossover landscape. Numerous studies have shown that the presence of crossovers at specific genome sites correlate with certain chromatin features as well as the DNA sequence context. Crossovers in maize are predominantly present in open chromatin regions characterized by reduced DNA methylation and nucleosome occupancy levels. In addition, CG-rich short sequence motifs have been identified at these sites. We previously found that chromatin and DNA sequence features can be used to identify CO sites with very high accuracy using machine learning. Here, we examined if CO sites can be identified based on DNA sequence alone. As presence of specific characters underlie the positions of crossover sites, identification of these sites resembles the object detection problem in natural language process. This approach considers the presence or absence of keywords as well as the syntenic relationship between words. Thus, the computational approach for crossovers detection treats DNA sequence as a sentence, and crossovers as keywords in the sentence to detect. We evaluated this approach using crossovers from the maize NAM population, and found that it exhibits good performance with the accuracy of 0.89. Cross-species testing of the maize-trained algorithm using genome sequences of Arabidopsis, rice, and tomato produced similar results. Using this method, we can not only recover the known crossover sites but can also predict crossover hotspots that have not been previously identified due of the limited size of the existing high-quality CO datasets. As it relies on DNA sequence alone, this pipeline can be generalized for cross-species studies in the many plant species where epigenome information does not exist. Furthermore, our result can provide insights into how sequence composition affects crossover landscape diversity among species.

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P11

Genomic history of maize as interpreted from whole-genome alignment of 26 lines

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The remarkable diversity of maize genomes allows for its broad adaptation to different environments. Here we surveyed the genomic architecture of 26 maize inbreds that were chosen to represent its great genetic variation. Whole-genome alignments and divergence-time analyses showed that the distinct genomic features of modern maize were contributed by structural changes accumulated over a span of 0.75 million years. Stratified haplotype patterns were observed in the pericentromeric areas, indicating the occurrence of multiple introgression, segregation and selection events in the last 0.5 million years. Sequence alignment of tandem repeat arrays further revealed the presence of haplotypes in centromeres, knobs and nucleolus organizer regions (NOR). Divergence times inferred from syntenic transposable elements in knobs are in concordance with the instances of major introgression events. Finally, analysis of unmethylated regions and differential gene expression suggested a phenotypic effect of the ancient genomic remnants in modern maize.

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P12

GrasVIQ: An image analysis framework for automatic quantification of veins in grass leaves

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Leaf veins facilitate transport and provide mechanical support to the leaf and have critical implications for the performance and productivity of the plant and the ecosystem. Computational image analysis programs have been developed to extract and quantify vein traits from the reticulate venation of dicots, but a dedicated program for the parallel venation of monocots, particularly grasses, has yet to be developed. To address the need for high throughput vein phenotyping in grass species, like *Oryza sativa* (rice) and *Zea mays* (maize), we developed the Grass Vein Image Quantification (GrasVIQ) framework which automatically segments and quantifies vein from images of cleared leaf pieces using classical computer vision techniques. Using image datasets from inbred lines and auxin mutants in maize, we demonstrate that GrasVIQ can perform high throughput quantification of vein traits, including vein density, vein width, and interveinal distance, with a precision on par with manual quantification. Further, we show that the framework can be used to recognize quantitative traits, identify previously undetected phenotypes, and measure vein patterning defects, which is advantageous for both basic and translational research. We envision GrasVIQ to be adapted for vein phenomics in maize and other grass species.

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P13

Identification of transposable element families that exhibit tissue-specific variation in chromatin structure as revealed by global MNase sensitivity profiling

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Transposable elements (TEs) make up a large portion of the maize genome. Some of these elements have been shown to be active in different tissues or developmental stages. Transposon activity, thought to involve changes in chromatin structure, is associated with more accessible or "open" states. We developed Differential Nuclease Sensitivity profiling (DNS-seq, Vera et al., 2014) to identify MNase-hypersensitive regions of open chromatin. We applied DNS-seq and peak calling to four distinct B73 tissues: root tip (RT), coleoptilar node (CN), earshoot (ES), and endosperm (EN) (Turpin et al., 2018; DOI 10.1016/j.dib.2018.08.015). Chromatin accessibility data is often used to study gene regulation; however, we used it to study transposon biology. We examined whether TE families exhibit tissue-specific changes in chromatin structure. We analyzed 205 abundant TE families by calculating the number of base pairs of each that intersected with open chromatin, classified as MNase hypersensitive footprints (DNS-seq positive peaks, iSeg-BC1). For comparison, we generated a probability distribution from a 100-iteration control in which the peak positions were shuffled. Then we obtained Z-scores using the mean and standard deviation of the random-distribution control simulations compared to the observed intersection scores. The resulting 820 Z-scores revealed the statistical degree of enrichment (positive Z-scores) or depletion (negative Z-scores) of open chromatin for each TE family in the four different tissues. We found that some TE families, such as puck, milt, bygum, iwik, ilyl, and ebel, appeared to have positive Z scores in only a single tissue, CN. Similarly, ubow and osed were enriched in only ES, whereas ibulaf was enriched in only RT, and none of them for only EN. This study reveals that TE families can exhibit tissue-specific chromatin dynamics, illustrating a novel approach to better understand transposon behavior in maize development.

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P14

Identifying environmental index (EI) for genome-wide association studies (GWAS) and genomic selection (GS) for current and future environments

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The phenotypic variation of living organisms is shaped by genetics, environment, and their interaction. Understanding phenotypic plasticity under natural conditions is hindered by the apparently complex environment and the interacting genes and pathways. With genetic mapping populations in sorghum and rice showing flowering-time phenotypic plasticity, we reported earlier that an integrated analysis enabled by the identification of an environmental index is powerful to reveal the genetic effect continuum and facilitate performance prediction across environments. With extensive field-observed complex traits, environmental profiles, and genome-wide SNPs in three major crops (maize, wheat, and oat), we further expanded the analytical framework to diverse genetics materials to pinpoint the genetic and environmental factors underlying phenotypic variation of quantitative traits. Our findings demonstrated that genes identified through GWAS for two reaction-norm parameters (*i.e.*, intercept and slope) derived for the flowering-time were less colocalized for a diverse maize panel than those for wheat and oat breeding panels. This finding agreed with the different diversity levels of genetic constitution of the panels. Besides, we showcased the potential for accurate, systematic performance forecasting of diverse germplasm panels in new environments. This general analytical framework and the companion analytical package (CERIS-JGRA: Critical Environmental Regressor through Informed Search – Joint Genomic Regression Analysis) should facilitate biologically informed dissection of complex traits and enhanced performance prediction in breeding for future climates.

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P15 @YinjieQiu

Maize pan-genome construction using 26 NAM genome assemblies

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Maize (Zea mays ssp. mays) is a highly diverse species with extensive structural variation between individuals within the species. In this study, we used the 26 NAM founder genomes and corresponding gene annotations to construct a gene space pan-genome using an approach that combines synteny and gene sequence homology. A total of 103,538 pan-genes were identified. When categorizing pan-genes into core (present in 26 lines), near-core (24-25 lines), dispensable (2-23 lines), and private (1 line) pan-gene types, the proportion of each pan-gene type in the pan-genome was 26.96%, 4.00%, 49.35%, and 19.69%, respectively. For each genotype, the proportion of genes classified into each of these pan-gene types was consistent, with an average of 58.39% (SE = 0.07%) belonging to the core genes, 8.22% (SE = 0.05%) to the near-core genes, 31.75% (SE = 0.09%) to the dispensable genes, and 1.64% (SE = 0.08%) to the private genes. When looking at the syntenic relationship between maize pan-genes and sorghum, the majority of core/near-core genes were syntenic (57.8%) whereas only 1.8% of dispensable/private genes were syntenic. The M1 and M2 subgenomes of maize were composed almost exclusively of core (87.23%) and near-core (6.19%) pan-genes. Finally, a total of 16,267 pan-genes had a putative tandem duplicate in at least one genome, and the average number of tandem duplicate copy numbers was 2.20 (SE = 0.01). The pan-gene matrix generated from this study provides a powerful resource for future comparative genomic studies and will facilitate a deeper understanding of the functional aspects of these different pan-genome fractions.

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P16

MaizeMine facilitates meta-analysis of diverse data sets through genomic data integration

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High-throughput genomic technologies have facilitated the generation of numerous high-quality genomic datasets. Maize researchers frequently need to conduct comparative analysis between their own datasets and published or publicly available data. MaizeMine (http://maizemine.maizegdb.org), MaizeGDB's data mining warehouse, allows 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. Currently, MaizeMine utilizes the InterMine data warehousing system to integrate genomic sequences from the B73_RefGen_v3 and B73_RefGen_v4 genome assemblies, three sets of gene annotations (AGPv3, AGPv4, NCBI RefSeq), Gene Ontology (GO), protein annotations (UniProt), protein families and domains (InterPro), homologs (Ensembl Compara), and pathways (CornCyc, KEGG, Plant Reactome). In our most recent update of MaizeMine (v1.3), we have added data sets for a SNP array (Illumina SNP50), whole genome EMS mutagenesis sites, three insertional mutagenesis collections (Vollbrecht, AcDs, Barken_Mu Illumina, and McCarty Uniform Mu), maize-Gamer annotations, and root and shoot transcriptional start sites. MaizeMine also provides pre-computed variant effects and expression levels based on RNA-seq data from the Zea mays Gene Expression Atlas (NCBI BioProject PRJNA171684). Database cross-references facilitate easy gene identifier conversion between AGPv3, AGPv4 and RefSeq. MaizeMine also provides simple and sophisticated search tools, including a keyword search, built-in template queries with intuitive search menus, and a QueryBuilder tool for creating custom queries. With the release of the significantly improved Zm-B73-Reference-NAM-5.0 assembly (aka B73_RefGen_v5) and Official Gene Model annotations on January 17th 2021, we will move forward in the coming year to update MaizeMine with B73-RefGen_v5 data sets as they become available. In addition to older, v5 updated data sets, new data sets (GWAS, Chromatin characterization, TF-binding sites, DNA methylation, Bonn-Mu insertions, etc.) will be incorporated and will contribute towards making MaizeMine an even more robust resource for the maize research community.

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P17

Methods to effectively leverage LC-MS for population level biochemistry phenomics

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Untargeted metabolomics offers profound opportunities to understand plant biochemistry. However, implementing metabolomics at the diversity population scale (which requires hundreds to thousands of samples) faces scalability challenges at the interface of data science, chemistry and biology. For example, the technology most often used to identify metabolites in an untargeted fashion, high-resolution liquid chromatography mass spectrometry (LC-MS), produces large data files that contain high levels of noise along with drift in both the LC and MS axes. These sources of noise can fluctuate within and between runs as well as over the long timespans (months to years) needed to process population sized datasets. Thus, while highly sensitive LC-MS technology makes it possible to detect thousands of metabolites without a priori knowledge, effective quality control methods that can be implemented at scale are needed. I will present pipelines and methods which leverage labeled standards and are being used to interrogate metabolic phenotypes water deficit in two C4 grasses (Setaria and Sorghum) through LC-MS driven untargeted metabolomics at a population level of 1800 individuals in each species.

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P18

Mining maize with Gramene

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Need to find orthologs for your favorite maize genes? How about expression? Would getting a sorghum mutant of your gene of interest accelerate your research? Maybe 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 93 reference genomes including Zea mays, and pathways for 107 plant species. Built upon the 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. Gramene also hosts genetic variation for 12 genomes including maize, and the USDA-ARS sorghum EMS collection. The Plant Reactome hosts 320 reference pathways curated in rice and projected by orthology to other species, including 269 maize projected pathways. In addition, visualizations of EBI Expression Atlas data from over 960 plant experiments (23 baseline studies with 1,165 assays in maize) are integrated into the search results panel, and both the genome and pathway browsers. Gramene is supported by NSF grant IOS-1127112, and partially from USDA-ARS 001-8062-505 002.

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P19

Modeling chromatin accessibility across angiosperms

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The DNA-binding domains of transcription factors (TF) and the motifs that they bind are known to be incredibly stable over evolutionary time. Functional TF binding sites are concentrated in the open chromatin regions of the genome, which are also known to be conserved across species. Convolutional neural networks (CNN), a type of deep learning model, are particularly well-suited for prediction tasks involving the spatial arrangement of motifs in a sequence and have been previously applied very successfully to biological problems. We trained a recurrent CNN to predict the binary (open or closed) chromatin state in leaf tissue over 300bp sequence windows. Using ATAC-Seq data from 13 angiosperms, we tested the performance of models trained within species versus models trained across species and saw that the across-species models consistently performed as well as the within-species models. This shows that spatial patterns of motifs inside open chromatin regions are conserved, suggesting that chromatin remodeling could employ similar mechanisms across angiosperms. Additionally, these models can be used to accurately predict chromatin accessibility in specific tissues directly from genome assemblies. We are now examining the model parameters and predictions to gain potentially novel insight into plant chromatin remodeling mechanisms.

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P20 @FabioGomezCano1

Multi-networks integration to prioritize regulatory genes of maize phenolic metabolism in maize

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Elucidating gene regulatory networks (GRNs) is a major area of study within plant systems biology. Intrinsic relationships exist between phenotypic traits and particular gene expression profiles. These expression patterns are largely defined by regulatory links between sets of transcription factors (TFs) and their target genes. In maize, 45 coexpression networks capturing >6,000 RNA-seq samples enabled prediction of high confidence TF-target associations. In addition, expression QTL (eQTL) mapping on a set of 304 diverse maize inbred lines was performed to link natural variation and gene expression. Finally, a set of ~12 million protein-DNA interactions (PDIs) involving 186 TFs were recently identified using ChIP-seq, DAP-seq, and Y1H assays. Here, we take advantage of all these sources of information to identify high confidence and functionally relevant regulatory interaction associated with maize phenolic metabolism. Initial analyses focused on the identification of eQTLs, PDIs, and co-expression links associated with phenolic-genes. In total, we found 275 trans-eQTLs and and 154 cis-eQTLs associated with maize phenolic-genes, of which 27/275 and 55/154 mapped to TFs and ChIP/DAP-seq peaks of the PDI collected, respectively. Comparison of predicted TF-target association with the co-expression network allowed us to identify several high-confidence interactions. These interactions are currently being experimentally validated by monitoring metabolic changes of phenolic compounds in maize protoplast transformed with the TFs candidates. The results to be presented highlight the importance of the integration of multiple layers of information to guide predictive biology towards the discovery of novel edges in GRNs. This research was funded by NSF grant IOS-1733633 to N.S, N.dL, and E.G.

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P21
Multi-omics dissection of the maize seedling cuticle

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The plant cuticle covers the aerial surfaces of plants and serves as a protective layer against environmental stresses. The cuticle is comprised of an insoluble cutin matrix intercalated with and laid atop by solvent-extractable lipids, including fatty acids, aldehydes, alcohols, hydrocarbons, and wax esters. The biosynthetic and genetic networks that underlie cuticle deposition in maize seedlings is not fully understood. In this study we aimed to: (1) interrogate how cuticle composition differs between plant organs and between genotypes; and (2) uncover the gene networks that underlie cuticle formation. To this end, we queried the compositional difference in the cutin monomer and cuticular lipid metabolomes for different seedling organs (roots, coleoptiles, and seedling leaves) from four genotypes (B73, Mo17, and the reciprocal hybrids). A suite of multivariate analyses was used to identify the genes whose expression exhibit a strong association with metabolome composition. Multivariate metabolome analysis demonstrated that organ differentiation exhibited a predominant influence on the cuticle compositions, as compared to different genotypes. Our transcriptome-metabolome integration pipelines identified approximately 1900 genes that were strongly associated with either or both cutin and cuticular lipid compositions across the seedling organs. Approximately 60 of these genes have previously been shown to be associated with the biosynthesis of cuticles. Based on gene annotations and gene ontology enrichment analyses, the remainder of the candidate genes are likely involved in responses to light stimulus, photosynthesis, shoot development, and cellular lipid metabolism. Weighted gene co-expression network analysis was also conducted and via a machine learning approach, two of nineteen expression modules were predicted to be tightly associated with cuticle composition. Collectively, this systems-based approach and computational pipelines permit a foundational dissection of the genetic and metabolic networks underlying cuticle composition in maize seedlings.

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P22

One-stop pangenome browser for exploring the rich genetic diversity in maize

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Continued advances in sequencing/assembly technologies are generating an abundance of high-quality reference assemblies within crop species, ushering a transition from single-genome to pan-genome research approaches. With this transition, communities will need ready access to pre-computed comparisons of genome assemblies to identify and characterize common and variable regions. To accommodate this need, the Gramene comparative genomics project is developing Gramene subsites, each dedicated to the study of individual crop groups. We will describe current status on maize pan-genome subsite(http://maize-pangenome-ensembl.gramene.org) that support recent work to develop reference assemblies for 26 maize accessions parents of the nested association mapping (NAM) population. A key feature of maize pan-genome subsite is the application of uniform annotation protocols to minimize methodological artifacts, and the application of Ensembl and Gramene infrastructures for comparative analysis and visualization. A total of over 50K GeneTree families were constructed comprising over 1 million individual genes from 35 genomes. In addition to the 26 NAM lines including B73 (v5) and two older version of B73 (v3 and v4), the following 7 species were used as outgroups: Arabidopsis thaliana (TAIR10), Chlamydomonas reinhardtii (v5.5), Drosophila melanogaster (BDGP6), Oryza sativa (IRGSP-1.0), Selaginella moellendorffii (v1.0), Sorghum bicolor (NCBIv3), and Vitis vinifera (IGGP_12x). Supporting data tracks for repeat masking features, gene model annotation, R gene loci for each NAM genome are available on the subsite. Additionally the pan-site will support the reference genome of teosinte, the wild ancestor of maize, to help explore the genetic basis for maize domestication.

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P23

Pangenome-guided super-contiging and scaffolding of long-read assemblies of diverse CIMMYT maize lines

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The maize genome is full of highly-repetitive and low-complexity sequences that complicate the genome assembly process. The development of long-read sequencing technologies has enormously simplified the generation of highly contiguous assemblies, recently enabling the creation of the reference assemblies of the 25 diverse NAM founders. To reach this high-quality chromosome level assembly, the contigs produced for these required scaffolding through optical mapping technologies, which can trim, orient, and sort the contigs before joining and merging them into their final coordinates. Here, to avoid the cost of generating optical maps, we develop and test a bioinformatic pipeline that leverages these high-quality references to improve assembly contiguity using contigs generated from long-read sequencing technologies of 11 diverse CIMMYT maize lines and six existing older assemblies. Through the alignment of the assembly contigs to each reference genome, we identify the potential edges between contig pairs by defining a set of mapping statistics. We then build a contig graph by selecting the most likely edges between contig pairs using a linear model trained on mapping statistics, allowing us to generate super-contigs, a series of contig nodes joined through edges composed of sequence from the high-quality reference assemblies. On our new assemblies, this helps in resolving the hard-to-assemble regions that limit the contiguity of the raw assemblies, while on older ones, we are able to replace some of the gaps in the chromosome sequences represented by Ns. These super-contigs are then scaffolded to generate an almost complete pseudo-chromosome level sequence that can be used for whole-genome level analyses.

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P24 @hwb333

Project update: Nuclease profiling in maize - MNase sensitivity and frenter profiles for B73v5.

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The goal of this project (referred to as NUPRIME) was to develop micrococcal nuclease (MNase) profiling as a foundational resource for the integration of maize epigenomic data (NSF PGRP IOS 1444532). Differential nuclease sensitivity (DNS) profiles for four tissues of maize have been produced (Turpin et al., 2018; doi: 10.1016/j.dib.2018.08.015) and now realigned to B73v5. DNS-seq, like other methods, including DNAse-seq, and ATAC-seq, make use of a relatively light endonuclease digestion to define accessible chromatin regions. Of these methods, MNase is particularly well suited for mapping of DNA-protein particles, classically nucleosomes. More recently, we and others have repurposed MNase to map sub-nucleosomal chromatin particles (MOA-seq), thereby producing biochemically defined candidates for cis-element occupancy within fixed, native chromatin. To further develop these resources, we have aligned the DNS-seq sequences to B73v5 and share them online via the UCSC genome browser at FSU, www.genomaize.org. Data tracks and peak segmentation files can be analyzed or obtained from this server. Peak segmentation was done using the versatile and robust peak-calling algorithm, iSeg (Girimurugan et al. 2018; doi: 10.1186/s12859-018-2140-3). Among the new data tracks are the highly informative "Frenters" (Fragment Centers) coverage plots. These greatly facilitate the visualization and detection of footprints of chromatin particles. We present three new classes Frenters computationally calculated from original heavy and light digest libraries and the iSeg peaks called at a range of stringencies. The three new Frenters for each tissue are (1) Heavy digest Frenters to visualize positioned nucleosomes, (2) Light digest large Frenters to visualize large particles or nucleosomes in accessible chromatin, and (3) Light digest small Frenters to highlight subnucleosomal particles, as illustrated, for example, for FEA4 by Parvathaneni et al., (2020; doi: 10.1186/s13059-020-02070-8).

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P25 @ajo2995

Ranked choice voting for representative transcripts with TRaCE

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Genome sequencing projects annotate protein-coding gene models with multiple transcripts, aiming to represent all of the available transcript evidence. However, downstream analyses often operate on only one representative transcript per gene locus, sometimes known as the canonical transcript. TRaCE (Transcript Ranking and Canonical Election) holds an election to choose such transcripts in which a set of RNA-seq samples rank the transcripts by annotation edit distance. These sample specific votes are tallied along with other criteria such as protein length and InterPro domain coverage. The winner is selected as the canonical transcript, but the election proceeds through multiple rounds of voting to order all the transcripts by relevance. Based on the provided set of expression data, TRaCE can identify the most common isoforms from a broad expression atlas, or it can prioritize alternative transcripts expressed in specific contexts. We ran TRaCE on maize NAM population founder lines and human gene models. Results were validated in B73 by student curators and in human by APPRIS.

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P26

Sequence, assembly and annotation of maize inbred B104

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B104 is a highly transformable maize inbred line which shows high sequence similarity with the reference maize line B73. B73 and B104 inbred lines share the same background; they were developed as part of the Iowa Stiff Stalk Synthetic breeding program. To understand the dynamics and tempo of maize genome evolution within a modern maize breeding program and enhance our knowledge of maize transformation, we have generated a high-quality genome assembly of 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 and *ab-initio* approach. Transposable Elements (TEs) were annotated in the B104 genome using a pipeline that combines homology based-annotations with the structure-based annotations to provide a complete summary of the transposons. We characterized both large-scale (>1Mb) and small- to medium- scale (50bp - 1Mb) structural variations using read and genome alignment based approaches. To characterize the differences in the contributions of stiffstalk founder lines to B73 and B104 and explore how this governs patterns of structural variation, transposon, and methylation variation, we painted the genomes with the founder haplotypes and identified regions of shared and distinct founder ancestry. We explored the gene content variation between B73 and B104, based on the complete set of structural annotations from B104, B73 and 25 NAM founder lines and identified the number of core (50%), dispensable (44.6%) and private genes (5%) in B104. We further identified genes which are differentially expressed (1.3%) between B73 and B104 across the 10 tissues. We present a summary of structural variations including genic presence/absence, variation in gene expression and TE families across regions of shared and different founder ancestry.

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P27

The NAM genome assemblies and 2021 release of their official annotations at MaizeGDB

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In January 2020, the NAM sequencing consortium released through MaizeGDB the high-quality genome assemblies of the 25 NAM founders and version 5 of the B73 assembly. These 26 assemblies were sequenced using consistent technologies and methods, creating a set of maize genome assemblies that is more amenable to comparative analysis than groups of assemblies of varying quality, generated through different technologies and methods. At the same time, preliminary annotations for all 26 genomes were also released. These annotations were provided so that maize researchers could begin to explore the new assemblies, but with the warning that they should not be used for published research. **In January, 2021, the official annotations for all 26 assemblies were released, replacing the preliminary annotations.** The 2021 data release also includes new methylation, TE annotations, structural variants, and RNA-seq data for the 26 assemblies. Here we describe the official annotations and how they differ from the preliminary annotations, the NAM founder assemblies, the TE, SV, and RNA-seq analyses, and where to find their information and data at MaizeGDB.

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P28

The maize PHG - a practical haplotype graph

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The maize practical haplotype graph (PHG) represents the pangenome as a graph of nodes and edges. The nodes represent haplotypes, and the edges represent physical connections between them. To construct the graph, the B73 V5 reference genome was divided into genic and intergenic intervals (called reference ranges). Each of the NAM parent assemblies was aligned to the reference ranges using mummer4. The sequence aligning to each reference range was stored as haplotypes. An important application of the PHG is to impute genomic sequence from skim sequence or SNP data by finding the most likely path through the haplotype graph, given the sequence data. To demonstrate the imputation method and gain insight into parameter settings, short read sequence data was simulated for B73, CML247, and Oh43. The resulting imputation accuracies are reported for different parameter combinations. With appropriate parameter choices, the haplotype accuracy was greater than 99%, and the SNP calling error rate was below 1E-6.

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P29

rTASSEL: an R interface to TASSEL for association mapping of complex traits

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The need for efficient tools and applications for analyzing genomic diversity is essential for any genetics research program. One such tool, TASSEL (Trait Analysis by aSSociation, Evolution and Linkage), provides many core methods for genomic diversity analyses. Despite its computational efficiency, TASSEL has limited means to use scripting languages for reproducible research and interacting with other analytical tools. Here we present an R package rTASSEL, a front-end to connect to a variety of highly used TASSEL methods and analytical tools. The goal of this package is to create a unified scripting workflow that exploits the analytical prowess of TASSEL in conjunction with R's popular data handling and parsing capabilities without ever having the user to switch between these two environments. By implementing this workflow, we are able to achieve performances ranging from 2 to 20 times faster than other widely used R packages for various functionalities.

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P30

Analysis of the interaction between proteins of the meiotic chromosome axis and AFD1 in *Zea mays*

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Synapsis is a very important process during meiosis that allow the two homologous chromosome axes to be closely linked longitudinally during the exchange of genetic information through crossovers. The synaptonemal complex has a zipper-shaped protein structure and is formed of several proteins identified in maize (AFD1, DSY2, ASY1, ZYP1) Arabidopsis and rice (P31, PCH2).
In this work, we evaluate the expression of five of the components of the orthologs of the synaptonemal complex (SC) components in maize (ZmASY1, ZmDSY2; ZmP31, ZmPCH2, and ZmZYP1) as well as the meiotic cohesin ZmAFD1. From the results obtained from the RT-PCRs and qRT-PCRs, we show that, although the structure of the chromosomal axis is exclusive to the meiotic process, its components are expressed in both somatic and meiotic organs at the transcriptional level. However, we observed an enrichment in the expression of these genes in female as well as male meiotic inflorescence. Two isoforms of ZmP31 were discovered. So far there are no reports of the existence of two or more isoforms of P31 in other organisms.
On the other hand, we selected four of the coding genes for proteins of the synaptonemal complex: ZmAFD1, ZmDSY2, ZmP31, and ZmPCH2. The complete coding sequences for ZmAFD1, ZmDSY2, ZmP31 (isoforms A and B), and ZmPCH2 were amplified and cloned into expression vectors for the yeast two-hybrid system (pGADT7GW and pGBKT7GW). Furthermore, employing yeast two-hybrid assays, we identify that the two isoforms of ZmP31 have different interaction properties. ZmP31B can interact with ZmAFD1, ZmDSY2, and ZmPCH2 while the shorter isoform ZmP31A do not interact with ZmAFD1. ZmAFD1 can interact with ZmDSY2, but neither of these interacts with ZmPCH2. We are checking these results using a BiFC independent test.

Gene / Gene Models described: *afd1, p31, pch2, asy1, dsy2, zyp1afd1, p31, pch2, asy1, dsy2, zyp1*; ZM06G32090, Zm00001d039133, GRMZM2G059037, ZM03G12660, Zm00001d040873, GRMZM2G701566, ZM10G18860, Zm00001d025687, GRMZM2G019621, ZM02G33240, Zm00001d006088, Zm00001d006089, GRMZM2G035996, ZM05G21020, Zm00001d015469, AC210848.3_FGT004, ZM10G17910, Zm00001d025575, GRMZM2G14359

P31

Cohesin subunits play important roles in the maize meiotic process

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Cohesin is an evolutionarily conserved multi-protein complex that plays a pivotal role in chromosome dynamics. The cohesin complex is essential for sister chromatid cohesion and for establishing higher-order chromosome architecture, and it also plays specific roles in various meiosis-associated chromosomal events. Cohesin complex comprises core proteins and regulatory proteins. The core cohesin ring includes SMC (Structural Maintenance of Chromosomes) proteins Smc1 and Smc3 and one kleisin alpha family protein SccC1 (Mcd1 or Rad21) and a stromalin domain protein Scc3 (SA1 and SA2). The regulatory proteins include sororin, Wapl, and Pds5. We have cloned the Smc3 in maize, and found that Smc3 was enriched in the centromeric region from leptotene to pachytene, a period of meiotic centromere pairing. These findings suggest that Smc3 takes part in the progression of meiotic centromere pairing. The smc3 mutants exhibited premature loss of sister chromatid cohesion, mis-segregation of chromosomes, and abnormal spindle morphology during mitosis and showed incomplete centromere pairing and altered chromosome structure during early meiotic prophase I. These loss-of-function phenotypes indicate that Smc3 functions not only in sister chromatid cohesion, but also in meiotic centromere pairing. We also cloned Smc1 and Pds5B from maize. Smc1 exhibited strong labeling along chromosome arms and was not enriched in the centromere regions during early meiotic prophase I. Pds5B was localized to chromatin as discontinuous dots and can be detected in the centromeric regions during early meiotic prophase I. However, the centromeric Pds5B signals were not enriched. These observations indicate that different cohesin subunits may play different roles during meiosis in maize.

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P32

Genetic mapping and mutagenesis analysis of the trans factor 1 required for the B chromosome non-disjunction in maize

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In maize, the drive mechanism of the supernumerary B chromosome allows it to maintain itself in populations. One of the components of the B chromosome drive is that it frequently undergoes nondisjunction at the second pollen mitosis, which produces one sperm with two copies of the B chromosome and the other sperm with no B. During this division, at least two trans factors are required for the unequal allocation of the B chromosome. Previous analyses located the trans factors on the B chromosome cytologically. Recently, a well-assembled B genome allowed us to map one of the trans factor (trans factor 1) to 2.7 Mb of distal euchromatin region that contains a total of 34 genes. Transcriptome analysis on lines with and without B chromosome using mature pollen showed that three out of 34 genes were expressed. Three EMS TB-9Sb recovered by Wayne Carlson in the 1970's that have lost nondisjunction were analyzed and found to have mutations or a deletion in each for Zm00044a000666, which is one of the genes expressed in mature pollen. An analysis of TB-9Sb mutant hybrids showed that these mutations do not recombine with each other indicating that they are likely in the same gene with no off-target lesions in another trans factor for nondisjunction. Finally, a Cas9 mutagenesis on Zm00044a000666 showed loss of non-disjunction in some kernels in the T0 progeny. Genotyping and genetic crosses for the mutated kernels from the T0 are underway to test whether Zm00044a000666 is one of the factors required for B chromosome non-disjunction.

Gene / Gene Models described: ; Zm00044a000666
Funding acknowledgement: National Science Foundation (NSF)

P33

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 is the same as in human cells. Cytogenetic observation in maize meiosis with a specific anti-ZWINT-1 antibody reveals that it localizes to centromeric regions from interphase to 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.

Funding acknowledgement: National Natural Science Foundation of China

P34

Bayer's SmartStax® PRO technology to launch in US in 2022 with industry first three modes of action for corn rootworm control, including a novel RNAi.

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SmartStax® PRO Technology is the next generation of corn rootworm protection, and the first product in Industry offering three modes of action for corn rootworm control. It combines the proven benefits of SmartStax® Technology corn rootworm protection with a novel RNAi-based mode of action, providing improved control of corn rootworm over a range of pressure. RNAi-based technologies are developed from a naturally occurring process in the targeted plant or pest to stop or decrease the production of a specific protein and can be used to target specific pests, like the corn rootworm. In addition to corn rootworm, SmartStax® PRO Technology will offer growers protection against European corn borer, southwestern corn borer, fall armyworm, black cutworm and corn earworm. Bayer plans to conduct on-farm grower market development trials in 2021, while ramping up volume to meet grower needs for a U.S. commercial launch in 2022.

P35

Building an educational network focused on community curation of plant genomes

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High-quality gene models with accurate gene structure are essential for scientists to design experiments (e.g., CRISPR) targeting specific genes. Traditionally, validating gene models requires manual curation, a labor-intensive and time-consuming process where individuals evaluate and correct computational predictions using experimental evidence. Undergraduate science majors are a largely untapped source of gene curators who are interested and motivated, especially when the curation is tied to a course activity. We have developed curriculum modules for students to curate computer-generated maize gene annotations. These modules range from one or two laboratory sessions to semester-long activities in the context of course-based undergraduate research experiences (CUREs) and senior thesis projects. Students learn about gene and genome structure, sequencing technologies, bioinformatics, and the natural variations of gene expression. We will describe examples of and reflections on teaching undergraduates to use and apply the Gramene Gene Tree visualizer and Apollo gene editor, report on the efficacy of this approach for community-based gene annotation, and provide recommendations for future use. This effort represented a timely opportunity to support teaching and research virtually. The method could be used as a means to enable undergraduate students to participate in the annotation of virtually any sequenced eukaryotic genome with biological evidence.

Funding acknowledgement: National Science Foundation (NSF)

P36

Employing the maize TFome to Foster the Integration of Research with Education (F.I.R.E.)

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Gene regulatory networks are central to all cellular processes including those that underly important agronomic traits. Maize is a model system for investigating the architecture of gene regulatory networks (GRNs) and the underlying gene regulatory grids (GRGs) in cereal crops. Previously we developed the Maize Transcription Factor ORFeome (TFome) to advance the study of regulomics in cereals. The first release of the maize TFome contained 2,034 clones corresponding to 2,017 unique Transcription factor (TF) and CoRegulator (CR) gene models in recombination-ready vectors. The maize TFome was first employed to build a protein-DNA-interaction (PDI) network for the phenylpropanoid pathway (Yang et al., 2017. Mol Plant, 10:498–515). While the maize TFome provides a powerful resource for basic research there is a growing recognition that in order to promote careers in the sciences it is important to let students have an opportunity to perform research during their undergraduate careers. At the University of Toledo we initiated an effort to *F*oster the *I*ntegration of *R*esearch with undergraduate *E*ducation (F.I.R.E.). Previously we involved undergraduate students in the development of the maize TFome collection. Here we describe the development of a new upper level undergraduate research lab that furthers the integration of research with education. In this lab, students first employed clones from the maize TFome collection to generate yeast-1-hybrid (Y1H) bait strains. They then conducted Y1H screens to discover and evaluate novel candidate protein-DNA interactions. The findings were then used by students to develop a group research proposal in the NSF format. Here we describe the course learning objectives and the experimental plans and resources required to implement this course. Student performance and reaction is evaluated. This project is funded by NSF grant IOS-1733633.

Funding acknowledgement: National Science Foundation (NSF)

P37

Maize genetics Committee on Outreach, Diversity, Inclusion, and Education (CODIE) 2020-2021 update

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During the 2020 Maize Genetics Meeting, the newly formed Maize Genetics Cooperation (MGC) gave a statement responding to the issues related to the Black Lives Matter movement, highlighting the need for active pursuit of decisive steps to counter systemic racism and injustice. Subsequent discussions within the maize community led to the formation of the standing Committee on Outreach, Diversity, Inclusion, and Education (CODIE) of the MGC. This committee of volunteers is charged with fostering a diverse and inclusive community of maize genetics and genomics researchers. CODIE seeks to do this by increasing the equitable representation of people based on race, gender, physical ability, sexual orientation, and any other identity attributes and acknowledging their important contributions, within the Maize Genetics Cooperation (MGC) and its leadership; within the area of Maize Genetics; and within the broader field of STEM education, research, and industry. In its first year, CODIE took initial steps towards its mission including (1) formal formation of the committee and charge document; (2) compiling a recommended reading list and funding opportunities, disseminated via MaizeGDB/webpage/internet; (3) developing two new community awards to recognize significant efforts in this area; (4) sponsoring a series of "21-Day Racial Equity Habit Building Challenges" (5) expanding funding and mentorship opportunities for students; and (6) creating diversity and inclusion events at the Maize Genetics Meeting including the "Fostering Diversity in the Maize Research Community" lecture. To learn more about CODIE, including how to get involved, please visit https://maizegdb.org/mgc/outreach/, stop by the virtual poster, or meet current members at the conference networking session.

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

P38 @tross_michael

3D reconstruction of sorghum plants enables leaf by leaf GWAS and identifies orthologs of maize leaf angle genes

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Plant canopy architecture influences light interception, photosynthetic productivity and yield potential. More erect leaves allow for denser planting of crops as well as distributing light through the canopy in such a way as to improve the efficiency of photosynthesis. A number of genes in maize have been identified as contributing to variation in leaf angle. However, due to the labor-intensive nature of manual measurements of leaf angle, significantly fewer genes have been linked to genetic variation in leaf angle in other cereal crops such as sorghum. Here we present the automatic measurement of leaf angles estimated from skeletons derived from 3D reconstructed sorghum plants using voxelization. Measurements derived from this approach is highly heritable, estimated at 70% for the measured population. The values measured from 3D reconstructions of sorghum plants are correlated with manual leaf angle measurements of the same genotypes at the same stage of development in controlled environment conditions. Genome wide association study using the median leaf value of leaves 1 to 4, detected SNP markers that significantly contribute to variation in leaf angle including the *Dwarf3* gene which is a known contributor of genetic variation of leaf angle in sorghum. Leaf by leaf GWAS of the first three individual leaves also detected the *Dwarf3* gene as well as a number of novel loci. Leaf angle genes in maize, a close relative of sorghum, are plausible candidate genes for regulating the same trait in sorghum. Two of the novel GWAS hits for leaf angle in sorghum are adjacent to the sorghum orthologs of maize genes known to contribute to leaf angle variation.

Gene / Gene Models described: *dwarf3*; Sobic.007G163800
Funding acknowledgement: United States Department of Agriculture (USDA), FFAR

P39

A drone-based high-throughput phenotyping pipeline to estimate time-series nitrogen responses in maize

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Advancements in the use of genome-wide markers and advanced statistical modeling have provided new opportunities for dissecting the genetic components that control phenotypic trait variation. However, cost-effectively characterizing agronomically important phenotypic traits on a large scale remains a bottleneck. Unmanned aerial vehicle (UAV)-based high-throughput phenotyping has recently become a prominent method, as it allows large numbers of plants to be analyzed in a time-series manner. However, there has been less focus on using this technology for plot-level, genotype-specific analysis. In this experiment, 233 lines from the maize association panel were grown in a replicated incomplete block design both following conventional agronomic practices and under nitrogen limited conditions. UAV images were collected during different plant developmental stages through the season. A pipeline for extracting plot-level images, filtering images to remove non-foliage elements, and calculating greenness ratings based on vegetation indices was developed. This time-series rating data can track a crop's development through the season, assess whether a given genotype is under nitrogen stress as a function of phenology, and may also provide correlations with key developmental stages, such as the onset of flowering, kernel maturity, and ultimately grain yield. The plot-level time-series phenotypic data obtained from this experiment provide great opportunities to advance plant science and to facilitate plant breeding.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), National Institute of Food and Agriculture (NIFA), Experimental Program to Stimulate Competitive Research (EPSCoR)

P40 @LabYerka

A new sorghum MAGIC population for genome-to-phenome investigations of grain end use qualities

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Public interest in gluten-free foods and beverages has increased dramatically in recent years. Grain sorghum [*Sorghum bicolor* (L.) Moench] is a close relative of maize [*Zea mays* L.] and a common ingredient in many gluten-free products. However, the genetics underlying various grain quality traits (e.g. protein quantity, quality, and digestibility; starch digestibility, aroma, pericarp color, endosperm color, endosperm texture, seed size), and the influence of specific alleles on end-use qualities (e.g. baking, malting, brewing, animal feed), are not well-understood. A grain quality MAGIC population was recently developed at the University of Nevada, Reno to facilitate genome-to-phenome (G2P) investigations aimed to clarify relationships between specific alleles, production environments, and grain end-use qualities of sorghum. The eight parents included Wheatland *waxy*, Tx430 *waxy*, Tx623, KS115, Dwarf White Milo, Sureño, P850029, and Early Hegari; and were selected to generate a set of progeny that would segregate for numerous combinations of alleles associated with different end uses. The population is now in the first selfing generation and a final set of ~1200 lines will be selected in Spring 2021. Multi-environment testing of some lines has already begun in Argentina and will continue in the U.S. in Summer 2021. The population is being genotyped using a novel whole-genome targeted resequencing strategy that includes both trait-linked markers and SSR loci to enable estimates of genetic distance among the selected lines. Phenotyping for grain end-use qualities includes wet chemistry and NIRS followed by micro-malting and micro-brewing at the University of California-Davis Department of Food Science and Technology. MAGIC populations are ideal for G2P studies because multi-parental alleles segregate in similar genetic backgrounds, which makes testing their impact on end-use qualities in different production environments possible. This new population is an important resource for sorghum molecular breeding and comparative genomics investigations of seed developmental pathways.

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

P41

A screen for parthenogenetic mutants in maize

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Apomixis, the asexual reproduction through seeds, offers a way to fix any complex genotype for an unlimited number of generations. If incorporated into modern breeding approaches, apomixis would allow the perpetual propagation of F1 hybrids, which otherwise have to be generated each year anew by crossing inbred lines. Apomixis does not naturally occur in major crop species or their relatives and can thus not be harnessed by introgression. A viable alternative is the engineering of synthetic apomixis in crops by knocking out key genes of the sexual pathway and/or ectopically expressing genes of the apomictic pathway. Gametophytic apomixis differs from the sexual reproduction in three major steps: (a) formation of an unreduced and unrecombined gametophyte – apomeiosis; (b) development of an embryo without fertilisation – parthenogenesis; and (c) functional endosperm development. While apomeiosis can be induced in various ways, no mutants causing parthenogenetic development have been identified to date , with the exception of *indeterminate gametophyte1*, which produces parthenogenotes at very low frequency. To close this gap, we performed a mutant screen to identify *parthenogenetic* (*par*) mutants in maize. We screened EMS mutagenized populations as well as populations in which *Mutator* activity was induced for the development of maternal haploid embryos in crosses with wild-type pollen. We identified a promising *par* mutant and will present its genetic characterization.

Funding acknowledgement: Swiss National Science Foundation (SNF)

P42

An efficient method to segment pictures from FASGA stained stem cross section to highlight variation of histological profiles between maize genotypes.

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Maize stem can represent up to 50% of the plant dry matter harvested at silage stage and it has been shown that stem digestibility is correlated to stover digestibility. Biochemically, *in vitro* digestibility of dry matter (IVDMD) is linked to cell wall composition and structure. Histologicaly, at the internode level, we recently shown that *in vitro* digestibility is also linked to distribution of lignified tissues. This has been achieved by analyzing images of FASGA stained cross sections of internodes, where Alcian blue stains poorly lignified tissues in blue whereas Safranin stains highly lignified tissues in red. Analyzing obtained images remains challenging to accurately segmented and characterized the different types of tissues (schlerenchyma, bundle, parenchyma) in rind and in pith regions of maize internodes. Herein, we present an ImageJ/Fiji plugin that segments images by attributing each pixel according to their HSV (i.e. Hue, Saturation and Value) values and their position in the cross section. Hence, each region is defined and characterized (area, number of particles, RGB & HSV values). Advantages of this method reside firstly in the fact that delimitation of the different regions (i.e. rind and pith) in the cross section is more accurate than in previously proposed methods; secondly, it allows to highlight differences of pixel characterization in each tissue; thirdly, the throughput of analysis is enhanced mainly due to the fact that no need for parameterization is required for each image, allowing also a direct comparison between different images acquired. All these advantages make this tool suitable for genetic analyzes carried out on populations of several tens / hundreds of individuals and to identify the histological traits in link with digestibility.

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P43 @chase_krug

Analysis of plant height in Zea mays

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The genetic diversity between two parental lines of maize can influence heterosis. Heterosis is the process in which traits in the offspring surpass those of parents. However, correlation between heterosis and the cross direction (i.e., which line is used as male and which as female) and genetic distance remains unknown. All possible combinations of four maize groups (flint, tropical, stiff-stalk and non-stiff stalk), spanning a range of genetic distance were crossed to one another, resulting in 16 distinct cross types. We directly tested the effect of maternal and paternal background and genetic distance on heterosis using a diallel experiment, which is a mating scheme in which lines are crossed in all possible directions. The diallel consisted of 158 crosses which were replicated in three blocks. Mid-parent and high-parent heterosis were calculated using both plant height and ear height data. Mid-parent heterosis is when plant height of the cross is greater than the average height of both parents. High parent heterosis is when height of the cross is greater than that of the best performing parent. Heterosis was compared using statistical contrasts which test the significance of the direction of the cross and different combinations of heterotic groups. Our results indicate significant mid-parent/high-parent heterosis across all heterotic groups. Additionally, specific reciprocal crosses show a significant difference in mid-parent/high-parent heterosis. Statistical analyses were conducted, and plots were created in the R programming language. Our increased understanding of the phenomenon of heterosis will facilitate prediction of its occurrence in, for example, cultivar improvement.

Funding acknowledgement: National Science Foundation (NSF)

P44

Constrained non-coding sequence provides insights into regulatory sequence and loss of gene expression in maize

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DNA sequencing technology has advanced so quickly that in order to understand how genomes work, it is necessary to identify key functional regions using evolutionary approaches. This research develops a sensitive sequence alignment implementation to identify functional constrained non-coding sequences in the Andropogoneae tribe. The grass tribe Andropogoneae contains several crop species descended from a common ancestor ~18 million years ago. Despite broadly similar phenotypes, they have tremendous genomic diversity with a broad range of ploidy levels and transposons. These features make the Andropogoneae a powerful system for studying conserved non-coding sequence (CNS); here we used it to understand the function of CNS in maize. We found that 85% of CNS comprise putative functional elements (e.g., intron, UTR, cis-regulatory sequence, chromatin loop anchor, non-coding RNA, several transposable element superfamilies). CNSs are enriched in DNA replication active regions in early S phase of the mitotic cell cycle and have different DNA methylation ratios compared to whole genome background. More than half of putative regulatory sequences overlap with identified CNSs. We show that CNSs regulate gene expression, variants in CNS are associated with phenotypic variance, and CNS absence contributes to loss of gene expression. Furthermore, we find the evolution of CNS is associated with the functional diversification of duplicated genes in the context of the maize subgenomes. Our results provide a quantitative understanding of constrained non-coding elements in maize.

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P45

Cooperate or Compete: modeling competitive traits in maize

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Competition for resources occurs across all ecosystems, as resource capture plays a fundamental role in the survival of an individual. The sessile nature of plants leaves them no exception: plant-to-plant competition plays a major role in the success of an individual in terms of survivorship and seed production. In agriculture, competitive plants can lead to yield loss due to shading of neighbors and uneven acquisition of resources. Maize has been selected for plot-level yield, and as a result, has led to increased stand densities and field uniformity. However, despite a century of breeding, extensive yield variation still occurs across a field of genetically identical individuals. The presence of specific neighbors can have a direct phenotypic effect on an individual within a population, potentially causing some of this variation. These measurable differences, called associated effects, can be interpreted as heritable environmental effects in quantitative genetics. The question remains as to what extent traits such as plant height, leaf angle, and root architecture play a role in a plant's competitive ability. In this study, we used data from the maize Nested Association Mapping (NAM) population across 11 environments to model which phenotypic traits have the largest impact on the phenotype of neighboring individuals.We hypothesize that plants will have varying degrees of competitive ability and impact on their neighbors' level of fitness. By understanding the impact of each trait, predictions can be made for competitive ability of other genotypes, and extended to additional populations. Understanding competitive ability can benefit plant breeders by identifying lines with negative or positive traits related to competition that could be leveraged to make greater selection gains.

Funding acknowledgement: United States Department of Agriculture (USDA)

P46

Development of an index for remotely sensing physiological maturity in maize (*Zea mays* L.)

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Physiological maturity in maize is reached at the developmental stage referred to as black layer, where an abscission zone forms blocking photosynthates from moving into the developing kernels from the vegetative tissues. Currently there is no high-throughput field-based phenotyping method available for detecting black layer, although remotely sensed spectral data may offer a solution to this problem. The aim of this project is to develop a vegetation index that can accurately predict when a plant reaches black layer that is repeatable across genotypes, different environmental conditions and different senescence patterns. Hyperspectral data was collected of the maize canopy during the late grain filling period until approximately 2 weeks after black layer formation for 16 genotypes exhibiting a range of senescence patterns over two growing seasons. This data was analyzed using a random forest machine learning algorithm, where important wavelengths were determined and used to predict physiological maturity with a high degree of accuracy. Using the wavelengths identified by random forest, several potential indices were examined, resulting in a vegetation index that has a greater than 80% accuracy of predicting if a plot has reached physiological maturity. The wavelengths used in the index can be easily adapted into current commercial sensors and attached to high-throughput phenotyping platforms such as drones and high clearance tractors to aid maize breeders in making selections based on physiological maturity.

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P47
Differential regulation of maize and sorghum orthologs in response to the fungal pathogen *Setosphaeria turcica*

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Host jumps are a threat to food security, and host resistance is critical for ensuring food security. *Setosphaeria turcica* infects both maize and sorghum and the isolates are host-specific, offering a unique system to examine compatible and incompatible interactions. We hypothesized that resistance mechanisms are conserved between hosts. We conducted transcriptional analysis of maize and sorghum in response to maize-specific and sorghum-specific *S. turcica* isolates and identified functionally related co-expressed modules. Maize had a larger transcriptional response than sorghum. *S. turcica* responsive genes were enriched in core orthologs in both crops, but only up to 16% of core orthologs showed conserved expression patterns. Most changes in gene expression for the core orthologs, including hub genes, were lineage-specific, suggesting that resistance in maize and sorghum evolved largely independently, possibly driven by regulatory divergent evolution. We identified several defense-related shared differentially expressed orthologs with conserved expression patterns between the two crops, suggesting a role for parallel evolution of those genes in both crops. Many of the differentially expressed genes during the incompatible interaction were related to quantitative disease resistance. This work can inform how to engineer an incompatible interaction and offer insights into how different hosts with relatively recent divergence interact with a common pathogen.

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P48 @DrSethMurray
Discovery of temporal loci controlling segregation of vegetation Indices through maize hybrid growth

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Field based high-throughput phenotyping has a potential to supply numerous temporal vegetation indices to plant breeding and genetics in maize that are calculated quantitatively depending on the reflectance of the green biomass of maize hybrids through the growth stages. This study used 280 maize hybrids from the maize Genomes to Fields (G2F) project grown in College Station, Texas 2017 as a randomized complete block design. Eight quality flight dates (27, 34, 41, 48, 55, 63, 88 and 103 days after planting) were used among the biweekly flights to extract 24 different vegetation indices. Breeding values of the hybrids were predicted within flight dates jointly (nested design; hybrids nested within flight dates) for each vegetation index to generate temporal breeding values. In total, 192 (24 indices times 8 time points) phenotype data were used in a genome wide association study (GWAS) to discover the loci controlling vegetation index segregation. Genotyping by sequencing data of 158 maize hybrids were associated with their 192 temporal vegetation index breeding values in GWAS. 63 loci belonging to chromosomes 1, 2, 3, 4, 5, 6, 7, 8 and 9 were discovered commonly. These loci were discovered at multiple time points of any one vegetation index, or in different times of more than one vegetation index. The remaining 184 loci were discovered for specific times during growth. Since temporal breeding values of vegetative indices were found to be correlated with grain yield in maize, underlying loci of vegetative indices can be used to manipulate grain yield in maize under different environmental conditions.

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P49 @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. Multi-parent 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.

P50

Diurnal fluctuations in turgor pressure impart negligible effects on sweet sorghum stalk strength

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Stalk lodging is a naturally occurring phenomena resulting from a structural failure of grain stalks leading to an estimated 5-20% reduction in annual, global grain yields. While our understanding of the driving forces of stalk lodging resistance is still developing, recent studies have primarily sought to explore how stalk composition and morphology drive stalk lodging resistance. Until this past year, there have been no studies examining how stalk physiological responses might also help drive lodging resistance. Using sweet sorghum as a model, a newly developed phenotyping device capable of accurately measuring stalk strength was used to measure stalk lodging resistance within three developmentally different lines of sweet sorghum. To better understand how naturally occurring diurnal fluctuations in turgor pressure drive stalk lodging resistance, stalk strength was measured over multiple 48-hour lodging intervals across key developmental stages spanning two years. Naturally occurring diurnal variation in turgor pressure (observed to range between 0.4MPa – 4.9 MPa) were found to impart a negligible, yet statistically significant effect on the mechanical attributes associated with stalk lodging resistance in fully mature sweet sorghum stalks. No such effect was observed in younger, developing stalks. The results of this study suggest that diurnal fluctuations in turgor pressure can safely be ignored in assessments of stalk strength.

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P51
Elemental profiling in maize to understand genotype-by-environment interactions

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The seed elemental profile of a plant is the cumulative result of an individual's genetic makeup, or genotype, and the environmental conditions throughout a growing season. Predicting the phenotypic response between environmental stimuli and genotype presents a major challenge to plant scientists and breeding programs. It is critical to understand these genotype-by-environment interactions (GEI) to improve genotype predictions ensuring highly productive lines are plant in each environment ultimately increasing crop productivity with fewer synthetic inputs. As part of the genomes to fields (G2F) initiative, seeds for ionomic elemental profiling were analyzed with high throughput inductively coupled plasma mass spectrometry (ICP-MS) from a diverse set of maize inbred lines across multiple years and locations. Patterns of genotype-by-environment interactions (GEI) were explored using a combination of modeling techniques to gain a holistic view of the relationships between phenotype components. Stable genotypes between locations and years were selected with the use of regression analysis and additive main effect (AMMI) models. Additionally, factorial regression modeling was applied to identify specific environmental terms influencing the phenotypic response. Future works aims to first validate genomic predictions obtained from the applied models with the use of an incomplete companion dataset, collected with the same years and locations. Second, this dataset will be applied to inform predictions made between mapping populations and multiple environment trials.

P52 @MerrittBurch
Elucidating the extent of pleiotropy in maize and its functional relevance towards trait prediction

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Pleiotropy, due to its magnitude and prevalence in maize, has been shown to have widespread effects in traits such as flowering time, leaf architecture, and inflorescence morphology. However, the genome-wide impact that pleiotropy has across all maize phenotypes is largely unknown. This project investigates the extent to which pleiotropy impacts phenotypes within maize through GWAS summary statistics reanalyzed from previously published physiological, morphological, metabolic, and expression phenotypes across the Nested Association Mapping population (NAM) and Goodman Association Panel. 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 in our association models. From our GWAS reanalysis of approximately 250,000 different traits in maize, we obtained over a billion SNP-trait relationships. We hypothesize that although pleiotropy is common across maize genomes, inferring true pleiotropy and casual relationships from statistical noise is confounded by linkage disequilibrium and other factors. Knowledge of how the pleiotropic nature of loci impacts traits has applications in plant breeding programs to further understand the genotype to phenotype landscape, uncover interactions between functional loci, and potentially improve trait prediction models.

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

P53 @SilvioSalvi2

Gaspé Flint 1.1.1, a small-size early-flowering maize inbred line

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In several plant species, early flowering genetic stocks have been selected and utilized as models for scientific, breeding, and educational purposes. We developed Gaspé Flint 1.1.1 (GF111), a new rapid cycle, diminutive maize inbred line. GF111 was developed by repeated selfing and selection starting from an open pollinated accession of the early flowering Canadian landrace Gaspé Flint. GF111 can be grown in 1-L pots, is characterized by short stature (0.4 - 0.8 m), small number of leaves (8.1 - 9.7), two ears per plant with only one being fertile, from zero to one tiller, early flowering (41 - 50 days after sowing and as little as 357 growing degree units), and very limited proterandry. High-density molecular marker analysis showed a high level of homozygosity (> 96%) and an approx. 30 Mb-long region shared with the maize reference line B73 on chromosome 10. Thanks to its high homozygosity, rapid generation cycle, easy hand-based pollination, good fertility, and limited growing requirements as to space and resources, GF111 has great potential to be adopted in genetic and gene functional studies, phenotypic screenings on phenomics platforms, and for teaching activities.

P54

Genetic analysis of field measured data from Genomes to Fields in Michigan

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In 2018 and 2019, as a collaborator of the Genomes to Field's initiative, we measured standard plant morphology and productivity traits in 500 partially replicated plots of hybrid maize. In addition, we assessed ear leaf number and total leaf number in the hybrids for eventually parameterizing physiological models. We investigated the genetics basis of these quantitative traits and the ability to predict them from genotypic information using a genome-wide association study (GWAS). We used the rMVP package to execute GWAS and the BGLR package with three different models to execute genomic prediction for these traits for 2018 and 2019. No associated SNPs were discovered in 2019, possibly due to decreased power from fewer lines or environmental effects. In 2018, several significant SNPs were found in ear height, plant height, ear leaf number, total leaf number, anthesis and silking. In genomic prediction, we have found similar performance of the three models in both years. The prediction accuracy was around 0.5-0.7 for both height and leaf number traits. The flowering traits showed surprisingly low prediction accuracy despite high heritability.

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P55

Genetic analysis of new maize hybrids for yield and resistance to aflatoxin accumulation

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Maize (*Zea mays* L.) is the most important cereal crop in sub-Saharan Africa which provides food for more than its 1.2 billion inhabitants. However, production is hampered by many factors including low yields and aflatoxin contamination. The toxin contaminates maize during pre-harvest as well as during storage. Grains with contamination levels above 20ppb are destroyed. Ghana lacks regulatory infrastructure for monitoring and detecting aflatoxin in grains prior to market, moreover most of the local maize varieties have been found to be susceptible to aflatoxin accumulation. Host resistance is envisaged as key approach in addressing the aflatoxin menace Sixteen aflatoxin resistant inbreds sourced from CHPRRU in Mississippi, USA CIMMYT, IITA, etc. were crossed as males to six local inbreds, two populations, in a North Carolina II design to generate 160 new hybrids and planted together with 9 checks using 13 x 13 lattice. The new hybrids were evaluated across six environments in two seasons. Five plants each per hybrid were inoculated with a local strain of *Aspergillus flavus* inoculum a week after 50% mid silking at a concentration of 9 x 10^7conidia/ml. Statistical analysis showed significant effect of environment and genotypes for all traits especially aflatoxin accumulation resistance and yield. The general combining ability effect of males for all traits were found significant (P

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P56

Genetic architecture of kernel compositional variation in a maize diversity panel

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Maize (*Zea mays* L.) is a multi-purpose row crop grown worldwide, which overtime has often been bred for increased yield at the detriment of lower composition grain quality. Some knowledge of the genetic factors that affect quality traits has been discovered through the study of classical maize mutants. However, much of the underlying genetic architecture controlling these traits and the interaction between these traits remains unknown. To better understand variation that exists for grain compositional traits in maize, we evaluated 501 diverse temperate maize inbred lines in five unique environments and predicted 16 compositional traits (e.g. carbohydrates, protein, starch) based on the output of near-infrared (NIR) spectroscopy. Phenotypic analysis found substantial variation for compositional traits and the majority of variation was explained by genetic and environmental factors. Correlations and trade-offs among traits in different maize types (e.g. dent, sweetcorn, popcorn) were explored and significant differences and correlations were detected. In total, 22.90-71.09 percent of the phenotypic variation across these traits could be explained using 2,386,666 single nucleotide polymorphism (SNP) markers generated from whole genome resequencing data. A genome-wide association study (GWAS) was conducted using these same markers and found 70 statistically significant loci for 12 compositional traits. This study provides valuable insights in the phenotypic variation and genetic architecture underlying compositional traits that can be used in breeding programs for improving maize grain quality. **disclosure/disclaimer** This study was funded by PepsiCo R&D. The views expressed in this abstract are those of the authors and do not necessarily reflect the position or policy of PepsiCo, Inc."

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P57

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. 800 total maize genotypes were screened and rated from the Wisconsin Diversity panel and Iowa State's Germplasm Enhancement of Maize (GEM) program. The experiment was planted in replicated field trials in 2019 in Michigan and 2020 in Michigan, Wisconsin, and Indiana using natural pressure as inoculum. Disease ratings were taken at six timepoints as a percent disease coverage of the ear leaf. 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 third year of field trial data to validate candidate resistance, while working to introgress resistance to tar spot into more elite temperate varieties.

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P58

Genetic variability of the expression of plasma membrane aquaporins in maize leaves: from eQTLs to characterization of cis- and trans-acting regulatory factors

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Aquaporins are channel proteins facilitating the movement of water and other small solutes across biological membranes. They are involved in multiple cellular and physiological processes and play indisputable roles in the adaptation to the environment. While the dynamics of aquaporin genes expression profiles over daytime, organs, development stages and in response to environmental cues, has been extensively reported, descriptions of some precise molecular components of this regulation are scarce.Measurements of gene expression of five plasma membrane aquaporins (*PIP* genes) in maize leaves (elongation and mature zones) across a 252-hybrid panel (DROPS panel) by RT-qPCR revealed the existing diversity and pointed at different profiles of variability that imply different effects of selection over time on different aquaporin isoforms. GWAS and covariate GWAS allowed mapping numerous eQTLs, both local and distant. The implementation of a double fluorescent promoter activity reporter assay, transformed by biolistic into entire maize plants, allowed the evaluation of both *cis* and *trans*-acting regulatory candidates. Among them, a MITE-containing indel within a *PIP* gene promoter region, and a distant NAC transcription factor, were successfully identified as actors of the *PIP* gene regulation. Further steps should be directed to reveal the physiological relevance of such regulations.

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P59 @Jonas_Ro_riguez

Genome-wide association reveals candidate genes for saccharification efficiency and stalk anatomical features in maize

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Previous work in plants, including maize (*Zea mays* L.), has demonstrated the potential for utilizing lignocellulosic biomass as a feedstock for biofuel production. Stalks are among the most recalcitrant tissues in physiologically mature maize plants and present complex anatomical features. The relationship between saccharification and anatomical traits have not been fully investigated. This study aims to assess the relative contribution of anatomical characteristics in maize stalk tissue on cell wall bound sugar release, and to identify genomic regions associated with these traits. Using a set of 652 diverse maize inbred lines and a nested association mapping population consisting of 2,700 recombinant inbred lines from 20 bi-parental families sharing a common parent, we performed enzymatic hydrolysis assays to quantify glucose and pentose release as proxies for saccharification efficiency in physiologically mature stalk internodes. Additionally, we measured ten stalk anatomical features in the internodes of the 652 diverse inbred lines by applying image analysis methods. We found substantial genotypic variation with moderate to high (0.53 – 0.89) entry mean heritabilities for saccharification efficiency and stalk anatomical traits. A multiple linear regression model with the stalk diameter of the long radial axis and rind area as explanatory variables was able to account for 16% of the observed variation for glucose release. For pentose release, a model including the long radial axis stalk diameter and vascular bundle area was able to account for 19% of the observed variation. Genome wide association studies for stalk saccharification and anatomical traits identified 76 significantly associated single nucleotide polymorphisms (SNPs) across all traits, with a single SNP explaining up to 10% of the phenotypic variance observed for a given trait. Of the SNP peaks identified, 7 were associated with more than one trait and at most four. This study demonstrates the utility of leveraging natural variation and mapping approaches to investigate key traits which define superior maize varieties.

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P60

Genomic selection to optimize doubled haploid-based hybrid breeding in maize

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Crop improvement, as a long-term endeavor, requires continuous innovations in technique from multiple perspectives. Doubled haploid (DH) technology for pure inbred production, which shaves years off of the conventional selfing approach, has been widely used for breeding. However, the final success rate of *in vivo* maternal DH production is determined by four factors: haploids induction, haploids identification, chromosome doubling, and successful selfing of the fertile haploid plants to produce DH seeds. Traits in each of these steps, if they can be accurately predicted using genomic selection methods, will help adjust the DH production protocol and simplify the logistics and save costs. Here, a hybrid population (N=158) was generated based on an incomplete half diallel design using 27 elite inbred lines. These hybrids were induced to create F1-derived haploid families. The hybrid materials, as well as the 27 inbreds, the inbred-derived haploids (N=200), and the F1-derived haploids (N=5,000) were planted in the field to collect four DH-production traits, three yield-related traits, and three developmental traits. Quantitative genetics analysis suggested that in both diploids and haploid families, most of the developmental traits showed high heritability, while the DH-production and developmental traits exhibited intermediate levels of heritability. By employing different genomic selection models, our results showed that the prediction accuracy ranged from 0.52 to 0.59 for the DH-production traits, 0.50 to 0.68 for the yield-related traits, and 0.44 to 0.87 for the developmental traits. Further analysis using index selection achieved the highest prediction accuracy when considering both DH production efficiency and the agronomic trait performance. Furthermore, the long-term responses through simulation confirmed that index selection would increase the genetic gain for targeted agronomic traits while maintaining the DH production efficiency. Our study provides an optimization strategy to integrate GS technology for DH-based hybrid breeding.

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P61 @chen_qiuyue

Harnessing knowledge from maize and rice domestication for new crop breeding

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Crop domestication has fundamentally altered the course of human history, causing a shift from huntergatherer to agricultural societies and stimulating the rise of modern civilization. A greater understanding of crop domestication would provide a theoretical basis for how we could improve current crops and develop new crops to deal with environmental challenges in a sustainable manner. Here, we provide a comprehensive summary of the similarities and differences in the domestication processes of maize and rice, two major staple food crops that feed the world. We propose that maize and rice might have evolved distinct genetic solutions toward domestication. Maize and rice domestication appears to be associated with distinct regulatory and evolutionary mechanisms. Rice domestication tended to select *de novo*, loss-of-function, coding variation, while maize domestication more frequently favored standing, gain-of-function, regulatory variation. At the gene network level, distinct genetic paths were used to acquire convergent phenotypes in maize and rice domestication, during which different central genes were utilized, orthologous genes played different evolutionary roles, and unique genes or regulatory modules were acquired for establishing new traits. Finally, we discuss how the knowledge gained from past domestication processes, together with emerging technologies, could be exploited to improve modern crop breeding and domesticate new crops to meet increasing human demands.

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P62

High throughput phenomics approach for developing drought resilience in maize inbred lines

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Phenomics is an emerging science aimed at non-destructive methods that allow large scale screening of genotypes, thereby complementing genomic efforts to identify genes relevant for crop improvement under both favorable and unfavorable environments. Thirty maize inbred lines from different sources (exotic and indigenous) maintained at Dryland Agriculture Research Station (SKUAST- Kashmir) were chosen for the study. In the automated conveyer for plant transport and imaging systems (the ICAR-NIASM LemnaTecScanalyzer system for large plants), top and side view images were taken of the VIS and NIR range of the light spectrum. The Lemnagrid Integrated Analysis software for high-throughput plant image analyses was used for image-based plant feature extraction.
Image processing is divided into two major parts: image segmentation and feature extraction. All thermal images were obtained with a thermal imager (Vario CAM hr Inspect 575, Jenoptic, Germany). The results introduced a dataset of 30 maize inbred lines. Images were collected daily for 11 days. Imaging started one day after shifting the pots from the greenhouse. Different surrogates were estimated in the study such as area, plant aspect ratio, convex hull ratio, caliper length, etc. A strong association was found between canopy temperature and above ground biomass under stress conditions. Lines showing promise in different surrogates should be crossed with locally adapted lines to develop mapping populations for traits of interest related to drought resilience, in terms of improved tissue water status and map genes/QTLs of interest.

Funding acknowledgement: United States Department of Agriculture (USDA)

P63

High-throughput phenotyping of a genetically characterized maize introgression library provides insight on the relationship between root system architecture and water use efficiency

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Water deficit remains one of the major factors limiting maize yield and its stability. Although genetic variation for water-use efficiency has been identified in breeding materials, its physiological components and genetic basis are still largely unknown. Root system architecture has a key role in determining the plant capability to intercept and uptake water from the soil. Furthermore, root/shoot allometric relationships are widely considered as one of the adaptive traits involved in cereal domestication. In this experiment, a high-throughput phenotyping platform was used to evaluate water balance traits in a maize introgression library originated from an elite × landrace cross (B73 × Gaspé Flint) grown to adult vegetative phase, at well-watered and water-stressed regimes. The heritability of biomass accumulation per day, water-use and water-use efficiency, transpiration, early vigor and leaf appearance rate ranged from 0.77 to 0.93. A negative correlation was evidenced between root/shoot ratio at seedling stage and biomass accumulation or water-use efficiency (r from −0.20 to −0.45, respectively). QTL mapping identified seven major QTL clusters affecting shoot growth and/or water-use efficiency, two of which (at chromosome bins 1.01 and 8.04-5) overlapped with previously mapped QTL for root biomass and are known to include root developmental genes. Correlation and QTL mapping results support the hypothesis that genetically-controlled, reduced root/shoot biomass ratio at the seedling stage positively affects maize grown in relatively high-input conditions with no or limited water constraints.

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P64

High-throughput phenotyping plant height in sorghum with UAV

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Plant height is a critical agronomical trait, and understanding its genetic control is important for breeding high-yielding cultivars. This requires the use of robust, efficient, and economical processes to accurately genotype and phenotype large plant populations. While recent advances in sequencing technology have made this possible for genotyping, the development of a low-cost and high-throughput field-based phenotyping technology is still a work-in-progress. In this study, we used a small unoccupied aerial vehicle (UAV) to acquire ultra-high-resolution RGB imagery and estimate plot-based plant height for a population of 243 recombinant inbred lines of sorghum. Aerial surveys were conducted at 42, 63 and 106 days after planting. Each image collection was orthomapped and used to build a digital surface model (DSM). Next, the elevation of bare ground was interpolated from the DSM with the least vegetation cover to obtain a digital terrain model that served as the base level for plant height estimation. Strong and positive Pearson correlation of up to 0.89 was observed between UAV and manual measurements. The 3D reconstruction of the field successfully captured the genotypic differences in height and genome scans of UAV-estimated plant height detected strong genetic signals. The signals of plant height genes *Dw1*, *Dw3* and q*HT7.1* were consistent throughout the season while a new signal detected on chromosome 2 was growth stage specific. These results suggest that the high spatial and temporal resolutions of UAV imagery combined with modern genomic technologies can be leveraged to decipher the genetics of a complex and dynamic trait such as plant height.

Funding acknowledgement: National Science Foundation (NSF), Iowa State University Raymond F. Baker Center for Plant Breeding, Iowa State University Plant Sciences Institute

P65

How does maize respond to heat stress: An old question with an expanded study scope

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Heat stress is a growing threat to agricultural production worldwide. It is imperative to understand maize heat response mechanisms to accelerate the development of heat-tolerant cultivars. The overall goals are: 1) to quantify natural variation of heat stress response and identify underlying regulatory mechanisms; 2) to identify genetic loci that contribute to various heat stress related phenotypes. To that end, we are combining whole-genome transcriptome profiling, lipidome profiling, and extensive physiological characterization of diverse maize inbred lines, and targeted genetic mapping with biparental populations to establish a clearer understanding of heat stress response. Twenty-seven representative heat tolerant and susceptible inbred lines were selected from a maize diversity panel following multiple years of extensive observations under well-watered conditions in Lubbock, Texas. For transcriptome profiling, 3' mRNA sequencing method was performed using 432 leaf samples from 27 inbred lines, collected at 4 time points from developing and mature leaves in a greenhouse heat stress experiment. Preliminary results identified differentially expressed genes (DEGs) ranging from 4000 to 7000 for two distinct heat response groups (tolerant and susceptible) at each time point. Gene ontology analyses uncovered shared and unique responding patterns for the two groups along the time series. Through motif enrichment analyses, 195 transcription factors were identified as potential regulators of heat response. In addition, 22 QTLs were identified within two existing populations (B73 × NC350 and B73 × CML103). Three inter-connected mapping populations (B76 × B106, B76 × NC350, and NC350 × B106) were further developed and phenotyped to map heat tolerance QTLs. Our study provided insights into the complex mechanisms of maize heat stress from molecular, biochemical, and whole-plant levels.

Funding acknowledgement: United States Department of Agriculture (USDA), National Institute of Food and Agriculture, Plant Science Institute of Iowa State University, Iowa State University Department of Agronomy, Chinese Scholarship Council

P66

Identification of QTLs for ear prolificacy and tillering in maize using two connected RIL populations

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The presence of multiple ears on the same stem node (Prolificacy) and number of tillers (Tillering) are important traits shaping maize plant architecture and affecting yield, specifically in silage maize. In this study, two connected F6:7 RIL populations sharing one parent line (B73 x Lo1016 and Lo964 x Lo1016) were evaluated in the field over two seasons for plant architecture and phenology traits. Lo964 is a high prolificacy - low tillering line, while Lo1016 is low prolificacy - high tillering. We performed quantitative trait loci (QTL) mapping by joint inclusive composite interval mapping (JICIM) using ICI Mapping software. Across populations, prolificacy ($h^2 = 0.47 – 0.67$) and tillering ($h^2 = 0.61 – 0.64$) showed nearly normal distribution suggesting polygenic control. Mild correlations were observed between plant height and tillering ($r=0.24$) and prolificacy and lowermost ear position ($r=0.26$) or number of leaves ($r=0.18$). Four QTLs for tillering were detected on chromosome 1, 2, 4 and 9. The tillering QTL on chr 1 corresponds to a known major tillering QTL formerly identified in maize x teosinte cross. One QTL was identified for prolificacy on chr. 2 explaining 14.0% of the phenotypic variance explained (PVE) have never been described before. Our study revealed novel loci associated to prolificacy and tillering, providing support for cloning the corresponding genes and shedding light on the genetic control of maize plant architecture.

Funding acknowledgement: KWS SAAT SE & Co

P67 @AmanArora_7

Identification of alleles affecting known pathways using a transcript accumulation index.

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The assignment of molecular identity and causality for quantitative trait from observations of phenotypic variation remains a major challenge in biology. Knowledge of genes' role in a process and their interactions can help in identifying the causal variants responsible for a phenotype. Expression-based genome-wide association studies (eGWAS) bypass some of this complexity to identify alleles responsible for natural variation in transcript accumulation. These eGWAS experiments generate SNP-trait associations for each gene but provide limited insights about phenotypic consequences or pathway impacts. Differential gene expression analyses identify sets of genes that are coregulated due to a treatment, condition, or genetic background. Here we demonstrate an index-based GWAS approach that turns gene expression pattern into a hypothesis test of SNP-to-pathway association. We calculate a unique value for each biological process of interest, called an index, from the transcript abundance data of all genes impacted by the process. These indices are quantitative trait values, that directly report on a known biological pathway. By incorporating prior knowledge in calculating an index, and capturing the information available from a large number of genes in a single index, this turns our GWAS result into a hypothesis-driven experiment. Alleles that alter the index are predicted to alter the status of a plant for the condition, treatment, or pathway that was used to build the gene set. Publicly available maize transcriptome datasets were used to calculate two different indices from gene expression studies of brassinosteroid (BR) biosynthetic mutants and BR treatments of maize seedlings. GWAS identified alleles in genes responsible for BR biosynthesis and signaling as the top associations for alteration in the BR-index, indicating the validity of the approach. These indices can be calculated for any group of co-regulated genes, pathways, co-expressed gene networks, transcription factor targets, or protein complexes.

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P68

Identification of loci influencing *Teosinte crossing barrier1 (Tcb1)* efficacy in maize by Quantitative Trail Loci (QTL) mapping and Genome-Wide Association Study (GWAS)

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Pollen cross-contamination has been one of the major problems for maize breeders. Various mechanical methods applied to avoid these contaminations are ineffective. The genetic information related to maize fertilization could be used as an effective method to prevent pollen contamination. One of such genetic systems that showed pollen rejection ability is *teosinte crossing barrier 1 (tcb1)*. Silks of a plant possessing dominant *Tcb1-s* reject 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-s*. To find those modifiers, we set up a QTL mapping experiment using Intermated B73 x Mo17 (IBM) recombinant inbred lines (RILs) for two consecutive years. We identified two significant QTLs at chromosome 4L and 5S for both years. They explained 16% to 17.60% of total phenotypic variation (R^2) and had negative additive effect with no interaction. The QTLs identified in respective chromosomes overlapped in both years and further study of the overlapped region resulted in the identification of 14 candidate genes (9 in 4L and 5 in 5S) possibly modifying the *tcb1* gene action. In order to have a further insight into the blocking effect across diverse maize germplasm, we also conducted GWAS using Ames 282 diversity panels. From this analysis, we identified six candidate loci (at 1L,3L, 4L, 5S, 7L, and 10S) that were associated with pollen blocking action. The candidate genes in these loci are being studied. The introgression of this genetic system, along with the appropriate modifying factors could be a novel and reliable solution for pollen contamination in maize breeding.

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P69

Identification of the favorable exotic alleles in controlling the ear morphological traits using Germplasm Enhancement of Maize (GEM) doubled haploid lines

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Maize ear morphological traits are critical components of grain yield, but the genetic basis in controlling their variation remains largely unclear. In this study, we used a diverse panel of the doubled haploid (DH) lines (N=243) derived from the Germplasm Enhancement of Maize (GEM). The GEM lines were developed using the U.S. temperate genetic materials and the diverse exotic races (N=52) that were mainly from the tropical areas of the world. The GEM-DH population was planted in the field according to an incomplete block design with four replications. After harvesting, the phenotypic data, including ear length (EL), ear diameter (ED), ear weight (EW), and kernel row number (KRN), were collected manually from the mature ears. Abundant phenotypic variation revealed the genetic complexity of these traits. After conducting genome-wide association studies (GWAS) using 60K SNP markers, 34, 10, 17, and 18 significantly associated loci were identified for EL, ED, EW, and KRN, respectively. Of these significant GWAS signals, 9/79 (11%) loci carried favorable alleles derived from tropical materials. These results suggested that tropical alleles can be leveraged to identify favorable alleles in controlling the ear morphological traits and maybe the untapped genetic resources for further temperate maize improvement.

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P70

Improving genomic selection through the addition of a competing diversity objective

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Genomic selection (GS) offers a method to estimate the effects of genome-wide molecular markers and select individuals based on their genomic estimated breeding values (GEBV). Compared to traditional phenotypic selection, GS enhances rates of genetic gain, often at the expense of genetic diversity. Since genetic diversity is crucial to long-term success in recurrent selection breeding programs, several alternative, single-objective selection strategies have been proposed to combat diversity loss and improve long-term genetic gains. Several proposed strategies include optimal contribution selection (OCS), weighted genomic selection (WGS), optimal haploid value selection (OHV), optimal population value selection (OPV), and expected maximum breeding value selection (EMBV). I propose a novel multi-objective selection strategy that includes genetic gain and genetic diversity as two competing objectives. I visualize the Pareto frontier for this novel strategy and compare its performance against OCS, WGS, OHV, OPV, and EMBV.

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P71

Integrating GWAS results to increase predictive ability for ear leaf area in Maize

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Ear leaf area is an important factor that affects metabolic processes and overall growth in maize (Zea mays L.). Due to the labor-intensive and time-consuming characteristics of destructive methods for ear leaf measurement, a non-destructive and efficient approach of estimation is highly desirable for breeders and crop modelers. In this study, a subset of 575 genotypes from the expanded WiDiv panel was employed to detect the genetic architecture underlying maize ear leaf area, and prediction models with high-density molecular markers were built. Phenotypic values from individual years and the best linear unbiased prediction value based on two years were used for the analysis. As a result, 23 significant SNPs were identified by three traits on seven out of ten chromosomes of maize. Comparison of 11 different prediction models which could be classified into four categories, say, single marker regression (SMR), penalized regression (PR), Bayesian regression (BR), and GWAS signal based regression (GSBR), showed that the model which integrated GWAS signals achieved the highest predictive ability. The signals identified from different environments are helpful to increase predictive ability further.

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P72 @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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Flowering time or photoperiod response has been a strong barrier to the adaptation of a crop to a new environment. In maize, tropical germplasm are well adapted to short day growing condition because of its proximity to the center of origin. However, tropical maize, if grown in long day condition will delay the flowering time and is not limited to increase plant height and disease-pest susceptibility. Adaptation of early maize in the long day environment should have been overcome by selecting maize lines for early flowering. This may have ended in masking the effect of beneficial alleles for some seed quality traits, and loss of some beneficial alleles at the cost of adaptation. To address these questions on pleiotropy and linkage drag, we created a set of near isogenic lines capturing an allelic series (NILAS). Near Isogenic Lines capturing Allelic Series (NILAS) are a set of inbred lines that contains one 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 flowering time QTL that were previously identified as a barrier for the adaptation of tropical germplasm in the temperate condition. Each of the four NILAS was developed by crossing seven tropical donor parents (DP) with two temperate recurrent parents (RP) each capturing 12 overlapping introgressions developing in total of 672 lines for each QTL x DP x RP combination. All the NILAS were accessed 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 in four seed quality traits; starch, crude protein, phosphorus and fat.

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P73

Leveraging structural variant information in GxE genomic prediction models

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Genomic prediction has revolutionized plant breeding by allowing breeders to predict plant performance solely based on genetic information, mostly using single nucleotide polymorphisms (SNPs). However, genotype-by-environment interactions (GxE), i.e., the differential performance of genotypes in different environments, decrease genomic prediction models' accuracy in multi-environment scenarios. Recent studies have addressed this shortcoming by improving the statistical models used in genomic prediction, but further improvements in prediction accuracy are needed. Here, we test a complementary approach that utilizes structural variants (SVs) – a source of genetic information largely neglected by current models – to boost prediction accuracy even more. Using real genotypic information from 330 maize recombinant inbred lines with SNP and SV information projected from their seven sequenced parental lines, we simulate traits with different genetic architectures in multiple environments using the R package *simplePHENOTYPES*. We vary the heritability, the number of quantitative trait loci (QTNs), the type of causative variant (SNPs and SVs), and the variant effect sizes. Weather data from 10 locations in the U.S. Midwest over two growing seasons is used to generate a residual correlation matrix among environments. Also, we add random effects to each QTN at each environment to simulate traits in a GxE scenario. Finally, GBLUP models with a factor analytical multiplicative mixed model are used to accommodate GxE and test different types of markers as predictors (SNPs, SVs, or SVs with varying levels of linkage disequilibrium to SNPs), and prediction accuracies are obtained using cross-validation. The overall goal of this study is to determine if there are genetic architectures and GxE levels for which the use of higher-cost SV markers would improve prediction accuracy enough to justify their inclusion in large scale genetic prediction modeling in a breeding program.

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P74

Mapping resistance to an Illinois isolate of anthracnose leaf blight in sorghum

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Anthracnose leaf blight is an economically important sorghum disease caused by the hemibiotrophic fungal pathogen *Colletotrichum sublineola*. The disease is reported to cause yield loss of up to fifty percent. Although qualitative and quantitative resistance have been identified, there is significant variability in pathogen strains virulence from different regions. We conducted a genome-wide association study (GWAS) complemented by mutant screening to understand the genetic architecture of anthracnose resistance in sorghum to an Illinois strain of *C. sublineola*. We evaluated 579 temperate-adapted sorghum conversion lines for disease resistance in inoculated field experiments in 2019 and 2020. This sorghum population has genotypic information available for 332,150 single nucleotide polymorphisms (SNPs). Mapping was conducted in the Genome Association and Prediction Integrated Tool (GAPIT) using Mixed Linear, FarmCPU, and K-chromosome models. Significant SNPs were identified with the FarmCPU model on chromosomes 1, 2, 3, 5, 7, and 10 with the 2019 data and on chromosomes 1, 2, 4, 5, 6, 9, and 10 with the 2020 data. We also screened a population of 417 ethyl methanosulfonate (EMS) sorghum mutants with the Illinois isolate of *C. sublineola* in the greenhouse. Thirteen mutant lines were significantly different from the background line BTx623 and had higher disease severity. Several of these mutants had disrupted genes with a known role in plant defense. Our results are useful in sorghum breeding to improve host resistance to sorghum anthracnose disease and understanding resistance to this important pathogen.

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P75
Mechanisms of resistance to bacterial pathogens in maize

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Identifying and understanding the mechanisms that have evolved in maize to combat pathogens is crucial to developing more robust disease resistant varieties. *Clavibacter nebraskensis* (*Cn*) and *Xanthomonas vasicola* pv. *vasculorum (Xvv)* are two bacterial pathogens of maize that have threatened maize yields in the Midwestern United States. *Cn* is a gram-positive vascular pathogen. *Xvv* is gram negative and primarily a nonvascular foliar pathogen in maize. We hypothesized that maize has evolved distinct resistance mechanisms to defend against vascular and non-vascular pathogens. Furthermore, we postulated that different mechanisms of resistance can be unveiled using different inoculation and phenotyping techniques. To test these hypotheses, we examined diverse maize lines using spray, wound, and infiltration inoculations. By measuring several different quantitative and qualitative phenotypes, we identified resistance that acts early in infection to block bacterial entry, resistance that acts to slow bacterial movement in the veins, and mesophyll-based resistance. We used florescence microscopy to track fluorescently labeled pathogens in plants with unusual qualitative phenotypes. *Xvv* is completely unable to colonize xylem of any maize line tested, with one exception. We found that *Xvv* behaves like an extremely virulent xylem colonizing pathogen in one line when inoculated via wounding. However, *Xvv* was unable to colonize the xylem when infiltrated via the stomata and was moderately resistant to infiltration induced disease. This same line was also the most susceptible line to *Cn*, suggesting that it lacks common vascular resistance mechanisms. In addition, we identified possible hydathode-mediated defense, as there were two lines with strong resistance phenotypes to *Cn* when it entered the leaf via the hydathodes. Resistance was characterized by leaf reddening or HR-like symptoms around hydathodes and a lack of lesion development. Hydathode-resistant lines showed typical lesions with wound inoculations. We plan to map the genetic determinates of these traits in the future.

P76 @ravi_mural
Meta-analysis identifies pleiotropic loci controlling phenotypic trade-offs in sorghum and maize

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Community association populations consist of diverse accessions that are assembled and genotypes and shared across a research community to enable investigations into the genetic basis of phenotypic variation for diverse traits. Some target traits may be controlled by simple architectures, e.g., large effect loci implicated in disease resistance, however association populations are most frequently employed to study traits controlled by complex genetic architectures. As widely adopted community association populations are ultimately scored for a diverse set of traits, including the same trait in multiple environments, these populations represent a unique resource to investigate both pleiotropy and genotype by environment interactions. Here we focus on three community association panels: The Sorghum Association panel (406 genotypes), the SAM association panel (394 genotypes) and the Wisconsin Diversity panel (942 genotypes). Through a combination of literature mining and field studies we assembled 234 separate trait datasets for sorghum and 224 separate trait datasets for maize. Comparison of individual GWAS results demonstrated limited evidence for pleiotropy while a multivariate adaptive shrinkage approach recovered both known pleiotropic effects of existing loci and new pleiotropic effects. In sorghum, known large effect mutations selected for their impact on plant stature – the dwarf genes – were also associated with changes in root architecture. Comparison of GWAS results employing independently generated marker datasets suggest that current genotype datasets do not achieve saturation in the Sorghum Association Panel, while in maize much higher marker densities were already available. These findings will further help inform breeding of these crops as well as further extension of these finding to other crop plants for their commercial benefits.

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P77

Molecular regulatory mechanism underlying genic male sterility and its application in maize

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As one of the most important crops, maize not only has been a source of the food, feed, and industrial feedstock for biofuel and bioproducts but also became a model plant system for addressing fundamental questions in genetics and molecular biology. Male sterility is a very useful trait for hybrid vigor utilization and hybrid seed production in crops. The identification and characterization of genic male-sterility (GMS) genes in maize and other plants have deepened our understanding of the molecular mechanisms controlling anther and pollen development, and enabled the development and efficient use of many biotechnology-based male-sterility (BMS) systems for crop hybrid breeding. Here, I will report the main progress on the identification and characterization of 14 GMS genes cloned in our lab, such as *ZmMs7*, *ZmMs20*, *ZmMs25*, *ZmMs30*, *ZmMs33*, *ZmbHLH51/122*, *ZmTGA9/10*, *ZmMYB84*, *ZmMYB33*, *ZmPHD11*, and *ZmLBD10/27* in maize, and construct a relatively conserved but diversified regulatory network controlling anther and pollen development by comparative genomics analysis on the GMS genes in maize, *Arabidopsis* and rice. Furthermore, I will introduce and appraise the features of more than a dozen BMS systems (e.g. MCS, DMS, SPT) for propagating male sterile lines and producing hybrid seeds in maize and other plants. Finally, I would like to provide my perspectives on the studies of GMS genes and the development of novel BMS systems in maize and other plants. The continuous exploration of GMS genes and BMS systems will enhance our understanding on the molecular regulatory networks controlling male fertility and greatly facilitate hybrid vigor utilization in breeding and field production of maize and other crops.

Gene / Gene Models described: *ZmMs7, ZmMs20, ZmMs25, ZmMs30, ZmMs33, ZmbHLH51, ZmbHLH122, ZmTGA9, ZmTGA10, ZmMYB84, ZmMYB33, ZmPHD11, ZmLBD10, ZmLBD27*; Zm00001d020680, Zm0001d029683, Zm00001d048337, GRMZM2G174782, GRMZM2G070304, Zm00001d053895, Zm00001d017724, Zm00001d052543, Zm00001d042777, Zm00001d012294, Zm00001d020938, Zm00001d025664, Zm00001d012544, Zm00001d013416, Zm00001d033335, Zm00001d013732

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P78

New approaches for rapid adaptation of tropical maize to temperate environments

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Increasing genetic diversity in U.S. maize germplasm is key for creating climate-adapted cultivars. Tropical maize is a superior resource of exotic alleles to increase the diversity of U.S. maize germplasm. However, tropical maize is poorly adapted to temperate environments because of sensitivity to photoperiod, resulting in unfavorable plant development, growth and yield responses under long daylengths. This maladaptive syndrome hinders breeders from incorporating tropical germplasm in breeding programs because it requires years of pre-breeding to overcome. This project capitalizes on a tropical synthetic (TropicS) population and employs multifaceted approaches to understand and facilitate adaptation of tropical maize. First, an unprecedented parallel selection experiment spanning a latitudinal transect from Wisconsin to Puerto Rico created a multidimensional dataset of selection response phenotypes related to the maladaptive syndrome. This dataset was used to find patterns of local adaptation, and whether direct selection within locations is always effective. Second, an *Adaptational Genomic Selection* approach was used to test if genomic selection can be used to more rapidly adapt tropical maize to temperate environments. Prediction models were trained on flowering time evaluated in two target environments (Delaware and North Carolina) contrasting two different panels (individuals versus lines) in 2019. Genomic selection was applied in the off-season in 2019 and performance was evaluated across all generations in both target locations in the summer of 2020. Preliminary results suggest that genomic selection in an off-season nursery was ~60% as efficient for North Carolina and Delaware as direct selection in the original environments for reducing late flowering in this tropical population. With this additional generation of response to selection, adaptational genomic selection allows for more rapid access to exotic alleles.

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P79

Novel loci for leaf blight resistance in sorghum aids in understanding of *E. turcicum* pathosystem

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Northern corn leaf blight and sorghum leaf blight, both caused by *Exserohilum turcicum*, are major diseases of maize and sorghum, respectively. Northern corn leaf blight has been ranked as one of the most damaging diseases in maize. In sorghum, yield losses as high as 50% due to sorghum leaf blight have been reported. Several major genes and numerous quantitative resistance loci have been mapped for northern corn leaf blight. Resistance to leaf blight in sorghum has been less well characterized. Understanding the genetic architecture of resistance in sorghum will lead to a better understanding of the relationship between resistance in maize and sorghum, which can ultimately enhance management options in both crops. In 2018 and 2019 we evaluated two sorghum recombinant inbred line (RIL) populations for resistance for *E. turcicum.* The BTx623 x IS3620C and BTx623 x SC155 populations consist of 235 and 81 unique lines, respectively. Resistance in both populations had moderate to high heritability. Through preliminary analysis, we saw evidence of different forms of resistance at work in each population. The phenotypic distribution of the BTx623 x IS3620C population indicated quantitative resistance, while for BTx623 x SC155 it appeared that there was a large effect locus. Using aHaley-Knott regression approach we identified a total of 7 QTL in the two populations. In the BTx623 x SC155 population we identified a QTL on chromosome 3 that explained more than 20% of the variation. In the BTx623 x IS3620C population a small effect QTL explaining 9% of the observed variation on chromosome 7 was stable across years. Understanding the relationship of defense across species furthers our understanding of the *Exserohilum turcicum*pathosystem.

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P80

Optimizing Leaf Morphology in Zea mays Using a QTL Mapping Approach to Identify the Genetic Regulators of Specific Leaf Area

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Specific leaf area, defined as the area of a leaf sample divided by its dry weight, is a proxy measurement of leaf thickness and cell density. Both traits can affect CO_2 diffusion rates resulting in potential changes to photosynthetic and transpiration efficiencies. Identifying the genetic regulators of SLA has the potential to inform us about the relationship between leaf morphology, photosynthetic capacity, and plant transpiration. Previous studies have shown variations of SLA in response to environmental factors, and how these changes can improve plant performance under severe stress. However, we currently lack an understanding of how plants are regulating SLA. Here we present the use of a biparental mapping population to identify QTL controlling SLA in *Zea mays*. A NAM RIL family containing 200 lines was grown in two separate field seasons using an incomplete randomized block design. Best linear unbiased estimators were calculated for each line. Using a publicly available genotypic data set composed of 7,386 markers available from Panzea we identified three significant QTL using the software package Rqtl. An additive genotypic effect was observed for each QTL, with the largest effect QTL located on chromosome 5 that accounts for 13.82% of the total phenotypic variance. Steps to further identify the genetic regulators of SLA and reduce the size of our QTL regions will also be discussed. We are pursuing the fine mapping of the identified QTL and will use cross section images to determine if any anatomical differences are correlated with the SLA phenotype. Dissecting the genetic regulators of SLA will provide new insights into the optimization of leaf morphology. Improving photosynthetic and transpiration efficiencies by selecting for improved leaf traits will allow for the production of more efficient crops.

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P81

Perennial relatives of maize may contain the key to effective nutrient recycling in maize production

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Maize production is one of the world's largest users of fertilizers at a significant environmental cost. Nitrogen fertilizers alone contribute almost 80% of the GHG emissions from maize production in the United States. Circular cycling of nutrients through our production systems will be critical to developing and maintaining sustainability. One way to do this would be focusing maize toward starch/carbon production to free up not only nitrogen, but other nutrients typically stored in the kernels for recycling in an unconventional manner. Intercepting and storing nutrients in the maize cob vasculature during grain filling is a potential solution wherein the cob becomes a co-product that serves as a nutrient source for the next year's crop. We examined cob nitrogen content at harvest in ex-plant variety protection maize lines from the Iodent and Stiff Stalk Synthetic heterotic pools and found no natural genetic variation. In hindsight this is expected as maize is an annual plant: there is no benefit to the offspring's survival for nutrients to be retained in the cob and, as such, conventional breeding will not be effective for this trait. We are continuing to examine possibilities around cob nutrient storage by looking to perennial species. Storage of nutrients in vascular tissues is common in perennial species and closely-related perennial species may provide the needed mechanisms by which to store nutrients in the cob. We are characterizing overwintering storage proteins in Tripsacum rhizomes to identify 1) if there are any homologous proteins in maize that could be co-opted for use in nutrient storage in the cob, and 2) potential storage proteins that could be used in transgenes to express nutrient storage in the cob vasculature. If interested in collaborating with us on this project please contact us, we are looking for partners with expertise in genetic modification in maize.

Funding acknowledgement: United States Department of Agriculture (USDA)

P82 @liu3zhen

Phenotypic association and prediction through integrative K-mer analysis

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Genome wide association study (GWAS) with single nucleotide polymorphisms (SNPs) has been widely used to explore genetic controls of phenotypic traits. Here we employed k-mer GWAS, which is based on counts of k-mers, short substrings from sequencing reads, for genetic association studies. Specifically, k-mer occurrence counts from whole genome sequencing reads of diverse maize inbred lines were used to associate with phenotypic data. Using cob color and kernel color traits, we demonstrated that k-mer GWAS can identify trait-associated k-mers from causal loci. In the case of kernel color genetic mapping, analysis of co-expression of associated k-mers and known pathway genes based on massive RNA-Seq data directly pinpointed two known causal genes, *yellow endosperm 1* (*y1*) and *carotenoid cleavage degradase 1* (*ccd1*). K-mer GWAS of kernel color also found a previously identified candidate gene *zeaxanthin epoxidase 1* (*zep1*). Analysis with diverse modern maize lines found that the three loci were under parallel selection, resulting in a predominant genotype combination of the alleles corresponding to yellow color. K-mer GWAS of flowering time resulted in thousands of associated k-mers. Both co-occurrence of k-mers among diverse maize lines and co-expression with known flowering time genes were constructed to facilitate identification of candidate genes. Further, k-mer data used as genotyping data for the prediction of flowering time achieved a similar accuracy to the prediction with SNP data. Our results demonstrated the use of k-mers for association mapping and genomic prediction in maize, and showed that the k-mer could be a hub element to integrate genomic, transcriptomic, and potentially epigenomic data for genetic analyses.

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P83 @HongyuJin5

Pollen-sequencing: A rapid and cost-effective method to construct genetic map using sequencing data from the hybrid pollens

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Meiotic crossover (CO), producing new intra-chromosomal recombination of different alleles, is one of the primary sources of heritable genetic variation. As the number of COs is not evenly distributed across the species or even among different genomic regions for a given species, it is crucial to characterize the distribution of the COs for the purposes of genetic research and application. In the plant species, the conventional method for COs mapping is expensive and time consuming, requiring genotyping of a large bi-parental population, such as the F2 or the recombinant inbred line population. Pollens of the flowering plants, carrying numerous recombinant gametes, is an accessible resource for the CO study. Here we proposed a method to construct the genetic map directly from the pollens of F1 hybrids through sequencing (pollen-seq). Our simulation study suggested that the accuracy of the reconstructed genetic map through pollen-seq could be affected by the sequencing depth and insertion fragment size. Using the pooled pollens from the B73xMo17 hybrid with a sequencing depth of 135x, we constructed a high-resolution recombination map of maize. The pollen-seq genetic map was largely in agreement with the existing map. Our results suggested pollen-seq is a powerful method for rapid and cost-effective genetic map construction.

P84

Presence/absence variations and SNPs equally contribute to the variations of protein and metabolite abundance.

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Understanding the mechanisms of adaptation to the environment in cultivated plants is a promising way to meet the challenge of maintaining food security in the context of global warming. In the case of maize, high-throughput sequencing has revealed that structural variations represent a large part of the genome and could have huge phenotypic effects. Among these, Presence Absence Variants (PAVs) may be involved in adaptation of maize to its environment, but their contribution to the genetic determinism of traits and genotype by environment interactions remains largely unknown. To address this issue, we performed a genome-wide association study between two types of polymorphisms, SNPs and Insetions/Deletions (InDels), and molecular traits obtained from proteomics and metabolomics analyses. The genetic panel used for this study was composed of 254 dent inbred lines genotyped with 978,134 SNPs and 72,041 InDels. The latter encompassed from 37 to 129,700 pb, including thousands of PAVs that are not present in the B73 reference genome. Proteins and metabolites were quantified by mass spectrometry in leaf samples from F1 hybrids obtained by crossing the inbred lines with one flint tester line. Hybrid plants were grown under two watering conditions (well-watered and water deficit) in greenhouse. In total, we detected 61,225 QTLs associated with proteome or metabolome variations. Among these, 4,766 QTLs were exclusively detected by InDels. To take into account the difference of marker density between InDels and SNPs, we used a re-sampling approach which showed that there is no difference for effect size distribution of QTLs between InDels and SNPs and for the number of QTLs detected by InDels or SNPs. Additionally, the QTLs detected by the two types of polymorphism were equally distributed in the two watering conditions. Our results suggest that InDels and SNPs equally contributed to molecular trait variation and response to drought stress.

P85 @SnehaAd1139933

Revealing the genetic diversity in teosinte introgressed maize population by morphometric traits and microsatellite markers

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Domestication and breeding bottleneck resulted in a rapid reduction of variation from cultivation. Teosinte (*Zea mays* ssp. *parviglumis*) is considered the biggest repository for the improvement of modern maize cultivars in terms of favorable allelic combination and enrichment of genetic base of existing breeding programs. In the present study, we evaluated morphological and molecular diversity among 100 lines derived from teosinte × maize (DI-103) hybridization. The high heritability coupled with high genetic advance for most of the traits elucidated the presence of additive gene effect in governing these traits, which offers reliable maize improvement through selection. Genotyping with 76 SSR markers produced 377 alleles with an average 5 alleles per marker loci. The polymorphic information content (PIC) ranged from 0.29 (bnlg197) to 0.86 (bnlg615, umc1726), reflected higher allelic variation and wide distribution in the teosinte derived maize population. Majority of the markers exhibited high PIC (0.60 to 0.86) values, which reflects the suitability of these markers for genetic diversity and relationship studies. Other genetic diversity parameters i.e. average gene diversity, heterozygosity, major allele frequency, and minor allele frequency were 0.48, 0.85, 0.58, and 0.48, respectively, supporting enormous genetic diversity among derived lines. Cluster analysis allocated the 102 lines including maize inbred (DI-103) and teosinte into 14 genetic groups, indicating genotypic uniqueness among derived lines. Linkage analysis using IciMapping4.2 revealed ten linkage groups in maize. Maximum allelic contribution from maize and teosinte parent was recorded in the case of MT-26 (65.2%) and MT-19 (59.4%) respectively. With 14 and 3 heterozygous segments MT-44 and MT-26 lines reflected maximum (37.5%) and minimum (2.4%) heterozygosity, respectively. The Maximum recombination (52%) was recorded in MT-40, whereas MT-81 expressed least recombination (30%). The results reflected a quite significant variability among derived lines and governed by introgression of teosinte alleles, which can be visualized by graphical genotype.

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P86

The effects of nitrogen stress on leaf angle in sorghum and maize

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In maize more erect leaf angles have been shown to be associated with increased yield under high planting density. This trait was a target of selection during breeding for maize improvement among US elite breeding programs but not Chinese elite breeding programs. Sorghum varieties with more erect leaf angles tend to have high yields and greater nitrogen accumulation in vegetative tissues. However, past studies of the impact of sorghum leaf angle focused on optimal growing conditions. As part of a greenhouse experiment in 2019 focused on differential responses to nitrogen stress among diverse sorghum accessions, eight out of ten sorghum accessions tested exhibited a decrease in adaxial leaf angle, e.g. more erect leaves, when grown under nitrogen deficient conditions. In 2020, a field experiment was conducted incorporating 346 sorghum varieties from the Sorghum Association Panel (SAP) grown under both nitrogen deficient and nitrogen replete conditions. Based on leaf angles scored in the field, the majority of sorghum varieties exhibited a statistically significant decrease in leaf angle under nitrogen deficient conditions (median of 2.7 degrees; p-value

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P87 @xiaoguanghuan

Unravel the roles of genetic regulation of transcriptional variation in temperate adaptation in maize

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Transcriptional regulation underlies the environment perception and adaptation of plants and, affect several agronomic traits of crops. Here we seek to uncover how gene expression and its regulatory elements co-opt with the adaptation of maize from tropical to temperate regions. A total of 572 unique RNA-seq datasets were generated from the seedling roots of 340 maize genotypes. Genes annotated as involved in cell division, chromosome organization and cytoskeleton organization exhibited statistically lower broad sense heritability of gene expression, while those annotated as involved in anti-oxidation activity exhibited significantly higher broad sense expression heritability. Expression GWAS identified 19,602 eQTLs for 11,444 genes and GWAS for alternative splicing identified 49,897 sQTLs for 7,614 genes. Similar to a previous study on cis-eQTLs, rare allele burden in genomic intervals with trans-eQTLs correlates with extremes of expression in target genes. A set of 663 genes was identified as harboring both cis-eQTLs and cis-sQTLs and the causal SNPs exhibited linkage disequilibrium greater than 0.6. This set of genes was enriched in transcription factors and genes annotated as responding to stresses. A comparison of expression heritability between 40 pure tropical lines and 55 elite temperate lines identified a subset of genes involved in cell proliferation, regulation of flower development, DNA replication and gene silencing with a significant reduction in the proportion of gene expression variation explained by genetic factors among the temperate lines relative to the tropical lines. Among a set of 932 genes identified as targets of selection during the adaption from tropical to temperate environments, 545 (Fisher exact test $p<0.05$) were associated with one or more cis regulatory elements. Further investigation on modes of actions of these regulatory elements will give insights on their evolutionary effects on the pre-existing expression and splicing variants segregating in tropical maize germplasm during the adaptation to temperate climates.

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P88

Variability in maize growth rates and utility in predicting end of season performance

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The corn breeding pipeline has the potential for gathering an immense amount of phenotypic data which could be used to evaluate plant productivity in changing environments. Conventionally, limited phenotypic data is collected due to time and labor requirements, but using tools such as unmanned aerial vehicles (UAVs) has increased the ease and speed of data collection. This increase in efficiency allows for collection of traits that would be time-consuming using traditional methods. Importantly, traits can be assessed throughout the growing season to determine rates of change. These traits, such as early season growth rate and rate of canopy closure, could provide breeders with important evaluation resources earlier in the season. In order to determine the utility and consistency of these traits, 500 lines of the Wisconsin diversity panel were evaluated with aerial imaging in 2018 (n=13 flights), 2019 (n=21 flights), and 2020 (n=10 flights) between planting and flowering. The rate of growth and canopy closure were characterized for each plot by fitting spline curves throughout the growing season. Variation in growth curves was then evaluated within and among years to test for consistency across environments. The growth curves were also used to assess whether yield could be accurately predicted by plant height, growth rate, or canopy coverage over various developmental stages. Finally, the genetic basis of growth rate and canopy closure at different points in the life-cycle were mapped using genome-wide association studies (GWAS) with ~2.5 million genome-wide SNPs. The collection of phenotypic data to provide growth curves can provide opportunities for breeders to make selection decisions earlier in the season and predict how genotypes will respond in different environments.

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P89 @snodgrasshopper

Variance component analysis of MOA-seq identified transcription factor binding sites for 143 maize traits

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Variation at particular regions of the genome have a disproportionate impact on phenotype, such as genes, promoter sequences, and transcription factor binding sites. Previous work has identified these regions by their accessibility, using methods such as ChIP-seq and ATAC-seq. The resolution of these methods can be hundreds of base pairs. Identifying these regions at higher resolution would narrow candidate loci and better identify genetic variants underpinning important traits. MOA-seq (MNase Open & Accessible) identifies transcription factor binding sites at high resolution (less than 50bp). Here we use MOA-seq data from 17 F1 maize hybrids to estimate the contribution of these regions to the phenotypic variation of 143 traits taken across the maize nested association mapping (NAM) RIL population. With the new NAM parental genome assemblies, we created and projected a new, dense set of SNPs called from whole genome alignments (~97 million SNPs) onto the NAM RIL population. We used these projected, alignment-based SNPs in a VCAP (Variance Component Analysis Pipeline), which split the genome into exclusive components and then estimated the heritability accounted for by each component for each trait. These components represented: (1) MOA peaks; (2) a null background matching MOA peaks for distance to nearest gene, allele frequency, and number of base pairs; and (3) the rest of the genome.

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P90

A WD40 repeat-protein under parallel domestication enhances grain yield in maize and rice

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Cereals are a closely related group of crops that collectively contribute up to 50% of human caloric intake worldwide. Yet, little is known about the extent of parallel selection among crops that, if more fully understood, could drive crop breeding programmes. Here, we compared two major cereal crops, maize and rice, that were domesticated independently ~10,000 years ago to show that at least 114 pairs of orthologous genes and several molecular pathways affecting different traits were selected in parallel during domestication and improvement. To molecularly dissect an example of a locus that had undergone parallel domestication, we cloned and characterized *KRN2*, a major quantitative trait locus controlling kernel row number within a maize domestication sweep, and showed that the orthologue in rice, *OsKRN2*, was also the target of a selective sweep. The encoded WD40 repeat proteins interacted with DUF1644 (of unknown function) to negatively regulate kernel row number by controlling inflorescence meristem size in maize and spikelet number in rice, and in turn, grain number in both crops. Knockout of *KRN2* in maize or *OsKRN2* in rice increased grain yield up to 10% and 8%, respectively, with no apparent trade-offs in other agronomic traits, suggesting potential applications of *KRN2* and its orthologues for crop improvement.

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P91

Chromatin structure regulates *mPing* excision and insertion

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Chromatin structure is known to function as the primary mechanism for preventing expression of transposable elements (TEs). Additionally, DNA methylation and element size have been shown to directly limit mobilization of some class II TEs. Our goal is to determine how altering chromatin structure directly affects excision and insertion of *mPing*, a class II TE from rice. We have performed yeast transposition assays in several chromatin structure mutant backgrounds (*swc2, snf2, isw1, rtt106, dot6,* and *hta2*). Excision frequency of a genomic *mmPing20:URA3 ADE2* reporter in the *rtt106, dot6*, and *snf2* mutants was significantly lower than the control, and excision frequency in the *swc2* mutant was significantly higher than the control. This suggests that reduced nucleosome density provides transposase proteins greater access to the terminal inverted repeats. Thus, chromatin packing may function to both repress transposase expression and inhibit transposase binding. In addition, the *URA3* selection marker allowed us to determine the frequency of elements reinserting after excision. Larger *mPing* elements inserted at a lower frequency, suggesting that element size may affect the stability of the transposition complex. In addition, insertion frequency was higher in the *snf2* mutant. This correlates with our observation that *mPing* has a high propensity for insertion into the yeast rDNA sequences, which are known to have reduced nucleosome density in the *snf2* mutant. The potential that increased nucleosome density increases the likelihood that a TE fails to reinsert suggests that physical inhibition of TE mobilization could have been a mechanism that shaped eukaryotic chromatin structure.

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P92

Cytosine methylation profiles distinguish different regulatory states of a paramutable *purple plant 1* allele

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Paramutation describes specific meiotically heritable changes in gene regulation dependent on trans-homologue interactions. In maize, paramutations have been documented at five distinct loci including *purple plant 1 (pl1)* which is required for anthocyanin production. A highly expressed state (termed *Pl-Rh*) of the *Pl1-Rhoades* allele can be heritably suppressed in trans by a transcriptionally and post-transcriptionally repressed state (termed *Pl′*), leading to reduced plant pigmentation in such heterozygotes and the persistence of weak pigmentation in subsequent progeny. Although the molecular mechanism remains largely unknown, forward genetic screens identify *required to maintain paramutation (rmr)* and *mediator of paramutation (mop)* loci affecting the paramutation process. Because most of the currently known RMR and MOP proteins are orthologs of *Arabidopsis* components that make up an RNA-directed DNA methylation (RdDM) pathway, it's hypothesized that a similar pathway underlies paramutation behaviors through 24-nucleotide RNA recruitment of chromatin modifications like cytosine methylation. To test this idea, we compared targeted 5-methylcytosine profiles of genomic DNA isolated from isogenic *Pl-Rh / Pl-Rh* and *Pl′ / Pl′*. All unmethylated cytosines were converted to uracils by TET2 and APOBEC enzymatic treatments and PCR amplicons from a tandemly repeated region of potential regulatory significance downstream of the *Pl1-Rhoades* coding region were Sanger sequenced. Here we show that all cytosines in *Pl-Rh* homozygotes are unmethylated while some cytosines in *Pl′ / Pl′* seedlings are partially methylated, consistent with the outcome of a RdDM-type process. By being able to distinguish cytosine methylation profiles between *Pl-Rh* and *Pl′* states we can begin to understand the temporal events during which *Pl-Rh* is changed to a *Pl′* state by investigating the methylation profiles of *Pl-Rh / Pl′* individuals at different developmental stages. These future studies will be critical for evaluating an RdDM-type working model for paramutations occurring in maize.

Gene / Gene Models described: *pl1*; Zm00001eb278680
Funding acknowledgement: National Science Foundation (NSF)

P93 @mcstitzer

Elevated transposable element copy number is associated with reduced fitness in maize

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The maize genomic sequence presents an incredible abundance and diversity of transposable elements (TEs). From genome assemblies and annotations of over 30 maize individuals, we see that while the proportion of the genome derived from TEs is broadly consistent; there is extensive variation in TE content between maize individuals. Both the abundance of TE families and the position of individual TE copies varies, with only about 20% of all TE copies found at the same position across individuals. Despite this great variation, it is unclear the degree to which these differences in TE content affect plant phenotypes and fitness. From whole genome assemblies of parental lines, we project TE copy number to genotyped recombinant inbred lines, and associate TE copy number to phenotypes. After correcting for parental ancestry, we find that total TE copy number is negatively associated with fitness-related traits like plant height. To investigate potential mechanisms, we associate TE insertion polymorphisms with expression of TE families and genes. Since genomes recognize and silence TE copies in a copy number dependent fashion, we associate TE copy number to TE family expression. A subset of TE families show patterns suggestive of copy number dependent silencing. Further, some TE families that are silenced in this copy number dependent fashion are associated with reduced expression of genes they are inserted near, suggesting possible cosuppression mechanisms. In organisms with smaller genomes, like yeast and Drosophila, there is strong experimental evidence for the deleterious impact of TEs on fitness, even when TEs do not directly disrupt genes. Yet, for larger genomes like maize with many more TEs, it appears these fitness costs are not as extreme, and limited to only some TE families. Altogether, while the maize genome can tolerate a large TE load, TEs still act as parasites.

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P94

Epigenetic adaptation of maize centromere in oat-maize addition lines

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Stably inherited alien maize chromosomes in oat-maize addition lines (OmA) can be produced by hybridizing maize and oat. Immunofluorescence shows that oat CENH3 can load on maize centromere regions in OmA. The mechanisms of oat CENH3 targeting to maize centromeres and the epigenetic changes at maize centromeres in OmA remain unclear. Here, we generated anti-CENH3 ChIP-seq data of maize, oat and OmA, and found that maize centromeres are expanded in the oat background. Packing DNA in maize centromeric nucleosomes exhibit different patterns between B73 and OmA. CENH3 binding to CentC satellites in OmA also showed lower sequence identity than that in maize. In addition, we identified several centromeric long terminal repeat retrotransposons of oat (CRO). Phylogenetic analysis reveals that these repeat sequences are different from the centromeric retrotransposons of maize, and the three subgenomes of oat can be distinguished by combining CRO1 and CRO2 fluorescence probes. The CROs contain specific sites with highly phased binding to CENH3 nucleosomes. Furthermore, maize CENH3 nucleosomes are highly phased around A or T dinucleotides, however, we did not find obvious specific dinucleotides that oat CENH3 phased. These results reveal that maize centromeric nucleosome patterns are mainly retained, but also undergo little remodeling, which sheds light on understanding the potential mechanisms of the adaptation of maize centromeres in OmA . Keywords: maize, OmA, centromere, CENH3, repeat sequences, nucleosome

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P95

Expanding BonnMu - The transposon-induced mutant resource in the European flint maize line F7

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The reference genome sequences of diverse maize germplasms have been rapidly assembled and updated, and facilitate genetics and genomics research (Schnable et al. 2009; Springer et al. 2018; Sun et al. 2018; Haberer et al. 2020). To uncover the underlying function of the predicted genes of a genome, various forward and reverse genetic resources in maize have been developed using transposon mutagenesis (Fernandes et al. 2004; Stern, et al. 2004; McCarty, et al. 2013; Liang et al. 2019). By using the well-described Mu-seq approach (McCarty, et al. 2013), we have recently established a European mutant collection for functional genetics, named *BonnMu* (Marcon et al., 2020). By now, sequencing of >4,000 Mu-tagged F_2-stocks in two US inbred backgrounds (i.e., B73 and Co125) detected 123,454 unique germinal Mu insertions, covering 23,000 of the annotated genes (52% of the B73v4 genome). BonnMu insertions and phenotypic seedling photographs of the Mu-tagged stocks are publicly available at MaizeGDB.org. Here, we expand our mutant collection in F7 (flint) genetic background representing an important genetic resource for central European maize research due to its stable growth properties under temperate climatic conditions. In order to create the mutant repository in F7 genetic background, four Mu-seq libraries consisting of 2,304 *Mu*-tagged F_2-stocks were sequenced by Hi-seq Xten. In total, 64,848 unique heritable *Mu* insertions were identified affecting the coding sequences of 18,548 (42% of the F7v1 genome) filtered gene set (FGS) genes with an average of 28 *Mu* insertions in each stock. To complement the reverse genetic analysis, visible seedling phenotypes of all segregating F_2-stocks were characterized in a forward genetic approach. Soon, Mu-insertions and respective photographs of the tagged stocks in F7 background will be integrated into MaizeGDB.org, to facilitate functional genomics study in this European flint maize line.

Funding acknowledgement: German Research Foundation (DFG)

P96

Exploring genetic and epigenetic contributions to imprinting in maize

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Imprinted genes are genes that are expressed dependent on which gamete the parent donates to the embryo, and are regulated by chromatin modifications. Though we have been aware of the presence of imprinted genes for half a century, they have remained difficult to study in depth. Recent advances in transcriptome sequencing have enabled us to explore imprinted genes with more depth and clarity than previously. We have been able to look into imprinted genes present in several maize lines with genome assemblies. This allows us to study the turnover of imprinting and identify imprinted genes and transposable elements displaying presence absence variation across genotypes. We have started to explore features of genome variation and epigenomic modification, focusing on finding associations between imprinted genes and putative regulatory regions with a particular focus on DNA glycosylases such as mdr1 which contribute to imprinting via demethylation activity in the central cell. This study provides insights into the role of presence absence variation and the regulatory regions involved in controlling imprinted genes in maize endosperm.

Funding acknowledgement: National Science Foundation (NSF)

P97

Functional characterization of R-loops in maize centromere

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R-loops are stable chromatin structures comprising an DNA:RNA hybrid and a displaced single-stranded DNA. R-loops have been implicated in gene expression and chromatin structure, as well as in replication blocks and genome instability. Here, we conducted a genome-wide identification of R-loops and identified more than 700,000 R-loop peaks in the maize (*Zea mays*) genome. We found that sense R-loops were enriched in promoters and transcription termination sites , which were different from the gene body localization of sense R-loops in *Arabidopsis* and *Oryza sativa*. At the chromosome scale, maize R-loops were enriched in pericentromeric heterochromatin regions and a significant portion of R-loops were derived from transposable elements. In centromeres, R-loops preferentially formed within the binding regions of the centromere-specific histone CENH3 and centromeric retrotransposons were strongly associated with R-loop formation. Furthermore, centromeric retrotransposon R-loops were observed by applying single-molecule imaging techniques, such as atomic force microscopy. Our results expand the understanding of the fundamental roles of R-loops in the maize genome, especially in centromeres.

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P98

Genetic regulation of sexual conversion of the terminal lateral inflorescence in maize during domestication

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The maize inflorescences (tassel and ear) experienced profound phenotypic changes during the domestication of maize from its wild progenitor teosinte (*Zea mays* ssp. *Parviglumis*), including sexual conversion of the terminal lateral inflorescence from tassel (male) to ear (female). Although a few key genes controlling this dramatic morphologic change have been cloned, recent study demonstrated that the natural variation of sexual conversion of the terminal lateral inflorescence in maize is dominated by large numbers of small-effect QTLs and the molecular mechanism underlying them remains largely unknown. Here, we report on an investigation of the genetic control of sexual conversion and identifying additional relevant genes. Quantitative trait loci (QTLs) were identified on six different chromosomes, three of which are novel QTLs (*STAM1.1*, *STAM1.2*, *STAM2.1*). Additionally, we also narrowed *STAM2.1* down to a 600 kb region containing 11 genes. The goals of this study are to (1) Clone and verify the underlying gene of *STAM2.1* that has a large effect conditioned on teosinte *tb1* allele on the male into female conversion; (2) Fine map and clone a second QTL called *STAM1.1* that has a similar effect; (3) Characterize the statistical and molecular interactions of *STAM1.1* and *STAM2.1* with *tb1*. Uncovering the molecular regulatory mechanism of QTLs that are responsible for the sexual conversion will enhance our understanding of maize inflorescence development.

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P99 @Clore_IV

Identifying putative LTR retrotransposons in maize insertions using LTR Predictor

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Long Terminal Repeat (LTR) retrotransposons are the most abundant type of transposable element (TE) found in the maize genome, characterized by having long terminal repeats on either side of a coding sequence or deleted coding sequence. The goal of this project was to write a script in python that can take a file of compiled insertions identified by long-read sequencing and pick out candidates that are likely to be LTR retrotransposons based on having structural characteristics expected of them. Using Biopython, we perform local alignments to estimate LTR length and location, and then a global alignment is performed to compare the similarity of the LTRs. The presence of primer binding sites are also noted. LTR Predictor can correctly identify LTR insertions in curated TE datasets, and can be used to screen for new LTR insertions in long-read sequencing datasets. Overall, LTR Predictor is an effective tool to identify candidate LTR retrotransposons based on defining sequence structure.

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P100

Insights into gene methylation through the NAM founder methylomes

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Variation in gene expression is key to diverse traits and can have epigenetic causes like gain or loss of methylation. The recently completed NAM genome assembly project included not only genome assemblies but also DNA methylomes for 26 inbred maize stocks that represent the genetic diversity of maize. Combined with the transcriptome data and gene and TE annotations generated in this project, we can profile maize methylation patterns and investigate the interplay between methylation, gene expression, and TEs. Genes can be generally divided into three categories based on methylation status: Gene body methylated genes have methylation in their exons specifically in the CG context and are typically constitutively expressed, while unmethylated genes lack methylation in exons and are typically tissue-specific. The third category is TE-like genes, which have both CG and non-CG methylation in their exons and are typically poorly expressed. We are quantifying the frequency that genes switch methylation status between NAM founders and their associated changes in expression. Additionally, we are investigating potential roles for TEs in and near genes in driving gene methylation changes in genes.

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P101

Patterns of natural selection in a plant transposable element

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Transposable elements (TEs) evolve in two dimensions. First, each insertion is subject to the processes of random genetic drift and selection across the host population, and second, the sequence itself is subject to these same processes. The pattern of evolution in the sequence has received little attention, particularly within a family of TEs. Studying the pattern of evolution and testing for the signature of selection within a TE family is difficult if the family is composed of hundreds or thousands of highly similar elements that are poorly resolved based on phylogenetic analysis. Here, we introduce a novel approach that leverages the variation between elements of a family within a single genome. Our method has the potential to detect both negative and positive selection acting on the sequence of the TE. We apply our method to the Sirevirus LTR retrotransposons that are found in maize in large copy numbers. These elements have intact and complete gag and pol coding domains. We find clear evidence of negative selection across all genes and in all five families of Sireviruses, but no evidence of positive selection. In the two largest families, our analyses suggest that most elements, although possessing intact coding sequences, are not capable of transposition. We also argue that our results imply that most of the gene product from an active element must be targeted to the transposition of the element from which it was produced. Our results shed new light on the selective processes affecting transposable elements in plants.

P102

Pl1-Rhoades paramutation is associated with molecular changes at downstream tandem repeats

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In maize, paramutations result in meiotically-heritable regulatory changes of certain alleles including *Pl1-Rhoades* 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 *Pl′*-like 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 influence 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 facilitate paramutation remain largely unknown. Here we report that a region downstream of the *Pl1-Rhoades* coding sequence conferring strong expression and paramutation behavior contains five ~2kb direct repeats while the weakly paramutagenic CML52 and nonparamutagenic B73 haplotypes have three and one, respectively. Preliminary transcription profiling and qRT-PCR show transcription and mRNA levels from these repeats correlate with *Pl1-Rhoades* expression. Small RNA profiling finds Pol IV-dependent 24nt RNAs are produced from sequences within the repeats indicating RMR proteins operate at this feature. Additional fractionation-based experiments revealed alternate nucleosome profiles within these repeats distinguishing *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 to facilitate paramutations remains an open question. We are currently identifying additional molecular features distinguishing alternate *Pl1-Rhoades* states and characterizing the processes controlling these regulatory transitions to determine how grass species generate, maintain, and transmit meiotically-heritable regulatory variation.

Gene / Gene Models described: *pl1*; Zm00001d037118
Funding acknowledgement: National Science Foundation (NSF)

P103

Rapid, heat-induced transgenerational reactivation of a silenced transposable element in maize

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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.

Funding acknowledgement: National Science Foundation (NSF)

P104 @Qi_Li_maize

Removal of imprints by a remote retrotransposition

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Genomic imprinting describes a phenomenon that primarily occurs in the endosperm wherein the differential expression of parental alleles depends on the allelic origin rather than the genomic dosage. Although hundreds of imprinted genes have been characterized and several epigenetic regulatory modes have been proposed in diverse species, how the imprint control is arisen or removed at a particular locus within a species is little understood. To dissect this, we design a genetic screen system using the dominant endosperm-filling mutant *floury3-reference* (*fl3-ref*) that exhibits maternally-inherited behaviors due to conserved paternal imprinting in the *Fl3* promoter. By crossing *fl3-ref* (as female) with over 300 inbred lines (as male), we screened two inbred lines, Qi205 (a Chinese Quality Protein Maize line) and Ms71 (a NAM founder line) that could alleviate the expressivity of *fl3-ref* phenotypes. The *Fl3-Qi205* and *Fl3-Ms71* alleles were not regulated by paternal imprinting, thereby allowing for a partial rescue of the floury phenotype when using them to pollinate *fl3-ref* plants. Map-based cloning revealed a distal locus in the upstream of the *Fl3* promoter responsible for the genomic imprinting. Through PacBio long-read sequencing, we determined the Qi205 genome sequence and created a long, continuous sequence at the *Fl3* locus. By comparing *Fl3-Qi205* and *Fl3-Ms71* alleles with those from 27 maize genomes (K0326Y, B73, Mo17 and other NAM lines), we identified that Qi205 and Ms71 contain a 12-kb *Xilon-Diguus* retrotransposon that is specifically inserted in ~109-kb upstream of the *Fl3* promoter, indicating that this retrotransposon functions as a "cis-acting imprinting intervener". 4C-seq further showed that the distal retrotransposon interferes in long-range interactions at the *Fl3* locus. Overall, this study reveals that a single retrotransposition event caused the removal of conserved genomic imprints at the *Fl3* locus from a region about a hundred-kilobase away. Our findings suggest that distal sequence variations could be a general trigger factor contributing to the variability of locus-specific imprint control within natural populations.

Gene / Gene Models described: *fl3*; GRMZM2G006585
Funding acknowledgement: Chinese Academy of Sciences

P105

The diversity and evolution of the cis-regulatory region of LTR retrotransposons in maize

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The *cis*-regulatory region of transposable elements (TEs) is important as it controls when and where TEs will activate. At the same time, it is also a prime target of host defences as methylating these regions can suppress TE activity. The epigenetic and evolutionary interactions between the TE *cis*-regions and host defences have often been studied, however, the sequence organization of the region itself is typically poorly resolved, which limits the better understanding of these interactions. Here, we attempted to systematically decode the regulatory region of TEs by focusing on the ten most abundant *Copia* and *Gypsy* LTR retrotransposon families in maize that collectively occupy ~50% of the genome. All five *Copia* families are Sireviruses, a plant-specific lineage of LTR retrotransposons. We show that the *cis*-region of these families is complex, characterized by a large number of palindromic motifs that form stable secondary structures. Despite their phylogenetic relatedness, every family contains a distinct set of palindromes. Furthermore, Sirevirus families that predate the maize/sorghum split now contain different palindromes within each host. Both findings reflect the rapid evolution of these sequences. The two most abundant families, *Ji* and *Opie*, contain the largest diversity of palindromes, suggesting that the emergence of new motifs may enhance activity or escape from silencing. The regions immediately flanking the palindromes are predicted recombination breakpoints, indicating how shuffling may occur during processes such as reverse transcription. In contrast to Sireviruses, the regulatory regions of *Gypsy* families are devoid of a similar complex organization, even though one family, *Xilon-Diguus*, shared striking similarities with one Sirevirus family. Overall, our findings show that the cis-region of TEs is highly diverse among families and superfamilies. In some lineages, however, it has been shaped into a complex, dynamic and non-randomly organized locus, possibly as a result of an evolutionary arms-race with host defences.

Funding acknowledgement: Royal Society

P106 @SigmaFacto

The evolution of 26 diverse maize genomes driven by transposable elements

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High-quality genomes have become increasingly available thanks to advances in sequencing technology. So far, there are over 30 maize genomes available, including the 26 recently released NAM founder genomes. Transposable elements (TEs) comprise over 80% of maize genomes and contribute to the extensive genetic variation found across lines. To study TE dynamics within the diverse NAM founders, we developed a pan-genome TE annotation module within EDTA (Ou et al. 2019) for consistent and accurate annotations in each genome. Our annotation revealed that approximately 31% of TEs are structurally intact, and 69% are fragmented. Annotation across all NAM genomes identified over 27,000 TE families, though the largest 500 families comprised 93% of the total TE space. We also identified 393,000 intact singleton TEs (2.2% of the TE space) that could not be classified. Comparing between NAM lines, we find that tropical maize genomes are ~54 Mb larger than temperate genomes, a difference that is mainly driven by LTR-RTs and Helitrons. This can be attributed to the combined effects of TE proliferation in tropical lines and removal in temperate lines. Further, we identified 25 active LTR-RT families contributing more new copies in tropical maize than temperate maize post domestication (~10 ka). Finally, we explore how methylation status, accessible chromatin regions, and patterns of expression in 10 tissues may contribute to the variable activity of these families contributing to the difference in genome sizes between tropical and temperate genomes.

Funding acknowledgement: National Science Foundation (NSF)

P107

The landscape and variation of the chromatin accessibility during maize domestication

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Maize (*Zea mays*) is one of the most important crop species, domesticated from its wild progenitor teosinte (*Zea mays* ssp. *Parviglumis*) about 9,000 years ago from central Mexico. Genomic regions that were under selection during the maize domestication process had been extensively studied. However, the landscape and variation of the chromatin accessibility and its potential contribution to phenotypic variation remain largely unclear. Here, using Assay for Transposase Accessible Chromatin with high-throughput sequencing (ATAC-seq), we profiled the genome-wide accessible regulatory sites in the teosinte (Ames8759) and modern maize (W22). We identified 60,176 and 80,847 open chromatin regions (OCRs) in teosinte and modern maize, representing 1% and 1.5% of the B73 reference genome. Among these OCRs, 37,957 (15.4 Mb) were shared between teosinte and maize. These shared OCRs were highly enriched in transcription start sites, suggesting their functional importance. Additionally, we identified 14,282 (8.7 Mb) differential OCRs, more than 60% (8,849/14,282) of which overlapped with genic regions, including the major domestication genes, such as *tb1*, *tga1*, *tru1*, and *ZmCCT10*. Our preliminary results suggested that differential chromatin accessibility maybe under selection during domestication and attributable to phenotypic variation.

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

P108

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-Rh*) or an epigenetically repressed paramutant state (denoted *Pl'*). *Pl1-Rh* alleles in the *Pl'* state often revert to *Pl-Rh* in plants homozygous for a null allele (*rmr6-1*) of 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 (sRNAs) in general, condition the inheritance of genome-wide regulatory information. To test this idea, seedling RNA-seq and sRNA-seq profiles of heterozygous BC_5 progeny from sibling *rmr6-1* mutant and heterozygous fathers were compared to identify heritable RNAP IV-dependent effects. RNA abundances of 140 genes were either significantly enriched or depleted in the progeny of mutant *rmr6-1* fathers as compared to progeny of heterozygous fathers. Additionally, 962 sRNA clusters were significantly enriched or depleted in an identical comparison. These differences point to other alleles, like *Pl1-Rh*, whose dysregulation in the absence of RNAP IV might persist through meiosis. Future studies aim to determine the role of environmental factors in effecting heritable changes at *Pl1-Rh* and other alleles 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.

Gene / Gene Models described: *pl1, rmr6*; Zm00001d037118, Zm00001d031459
Funding acknowledgement: National Science Foundation (NSF)

P109 @PaulSharu

Transposon-induced rearrangements activate gene expression in maize

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DNA transposons such as *Activator (Ac)* elements can have considerable biological impacts such as disrupted gene function, change in gene expression, or chimeric gene formation by causing structural rearrangements of the genome. The molecular structure of genomic rearrangements caused by alternative transpositions between *p1* and *p2* genes responsible for floral pigmentation in maize was investigated. In maize line *p1-wwB54*, the *p2* gene is expressed in anther and silk but not in pericarp, and the *p1* gene is partially deleted, making the kernels white. We identified complex rearrangements in this region caused by the movement of an *Ac* and a *fractured Ac (fAc)* element. These rearrangements change the position of a *p1* enhancer and activate the expression of *p2* in the kernel pericarp, resulting in red kernel color. We hypothesize that the decrease in distance of the *p1* enhancer from the promoter of *p2* activates *p2* expression in the kernel pericarp. Here we describe the detailed structure of rearrangements with single and multiple copies of the *p1* enhancer. Multiple copies of enhancer correspond to darker red kernel phenotype compared to alleles with a single copy. Chromosome conformation capture would reveal how the enhancer interacts with the promoter and the effect of enhancer copy number and location on gene expression in these cases.

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P110 @shanwai1234

Variation of transposable element expression across maize genotypes and stress conditions

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Transposable elements (TEs) makes up the majority of maize genome. Although most of TEs are silent, we explored a subset of TEs could be activated during abiotic stress (cold and heat). DNA methylation profiles and comparative genomics within three maize genotypes (B73, W22 and Mo17) were used to probe the variability of TE expression responses. Substantial variations were detected in both TE family and TE element level. Small regions lacking DNA methylation (UMRs) within TE elements tend to be associated with TE expression. A comparison of the expression of specific TEs in different maize genotypes reveals high levels of variability that can be attributed to both genome content differences and epigenetic variation. This study explores potentials of the genetic and epigenetic factors that influence TE regulations in both normal and stress conditions.

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P111

A circRNA from retrotransposon regulate centromeric transcription by interacting with chromatin state-related proteins

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Centromeres, chromosomal regions that determined by centromeric histone 3 (CENH3) and physically linked to the spindle during cell division, ensuring partition of genetic information to daughter cells. Centromeres have conserved function with an extreme variability in its DNA sequence. However, the key factor determining centromere localization remains mysterious. Recent findings on centromeric RNAs have aroused an interest in the role of RNA in assembly of centromeric chromatin and kinetochore. Our previous study demonstrated that the circular RNA from centromeric retrotransposon (circular CRM1 RNA) affected the loading of CENH3 by forming chromatin loops. Here, we found three proteins (hda106, nfa104 and nfc103) that interact with circular CRM1 RNAs by ChIRP –MS (Chromatin Isolation by RNA Purification). In detail, hda106 belong to HD2 type histone deacetylase, nfa104 and nfc103 belong to nucleosome/chromatin assembly factor, respectively. Further, we found that the transcription levels of CRM1 and LTR32 were decreased in hda106, nfa104 and nfc103-RNAi plants. So, we hypothesis that circular CRM1 RNAs regulate centromeric transcription by interacting with chromatin state-related proteins. In order to draw exact and exhaustive conclusion, the continuing experiment is needed. Further detailed analysis of the hda106, nfa104 and nfc103-RNAi plants will provide new insights to the function of centromere

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P112

A novel DNAJ-like protein is required for sugar export from source leaves

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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. As all heterotrophic life on earth relies on carbohydrates produced by plants as their primary source of energy, understanding how plants control the allocation of these compounds is crucial. The physiological, anatomical, and biochemical processes governing carbohydrate partitioning is well understood, but the underlying genetics is still poorly characterized. Towards understanding this process, we identified two allelic recessive mutants from EMS mutagenized populations*, carbohydrate partitioning defective13 (cpd13)* and *cpd35,* which demonstrate carbohydrate partitioning defects, including reduced plant growth, chlorotic leaves, and hyperaccumulation of soluble sugars and starch in mature leaves. Intriguingly, the mature mutant leaves start out with a unique crossbanding pattern of chlorotic and green regions and occasionally exude sugary droplets. By using polymorphic markers and whole genome sequencing-based approaches, we mapped the causative mutations to a gene encoding a protein containing DNAJ-like and thioredoxin-like domains. While these are not well studied in plants, some DNAJ-like proteins have protein folding activity and are known to be involved in protein quality control in other organisms. In tobacco leaves, translational fusions of CPD13-red fluorescent protein localized to the endoplasmic reticulum (ER). We hypothesize that the sugar export defective phenotype of the mutants is due to the inability of the defective CPD13 protein to properly process other interacting proteins. Ongoing studies will help explore this hypothesis and elucidate the function of this novel protein in carbohydrate partitioning and the peculiar crossbanding leaf phenotype.Funding provided by a grant from NSF PGRP to DMB (IOS-1025976)

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P113

A novel method for mapping crossing-overs in maize using MLH3 Chromatin immunoprecipitation.

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Meiotic recombination is the main driver of evolution and adaptation. It is also the vehicle of plant breeding, generating the variation on which breeders apply selection. Elucidating the patterns of distribution of recombination events along chromosomes will facilitate designing crop varieties with desired combinations of traits, such as increased yield or pathogen resistance. The most accurate current approach to map meiotic crossing-overs (COs) relies on genotyping hybrid progenies. It requires segregating populations and high-quality reference genome maps of parental lines. The major inconvenience of this method are high genotyping costs, which limit the number of individuals that can be examined, the time required to build mapping populations, and poor CO mapping resolution in genome regions with limited DNA sequence polymorphism. To overcome these limitations, we developed a new high-resolution CO mapping method based on chromatin immunoprecipitation (ChIP) with a CO marker protein MLH3. We generated two antibodies targeting the N and C termini MLH3 that colocalize to CO sites on pachytene and diplotene chromosomes in cytological preparations. These antibodies were used to perform ChIP experiments on late meiotic prophase I tassels in the B73xMo17 hybrid. Sequencing of ChIP products identified CO hotspots whose overall distribution across the genome resembles that of the genetically-identified COs. However, nearly half of the MLH3 ChIP peaks correspond to previously unidentified CO hotspots (Kianian et al., 2018). Furthermore, roughly 50% of MLH3 ChIP peaks map to sites located in regions of 10kb or more that lack DNA sequence polymorphisms. Such hotspots cannot be identified using the classical genotyping approach. Overall, our method allows high-resolution CO mapping regardless of the level of inter-parental DNA sequence polymorphism and samples hundreds of thousands of meioses as opposed to just a few hundred that are normally examined using the genotyping approach.

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P114

An improved protocol for increasing the concentration of high quality DNA from frozen maize tissues

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We have used the Qiagen DNeasy Plant Mini Kit to extract and purify maize DNA from husk tissue that has been stored at -80 deg C for whole-genome sequencing. Our campus Genomics Core Facility requires a minimum concentration of 50 ng/ul of DNA for genome sequencing. But our yields were usually 25-35 ng/ul.
We have recently used the Qiagen DNeasy Plant Maxi Kit to process larger tissue samples. The Maxi Kit also yielded concentrations in the 25-35 ng/ul range. We have developed a hybrid protocol that combines the use of both kits that gives sufficient DNA concentrations:
Approximately 1 g of frozen husk tissue is pulverized in a mortar and pestle in liquid N2, grinding 100 strokes of the pestle, and transferred to a 15 ml centrifuge tube. The DNeasy Plant Maxi Kit protocol is followed, finally eluting the DNA from the spin column 2 times with 0.75 ml of Buffer AE each time. This produced 1.3 ml with DNA concentrations of 20 to 25 ng/ul. A volume of 520 ul of this product was then transferred to a new 2 ml tube and mixed with 780 ul (1.5 volumes) of buffer AW1, which promotes binding of DNA to a silica membrane. This solution was then run through a DNeasy Mini spin column in two 650 ul aliquots, discarding the flow-through. The column was then washed 2 times with 500 ul of Buffer AW2 and the DNA finally eluted with 75 ul of Buffer AE. The average starting concentration of 2 samples was 23 ng/ul. The average final concentration was110 ng/ul. An agarose gel showed the DNA to have a molecular weight well in excess of 10Kb. The A260/280 reading of the purified DNA was 1.82 with an A260/230 reading of 2.43.

P115

An integrated biochemical and genetic approach to assess the roles of *Glossy2* and *Glossy2-like* in maize cuticle formation

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The cuticle is a hydrophobic barrier that covers all surfaces of the aerial organs of plants. It provides the first line of defense from many biotic and abiotic stresses that are detrimental to plant health. The cuticle is composed of a network of unique lipids that are solvent extractable, and intercalated within and laid atop an insoluble cutin matrix. The solvent extractable cuticular lipid mixture is comprised of very long chain fatty acids (VLCFAs) and their derivatives, including hydrocarbons, alcohols, aldehydes, ketones, and wax esters. Classical genetic strategies have identified approximately 30 *glossy* genes required for normal cuticle deposition in maize, and molecular characterization of these genes is providing new insights on cuticle formation. This study is focused on the maize *Glossy2* (*Gl2*) gene, which encodes a protein that is archetypal for the BAHD class of acyltransferases. Although *Gl2's* biochemical function remains uncharacterized, homozygous *gl2* mutant seedlings exhibit altered cuticle composition, presumably due to the inability to elongate VLCFAs to specific chain lengths. Recently, a close homolog of *Glossy2*, termed *Glossy2-like* (*Gl2-like*) was also characterized. To assess the *in planta* physiological function of *Gl2-like,* three unique mutant alleles have been generated via CRISPR-Cas9 genome editing. These *gl2-like* mutants, in combination with *gl2* mutants, will enable the characterization of the functional relationship between *Gl2* and *Gl2-like*. In parallel, the biochemical functions of GL2 and GL2-LIKE proteins will be investigated by characterizing recombinantly expressed and purified protein preparations. This combination of genetic and biochemical strategies will provide insight into the roles that *Gl2* and *Gl2-like* serve in maize cuticle biosynthesis.

Gene / Gene Models described: *Glossy2, Glossy2-like*; Zm00001d002353, Zm00001d024317
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P116

Analysis of stalk architecture and metabolic phenotypes underlying maize stalk bending strength

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Stalk lodging is a major bottleneck for improving productivity in maize (Zea mays L.). Genetic improvement of stalk lodging resistance has been limited due to a lack of reliable and field-deployable phenotyping approaches. In this study, we examined the key intermediate traits that contribute to stalk strength. These intermediate phenotypes can provide vital clues for understanding the biomechanical determinants of stalk strength. To this end, we generated 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 (Device for Assessing Resistance to Lodging IN Grains) revealed substantial phenotypic variation among hybrids. We are currently examining several other biomechanical properties of these hybrid stalks including rind penetrometer resistance, stalk linear density, high-resolution near-infrared spectral data, rind thickness, diameter, etc. These data will be combined using advanced structural engineering analyses and statistical/machine learning techniques to identify the most informative phenotypes underlying stalk strength. These data will enable a more comprehensive picture of the genetic architecture of stalk lodging resistance.

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P117

Anthocyanin3 negatively regulates anthocyanin synthesis in maize

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The *Anthocyanin3* (*A3*) locus when recessive is known to enhance pigment levels in mature aerial plant tissues such as husks, stalks, and tassels. It is known through previous genetic studies that *A3* resides downstream of *Anthocyanin1 (A1)* on Chromosome 3L. In order to decipher the location of *A3*, a Dissociation transposable element insertion in *A1* was mobilized in a cross to the *a3-ref* mutant stock. In a population of approximately 25,000 plants, one plant returned the intensely purple *A3* phenotype indicating a *de novo* insertion into *A3*. It was found through inverse PCR of the insertion that the gene responsible for the *A3* trait is *Mybr97*, a Myb-repressor gene with homology to a known negative regulator of anthocyanin synthesis in Arabidopsis. Protein interaction experiments demonstrated that *Mybr97* is capable of associating with *B1*, a canonical activator of anthocyanin synthesis. *Mybr97* was also shown to be transcribed in the roots, shoots, stalks, and pericarp of B73, with the highest expression in husks. A population segregating for the *a3-ref* mutant was created to determine what kind of effect this gene has on the transcriptome. All known anthocyanin biosynthetic genes were significantly upregulated in mutants versus wild-type plants as expected. GO-term enrichment analysis discovered an increase in stress response and secondary metabolism pathways with a decrease in photosynthesis-related transcripts in mutant plants. *Mybr97*, or *A3*, has a profound effect on the anthocyanin pathway in maize and enhances our understanding of how this pathway interacts with other regulatory networks in maize.

Funding acknowledgement: DDW The Color House

P118

Assessing PME activity levels in pollinated silks of compatible and incompatible crosses controlled by *Ga1*

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Maize is a monoecious plant and can be self or cross pollinated easily. There are some maize genotypes which do not set seed on pollination by crosses with plants of certain genotypes, however reciprocal crosses function normally. .. This unilateral cross incompatibility is governed by loci called gametophytic factors. Several gametophytic factors are known, of which, the *Gametophytic cross incompatibility factor1-s (Ga1-s*), *Gametophytic cross incompatibility factor2-s* (*Ga2-s*) and the *Teosinte crossing barrier1-s* (*Tcb1-s*) from maize and teosinte have been studied in detail. The pollinations in these systems are described as compatible or incompatible based on the success of fertilization. Cross incompatible reactions mediated by the *Ga1* locus are due to the activity of male and female functions which are expressed in the maize pollen and the silks, respectively. Pectin methyl esterases, a class of aspartyl esterase enzymes are the mediators of both these functions. ZmPME3 specifically expressed in *Ga1-s* silks may be responsible for the female function (Moran et al. , 2017) and ZmGa1P from the ZmGa1P- ZmPME10-1 complex in *Ga1-s* pollen has been shown to have the male function. (Zhang et al.,2018). The present study focusses on PME activities in pollinated silks of compatible and incompatible crosses. Preliminary PME activity assays reveal that pollinated silks of incompatible crosses show significantly lower PME activities as compared to activities in pollinated silks of compatible crosses. The reduced PME activity may be an effect of the female function on the growing pollen tubes during incompatible crosses.

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P119

Bacterium-enabled transient gene activation by artificial transcription factor for resolving gene regulation in maize

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Cellular functions are diversified through intricate transcription regulatory networks, and an understanding of individual transcription factors and their downstream genes are essential to elucidating many developmental processes and environmental responses. In nature, some pathogenic bacteria have evolved transcription factor-like proteins to directly manipulate host cell regulatory networks. Here, we employed the Transcriptional-Activator Like effectors (TALes), which represent a family of transcription factors that are synthesized by members of the γ-proteobacterium genus *Xanthomonas* and secreted to host cells for activation of targeted host genes. To characterize the regulatory network for cuticular wax production in maize, we constructed designer TALes (dTALes), which play a role as an artificial transcription factor, to specifically activate the maize gene *gl3*, a MYB transcription factor involved in the cuticular wax biosynthesis. Upon treatments with dTALes, delivered by the maize pathogen *Xanthomonas vasicola* pv. *vasculorum* (Xvv), *gl3* expression was induced significantly. RNA-Seq analysis of leaf samples from two treatments with each dTALe identified a total of 146 *gl3* downstream genes, including 92 up- and 54 down-regulated by both dTALes. Eight of the nine known genes that are known to be involved in the cuticular wax biosynthesis were up-regulated by at least one dTALe. Further analysis with the top-down Gaussian graphical model predicted that 68 of the 146 *gl3* downstream genes are directly regulated by the GL3 transcription factor. A chemically induced mutant of the gene Zm00001d017418, a candidate *gl3* downstream gene and encoding aldehyde dehydrogenase, exhibited a typical glossy leaf phenotype. The bacterial protein delivery system and artificial transcription factors, dTALes, proved to be a straightforward and powerful approach for the revelation of gene regulation in plants.

Gene / Gene Models described: *gl3*; GRMZM2G162434, Zm00001d017418
Funding acknowledgement: National Science Foundation (NSF)

P120

Biochemical and physiological variability among hybrids in response to varying N fertilizer application

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The application of nitrogen (N) fertilizer is a standard practice in field settings in the U.S. and strongly promotes productive and resilient maize [1, 2]. However, maize hybrid lines can display widely varying phenotypic responses to N application with differing outcomes on yield. Much remains unclear about the nature of these varying physiological and biochemical responses and how they influence maize productivity. A comprehensive understanding of the effects of N fertilizer and potential synergistic effects is important to effectively manage agricultural inputs for maximal yield. Toward this end, we are characterizing variation among a suite of biochemical and physiological traits across five maize hybrids grown in the field under two N application regimens. Initial results demonstrate prominent N- and hybrid-dependent effects on several photosynthetic pigments and strong developmental stage effects among multiple phenolic compounds. Further, we identify significant differences in chloroplast and cellular ultrastructure among hybrids that may, in part, account for differing responses to N. A significant correlation between hybrid lines and the nitrogen treatment was observed in prenyl lipid analysis. Our emerging results shed light on the important changes in the molecular mechanisms among hybrid lines in response to nitrogen fertilizer application. This research is supported by AgSpectrum, LLC. to P.L, A.T, and E.G. 1. Su, W., et al., *Nitrogen fertilization affects maize grain yield through regulating nitrogen uptake, radiation and water use efficiency, photosynthesis and root distribution.* PeerJ, 2020. **8**: p. e10291.2. Liang, H., et al., *Optimal Nitrogen Practice in Winter Wheat-Summer Maize Rotation Affecting the Fates of 15N-Labeled Fertilizer.* Agronomy, 2020. **10**(4): p. 521.

Funding acknowledgement: Ag Spectrum

P121 @alexsilvacor

Biosynthesis of maysin in early vegetative stages of maize, where and what for?

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Maysin is a *C*-glycosyl flavone present in silks of maize, broadly known as a key molecule to confer resistance to corn earworm (CEW), a critical pest in maize. Prior studies have shown the accumulation of maysin in leaves of high-altitude landraces of maize seedlings, conferring UV-B protection. Nevertheless, the maysin accumulation pattern in early vegetative stages and genes associated with its biosynthesis in leaves has not been well determined. In this study, we determined the temporal dynamics of maysin accumulation using a set of maize lines. Plants were grown both in a growth chamber and field conditions at Michigan State University in the summer of 2019. Our results demonstrated that levels of maysin increase in the youngest leaf from stage V1 to V5 and that this pattern is interrupted in stage V6, where maysin levels decrease. The amount of maysin found in plants grown under field conditions is significantly higher than in growth chamber, showing the environment's effects on maysin biosynthesis. *P1* is a MYB transcription factor known to control the expression of genes involved in the synthesis of maysin in silks. However, we identified large amounts of maysin in leaves of a maize line with a non-functional allele of *P1* (*P1-ww*), suggesting the presence of another master maysin regulator. Combining gene expression based on available RNAseq data, metabolic data, and gene identity analysis, we identified another R2R3-MYB transcription factor unlinked to P1, which is expressed mostly in the leaves of young plants. A set of experiments are being conducted to evaluate the function of this transcription factor in maysin biosynthesis. Using a set of maize mutant lines, we confirmed the role of some genes involved in maysin pathway in silks, suggesting a common set of genes in both organs. Finally, recent results indicate the role of maysin in conferring resistance to fall armyworm, bringing evidence about the multiple functions of maysin in leaves.

Gene / Gene Models described: *MYB154*; Zm00001d047671
Funding acknowledgement: National Science Foundation (NSF)

P122 @KhanguraRajdeep

Characterization of a novel dominant lesion mutant *Bella fleck1* which is modified by natural variation in maize

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Lesion forming mutants of maize are characterized by the formation of spontaneous lesions or speckles on the leaf blade and sheath. These mutants can be phenotypically characterized by their unique lesion morphology, color, inheritance, and mechanism. Over 30 complementation groups of these mutants have been recovered, and known molecular functions include enzymes in porphyrin metabolism, chlorophyll degradation, and an auto-active NBS-LRR protein. Thus far, all show lesion progression that gets more severe as the leaves age. The novel dominant lesion mutant *Bella fleck1* (*Bfl1*) is the only one of these mutants that displays leaf flecking in the newly emerging leaves, but not progressive lesion severity over development. *Bfl1* mutants are stunted and also exhibit root defects. An advanced backcross line was used for RNA-seq analysis of gene expression and variant calling. *Bfl1* was mapped to the long arm of chromosome 2 using bulked segregant analysis of mRNA sequencing data. Association mapping was used to identify modifiers of *Bfl1* by generating F1 hybrids with the members of a maize association panel. This identified a significant association in the region encoding *Bfl1* and consistent with a cis-effect. Remarkably, the mutants in the F1 hybrid association panel were resistant to common rust and other foliar fungal diseases. Differential gene expression and metabolite analyses of *Bfl1* were similar to the autoimmune *Rp1-D21* mutant of maize. *Bfl1* accumulated high levels of salicylic acid, disease-responsive metabolites, and defense-responsive genes. The molecular identity of the gene underlying the *Bfl1* mutation is currently being investigated.

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P123

Confirmation of alternative splicing for the auxin repressor gene ZmIAA1 in Zea mays

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Auxin is well-known for its important roles in plant body development and growth processes. The gene ZmIAA1 codes for a repressor (of the Aux/IAA gene family) in the nuclear auxin signaling pathway. Previously published data on ZmIAA1 gene expression suggested differential levels of expression in different tissues and evidence of alternative splicing. To confirm these observations, ZmIAA1 was amplified by RT-PCR from RNA collected from seedling roots, embryos and immature tassels. ZmIAA1 expression was observed in roots and tassels, but no observable expression was seen in embryos, which seemingly contradicts existing data. Additionally, alternative splicing was observed, but the sizes of three out of the four amplified PCR products appeared to be vastly different from any of the three expected amplicon sizes, suggesting that the existing available data on splicing might not be well understood. Further verification of the gene sequence and evidence for intron incorporation in the PCR products are needed to validate our findings. Phenotypic characterization of ZmIAA1 in vivo can also contribute to the understanding of the overall function of ZmIAA1 in plant development.

Gene / Gene Models described: *ZmIAA1*; GRMZM2G079957
Funding acknowledgement: National Science Foundation (NSF)

P124

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). 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 understanding the regulation of the phenylpropanoid genes that are most highly expressed during maize development. We also are conducting Y1H screens to discover upstream TFs that regulate the TF genes previously shown to bind phenylpropanoid promoters in maize. We have found candidate "Master TFs" that include novel members of the MYB, IAA, TRAF, LIM, GLK, NAC and ARF TF and coregulator families. This project is funded by NSF grant IOS-1733633.

Funding acknowledgement: National Science Foundation (NSF)

P125

Dissecting the genetic architecture of source-sink regulated senescence in maize

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Senescence is a complex developmental process regulated by a number of internal and external cues. The onset of premature senescence due to the absence of a strong sink, termed source-sink regulated senescence (SSRS), offers a unique opportunity to tease apart the role of sugar partitioning and signaling in senescence. To understand the genetic architecture and the underlying molecular mechanisms, we have completed a systems genetic analysis of SSRS in maize. Through characterization of the natural diversity for SSRS in a diversity panel and a biparental population, we have identified several genomic regions and the underlying candidate genes. To further confirm these findings, we performed time-course transcriptome and metabolic characterization of SSRS sensitive (B73) and SSRS resistance (Mo17) inbred lines. The metabolic analysis revealed that SSRS is associated with differential accumulation of sugars, cytokinin and abscisic acid activity, and hexokinase activity. We have validated two of the quantitative trait loci (QTL) in near-isogenic lines and fine mapping of these QTL is underway. Remarkably, natural diversity analysis resulted in the identification of a cathepsin B-like protease encoded by *ccp4* as an important gene regulating SSRS. Further support to such a role was provided by the analysis of natural allelic variation of *ccp4* in diverse maize inbred lines and by overexpression of maize *ccp4* in Arabidopsis. Confirmation of these findings through antibodies targeting CCP4 is currently underway. Finally, we have developed a novel clustering algorithm inspired by the features of hypothesis testing that allows the identification of significant genomic regions through the integration of the data from different omics systems. Characterization of the novel genes and pathways from this study will enhance the mechanistic understanding of senescence.

Funding acknowledgement: National Science Foundation (NSF)

P126

Elucidating the strigolactone biosynthetic pathways of maize (*Zea mays*)

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Strigolactones are plant hormones regulating a.o. shoot branching, root architecture and secondary stem growth, and are also exuded from the roots into the rhizosphere where they serve as host detection signals for beneficial arbuscular mycorrhizal (AM) fungi. On the other hand, strigolactones have been hijacked by parasitic plants, members of the Orobanchaceae, as germination signal to start their parasitism. Parasitic plants such as Striga are a major threat to maize cultivation in the African continent and are difficult to control. In maize root exudate, two canonical strigolactones - 5-deoxystrigol and sorgomol - have been reported (Yoneyama et al., 2015). Recently, we demonstrated the occurrence of seven new strigolactones in maize root exudate, of which two were structurally characterized as zealactone (Charnikhova et al, 2017) and zeapyranolactone (Charnikhova et al, 2018). The role of these structurally different strigolactones in *Striga* infection and/or other biological processes is unknown. Unraveling their biosynthesis pathway should help elucidating this.
Therefore, as a first step, we identified the genes encoding the core pathway of strigolactone biosynthesis, up to carlactone, based on the homology with the core pathway in other species (Alder et al, 2012). Agroinfiltration of these core pathway genes (*D27*, *CCD7* and *CCD8*) in *Nicotiana benthamiana* resulted in the production of carlactone.
Subsequently, candidate genes involved in the downstream strigolactone biosynthetic pathway were identified using co-expression analysis using the identified core strigolactone biosynthetic pathway genes as bait. A combination of heterologous expression (transiently in *Nicotiana benthamiana* and yeast), metabolomics and mutagenesis approaches are used to elucidate the downstream steps in the biosynthetic pathway of strigolactones in maize and study the biological relevance of the structural diversity in these (rhizosphere) signaling molecules.

Funding acknowledgement: ERC Advanced Grant, CSC Scholarship

P127

Engineered 6-phosphogluconate dehydrogenase assessed in field corn hybrids for mitigation of grain yield loss under heat stress

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Heat stress reduces maize grain weight and quality. Starch synthesis in the endosperm is sensitive to high temperature stress and has the potential to be a limiting pathway for grain yield under heat stress. In addition to enzymes directly involved in starch biosynthesis, chloroplast-localized 6-phosphogluconate dehydrogenase (PGD3) is critical for starch accumulation. PGD3 is one of three enzymes in the oxidative section of the Pentose Phosphate Pathway (PPP). Maize encodes two cytosolic isozymes, PGD1 and PGD2. Cytosolic PGD1 and PGD2 isozymes are heat stable, while the amyloplast-localized PGD3 is heat labile under in vitro and in vivo heat stress conditions. A heat stable 6-phosphogluconate dehydrogenase localized to amyloplast was previously developed by fusing the waxy1 N-terminal chloroplast targeting sequence to the Pgd1 and Pgd2 open reading frames. Previous work shows that The WPGD1 and WPGD2 transgenes complement the pgd3 defective kernel phenotype suggesting the fusion proteins are targeted to the amyloplast. Initial field trial suggests the WPGD1 and WPGD2 mitigate part of the yield losses due to high nighttime temperature stress. The *Wpgd* transgenes were introgressed into either B73 or color-converted W22. We generated B73 x W22 hybrids to assess transgenic and non-transgenic plants in a field trial comparing heat-stressed and non-stressed planting dates. Heat stressed plots showed yield losses and preliminary analyses of the hybrid transgenic plots show mitigation of these yield losses.

Funding acknowledgement: National Science Foundation (NSF)

P128 @PaulaCasati

Flavone synthesis and its connection with salicylic acid metabolism in maize plants

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Flavonoids are one of the main groups of specialized metabolites in plants. Flavones, one of the most important flavonoids, are synthesized by enzymes known as flavone synthases (FNSs). There are two different types of FNS enzymes, flavone synthase I (FNSI) enzymes are soluble Fe^{+2}/2-oxoglutarate-dependent dioxygenases; while flavone synthases II (FNSII) belong to a family of NADPH- and oxygen-dependent cytochrome P450 membrane-bound monooxygenases. In *Arabidopsis thaliana*, *Downey Mildew Resistant 6* (*DMR6*) encodes an FNSI enzyme. *dmr6* mutants show increased resistance against the multiple pathogens, including *Pseudomonas syringae*. This particular phenotype can be associated to an accumulation of the hormone salicylic acid (SA) in *dmr6* mutants. Furthermore, there is a restoration of susceptibility to the pathogen attack when *dmr6* plants are complemented with FNS I and II from maize. The aim of this work is to study the possible interconnection between flavone synthesis and salicylic acid metabolism. Thus, we analyzed the susceptibility against the attack of the pathogen *Pseudomonas syringae* in Arabidopsis wild type (Col-0 ecotype) and mutant plants in *SALICYLIC ACID 3-HIDROXYLASE* (*S3H*) gene. Salicylic acid 3-hydroxylase enzyme catalyzes the conversion of salicylic acid to 2,3-dihydrobenzoic acid. Therefore, *s3h* mutants accumulate higher levels of salicylic acid that results in enhanced resistance to infection by pathogens. The infection experiments were also carried out in *s3h* mutant plants expressing FNS I and II enzymes from maize. Transgenic lines exhibited restoration of susceptibility to Pseudomonas infection. We also quantified the level of salicylic acid in transgenic lines post-infection. Results show that expression of either ZmFNSI or II restore pathogen susceptibility and also increase SA levels. In addition, we analyzed the possible regulatory effect of the flavone apigenin on the expression of genes associated with SA metabolism using RT-qPCR. Together, our results suggest that there is a connection between flavone synthesis and SA metabolism.

Gene / Gene Models described: *fnsI, fnsII*; Zm00001d024946, GRMZM2G475380
Funding acknowledgement: FONCYT

P129 @LinaCastano_D

Flavonoids play a role in resistance to accumulation of aflatoxin in corn

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Aspergillus flavus is a facultative pathogen capable of producing aflatoxins (AF), potent carcinogens that accumulate in corn kernels, peanuts, cottonseed and tree nuts. To understand resistance mechanisms in corn to AF accumulation we performed a high-throughput genomics study using an in vitro kernel screening assay with *A. flavus* 3357, resistant corn hybrid TZAR102 and susceptible corn hybrid Va35. We determined that corn genotype, fungal treatment and duration of infection significantly co-vary to influence the overall gene expression patterns. We performed gene ontology enrichment analysis on highly significant genes and found enrichment of pathways linked to fungal and microbial responses such as Pathogenesis-related (PR) proteins. To determine additional genes of interest using field and gene expression data, we linked genome-wide association analysis results with gene expression data, allowing us to detect significant expression quantitative trait loci (eQTL). Our results showed that resistance to aflatoxin contamination is associated with specific flavonoid biosynthetic pathway genes. Additional experiments including functional genomics analyses and fungal bioassays to identify the role of flavonoids and their contribution to corn resistance to *A. flavus* growth and AF production will also be presented.

Funding acknowledgement: United States Department of Agriculture (USDA)

P130

Genome-wide dissection of the regulation of epicuticular wax in sorghum: the first defense line of plants against environmental threats

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The epicuticular wax on the surface of plants is the first defense line against abiotic and biotic stress. Sorghum, an important bioenergy and versatile crop, is also an excellent model to study the regulation of epicuticular wax. Compared with major grass crops including maize and rice, sorghum accumulates a higher level of wax, which contributes to its high water-use efficiency, and tolerance to drought and heat. Our previous work identified a key gene *BLM40* involved in the biosynthesis of epicuticular wax in sorghum. This gene was not reported in other species and does not have homologs in Arabidopsis, which indicates that sorghum harbors its unique mechanism to maintain the high load wax to resist the stresses from the environment. There is still a lack of system knowledge about the regulation of epicuticular wax in sorghum. To have a comprehensive understanding of the molecular mechanism of epicuticular wax in sorghum, we are performing a genome-wide dissection by combining large-scale genomics and classical genetics. From our large mutant population, over 100 sorghum bloomless mutants with largely reduced wax load were identified. So far, a total of 16 causal genes including three novel genes for bloomless phenotype have been identified by the bulk segregation analysis and whole genome sequencing. The analysis of the causal gene of the rest 30 bloomless mutants is undergoing. The causal genes will be linked together as a network according to the transcriptome data and the change of the wax composition of each mutant. More data will be presented at the conference.

P131

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 varying the dosage of complete sets of chromosomes (ploidy). Previous work from the laboratory had examined individual genes for effects of changes in chromosomal dosage. The predominant effect was an inverse modulation in the unvaried portion of the genome (*trans*), but positive modulations were also observed. Here we performed global RNA-seq studies of such effects in maize mature leaf tissue by examining the impact of both increased and decreased dosage of fifteen distal and one interstitial chromosomal regions. Monosomies, trisomies, and tetrasomies 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 chromosomal regions, although positive modulations also occur. Comparisons across one to four ploidies show much less modulation. Furthermore, this analysis revealed the existence of increased and decreased effects in which expression of many genes under genome imbalance are modulated towards the same direction regardless of increased or decreased chromosomal dosage, which is predicted from kinetic considerations of multicomponent molecular interactions. This study provides novel insights into the underlying molecular mechanisms involved in genomic balance and how regulatory dosage effects operate. Funding from NSF IOS-1545780.

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P132

How does the structure of the phloem cell wall contribute to whole-plant carbohydrate partitioning?

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Carbohydrate partitioning is fundamental to plant growth, but poorly understood at the genetic level. Carbohydrate partitioning is the process by which sugars move from source tissues, e.g., mature leaves, to sink tissues, such as the roots. These sugars are transported in the form of sucrose and later turned into glucose polymers to form cellulose or other cellular building blocks and metabolites. In plant cells, cellulose is the largest form of carbon storage and is deposited in plant cell walls. The *COBRA* gene, has been identified to affect the deposition of cellulose in *Arabidopsis thaliana*. COBRA proteins help assemble the cellulose microfiber structure of the cell walls during plant development, and *cobra*mutants have been characterized to have root defects. A gene that functions in carbohydrate partitioning in maize encodes a *COBRA* gene: *Brittle stalk2-Like3 (Bk2L3)*. To examine if there are cell wall changes between the wild type and mutants in the maize *Bk2L3* gene (first identified as the *carbohydrate partitioning defective28* mutant), I am conducting immunolocalization experiments using antibodies raised against cellulose and other cell wall epitopes. The immunostaining results are visualized using fluorescence microscopy to compare mutants to the wild type. Early promising results include possible alterations in the cell wall structure of the phloem tissue in the mutants. These and other data will help illuminate how BK2L3 contributes to phloem cell wall architecture and ultimately contributes to whole-plant carbohydrate partitioning.

Gene / Gene Models described: *Bk2L3*; Zm00001d034049
Funding acknowledgement: National Science Foundation (NSF)

P133

Investigating differential splicing of the 5' UTR of Zea mays Auxin Response Factor 27 in root, shoot, and embryo tissues

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The auxin response pathway relies on delicate regulation of the expression of key players from large protein families. We investigated whether alternative splicing of the 5' UTR of the auxin responsive transcription factor ZmARF27 occurs in a tissue-dependent manner in hopes of illuminating some of the pathways by which these proteins are regulated. RT-PCR performed on Zea mays shoot, root, and embryo tissues revealed a lack of ZmARF27 expression in embryo tissue and no tissue-specific differential splicing in shoot and root tissues. Further investigation of ZmARF27 expression in maize embryo tissue is needed to evaluate our findings, which contradict available ZmARF27 expression data. More robust RNA-seq experiments or removal of the ZmARF27 5' UTR may be fruitful in further examining its regulatory importance.

Gene / Gene Models described: *ZmARF27*; GRMZM2G160005
Funding acknowledgement: National Science Foundation (NSF)

P134 @cutebyul81

Investigating the molecular interactions of maize and common rust during pathogenesis

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Common rust, caused by the obligate biotroph *Puccinia sorghi*, is one of the most destructive diseases of maize. The *Rp1-D* gene confers resistance against *P. sorghi* IN2 isolate, mediating a hypersensitive cell death response (HR). To identify differentially expressed maize genes (DEGs) and metabolites associated with the compatible (susceptible) interaction and with *Rp1-D*-mediated resistance, we performed transcriptomics and targeted metabolomics analyses of *P. sorghi IN2*-infected leaves from the near-isogenic lines H95 and H95:Rp1-D. We observed a strong induction of the phenylpropanoid pathway (PPP) as well as the phytoalexin pathway upon infection in both lines, coinciding with increased expression of several genes encoding enzymes that catalyze these metabolic pathways. Several PPP compounds, including 4-coumaric acid and naringenin, specifically accumulated in the H95:Rp1-D resistant line. We identified a common response in H95:Rp1-D and H95 with an additional H95:Rp1-D specific resistance response observed at early time points at both transcriptional and metabolic levels. To better understand the mechanisms underlying Rp1-D-mediated resistance, we inferred gene regulatory networks (GRNs) occurring in response to *P. sorghi* infection. A number of transcription factors including WRKY53, MYB100 and NAC42 were identified as potentially important signaling hubs in the resistance specific response. This study provides a novel and multi-faceted understanding of the maize susceptible and resistant responses to *P. sorghi*.

Funding acknowledgement: National Science Foundation (NSF)

P135

Maize RNA Binding Motif Protein8a (RBM8a) is a candidate U12 splicing factor

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The precise recognition and splicing of non-coding introns from precursor mRNA is vital to eukaryotic gene expression. Most eukaryotes harbor two classes of introns in their genome, known as major U2-type and minor U12-type introns. U12-type introns represent less than 1% of all introns and are spliced by a distinct minor spliceosome. The biological implication of the simultaneous existence of two groups of introns remains largely unknown; however, mutations of minor spliceosomal proteins lead to aberrant splicing of U12-type introns, and developmental defects in both plants and animals. Here we report a maize mutant *rough*-414* (*rgh*-414*) displaying severe defects of endosperm development. The late development stage of mutant endosperm shows proliferative growth upon tissue culture and mimics phenotype of two well characterized minor splicing factor maize mutants *rbm48* and *rgh3*. The mutant is genetically mapped to a region spanning chromosome 10. None of the known minor splicing factors map in this region indicating that *rgh*-414* is likely a novel U12 splicing factor. Using a candidate gene approach, an RNA Binding Motif protein 8a (RBM8a) was identified as a putative causative gene for the mutant phenotype. The PCR amplified genomic sequence of the mutant *rbm8a* comparison with the cognate B73 and W22 inbred failed to detect any functional mutation bestowing the mutant phenotype. We also performed RT-PCR expression analysis of *rbm48* in different tissue and compared to both B73 and W22 inbred lines. Our data support an RBM8a expression polymorphism as a candidate molecular cause for the *rgh*-414* phenotype.

Funding acknowledgement: National Science Foundation (NSF), Oakland University

P136

Maize root hydraulic architecture and its response to water deficit

(submitted by virginia protto <virginiaprot@gmail.com>) (**Presenter: Virginia Protto**, *Undergraduate Student*)
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Water availability is the most limiting factor affecting plant growth and crop productivity worldwide. Understanding and controlling the mechanisms that underlie plant responses to drought has become essential for agriculture especially in the context of global change. Considered as a water-saving plant due to its C4 metabolism, maize is a suitable model for studying the physiological and genetic bases of cereal responses to drought. Here, we investigated maize root responses to water deficit and focused on three major aspects: hydraulics, growth, and signaling. These three functions shape root hydraulic architecture. We adapted a pressure chamber device to measure root hydraulic conductivity (L_{pr}) of hydroponically grown maize seedlings, using a whole embryonic root system or individual primary or seminal roots. Root system architecture (RSA) and L_{pr} were analysed in plants subjected to water deficit induced by various polyethylene glycol (PEG) concentrations. Moderate water stress (50 g/L PEG; Ψ = - 0.070 MPa) had no effect on RSA but slightly inhibited L_{pr}. In contrast, a higher water deficit (150 g/L PEG; Ψ = - 0.332 MPa) had inhibitory effects on root growth, lateral root formation and L_{pr}. Slight quantitative differences in both architectural and hydraulic responses to water deficit were observed between primary and seminal roots. In parallel, a genetic dissection of maize root hydraulics (L_{pr}) was initiated using Genome Wide Association Studies in a panel of 316 Dent lines. Associated SNPs and underlying candidate genes are currently under study. In the future, improved water transport measurements and root image analyses will be coupled to mathematical modelling to represent effects of water deficit on root hydraulic architecture of contrasting maize genotypes.

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P137

Major determinants of above ground architecture and flowering time affect brace root node number in sorghum

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Flowering time, height, chlorophyll content, flexural stiffness, and brace roots data were collected from a sorghum association population and a mutant population of the sorghum accession BTx623. GWAS detected alleles at *dw1*, *dw2*, and *dw3,* which are responsible for major effects on plant architecture, as determinants of brace root node number. These alleles also affected flexural stiffness, demonstrating that determinants of above-ground architecture and biomass also alter brace root node number and plant anchorage. Comparisons between isogeneic dwarf *dw3* and tall *Dw3* plants demonstrated that tall plants had lower flexural stiffness validating the GWAS result. Remarkably, tall *Dw3* plants appeared lighter green and quantitative measures confirmed this, though no GWAS hit for leaf chlorophyll content was observed in the association panel. An allele at the sorghum ortholog of *zcn8*, a known brace root regulator and flowering time determinant of maize, encoded the lowest p-value SNP in the genome affecting flexural stiffness of sorghum. Thus, the link between brace root node number and flowering time control is conserved between maize and sorghum. To accelerate sorghum research, we are culturing sorghum to maturity in standard greenhouse trays at high plant densities. We are currently growing sorghum at a variety of densities and tray depths to optimize seed-to-seed culture of sorghum in standard greenhouse conditions. This approach was successfully used for the production of a 1000 individual single-seed descent EMS population of BTx642 sorghum. At densities of 12 plants per (53x27x7cm^3) half of the EMS-treated plants generated seeds. High-density standard greenhouse tray culture of sorghum will enable any research institute with a greenhouse to study sorghum.

Funding acknowledgement: Department of Energy (DOE)

P138

Messengers Assemble: Building a hormone sensor toolkit for the study of cereal grains

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Phytohormone signaling pathways play an indispensable role in shaping every organ of a plant body, as well as contribute to how plants interact with their environment. In maize, for instance, auxin polar transport predetermines locations of new organ primordia, patterns vascular development, and mediates response to light and gravity. Cytokinin signaling regulates stem cell homeostasis, thus affecting extension of the shoot and the root, as well as size and morphology of organs such as leaves and inflorescences. Gibberellic acid is involved in maturation, floral induction, while mediating seed development, in coordination with abscisic acid, and response to stress, in coordination with auxin. These are only a few examples of the many phytohormones and small signaling molecules produced by plants, whose pathways inevitably cooperate to conduct all aspects of plant development. While many hormone sensing constructs have been created and studied in eudicot models such as Arabidopsis, less have been studied in maize, and less still in other grass systems.
We are currently gathering, building, and testing an expanding set of sensors for different hormone signaling pathways, currently including auxin, cytokinin, and gibberellic acid. The current roster of model systems include maize, sorghum, wheat, barley, and setaria. Functionality of the constructs are first verified using particle bombardment, with stable transformants to follow. Our goal is to provide the community with a comprehensive toolkit to visualize hormone signaling in various aspects of grass development, to examine how variations in hormone dynamics correlate with morphological and physiological changes, and to deploy them as markers to study the genetic pathways that shape organ architecture.

P139

Phenotypic analysis of maize and maize-teosinte lines following *Ustilago maydis* infection and tumor formation

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The corn smut fungus *Ustilago maydis* (*U. maydis)* is responsible for significant corn yield losses infecting all aerial parts and locally inducing tumors. The lack of resistant maize cultivars necessitates the identification of new sources of resistance. The near-isogenic lines (NILs) obtained from the cross between maize (B73) and teosinte (*Zea parviglumis*), have shown some evidence of resistance to corn smut. However, little is known about the varietal effects of *U. maydis* on tassel and ear-gall development. A randomized complete block design experiment was conducted in the greenhouse to investigate the resistance of four maize genotypes and two NILs (B73, H95, Golden Bantam, Mo17, NIL1, and NIL2) against different treatment levels of *U. maydis*. The six genotypes (180 plants for each genotype) were inoculated with 10^6 cells/ml of *U. maydis* at four different treatment levels (1ml, 2ml, 3ml, and 5ml). To determine the level of resistance for each genotype, corn smut disease incidence (number of plants infected) and disease severity (number and weight of galls in ears and tassels) were assessed 7, 10, 14, and 21 days post-inoculation. The preliminary results indicated that the H95 genotype was more susceptible (highest average ear-gall weight and size) than the other genotypes, while the two NILs were more resistant (lowest average ear gall weight and size). The statistical data analysis is ongoing.

Funding acknowledgement: Georgia Association of Corn Commission

P140

Potential alternative splicing in 3' UTR of Auxin Receptor ZmAFB2/3 b2

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ZmAFB2/3 b2 protein is an auxin-signaling F-box receptor in Zea mays. In the presence of auxin, this complex binds auxin repressor proteins and promotes their degradation, thus permitting auxin-related gene transcription by the ARF family of transcription factors. Preliminary gene models suggested tissue-specific expression and alternative splicing in the 5' and 3' UTR of ZmAFB2/3 b2. Primers were designed to test for the presence of splice variants and tissue-specific expression patterns, and RT-PCR was performed on RNA isolated from maize shoot, root, and embryo tissue. In contrast to predictions based on expression databases, we detected ZmAFB2/3 b2 expression in all tissues tested, but did find evidence of intron inclusion. More research is necessary to confirm the intron inclusion and to look for possible low levels of other splice variants. Studying ZmAFB2/3 protein expression levels will give additional insight to auxin control and response mechanisms in different conditions and tissue types.

Gene / Gene Models described: *ZmAFB2/3 b2*; GRMZM2G137451
Funding acknowledgement: National Science Foundation (NSF)

P141

Profiling of phenolic compounds in diverse maize kernels

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Maize is bred for different phenotypic traits to combat diseases and pests, increase yield, and survive in harsh environmental conditions. Many of these resilience traits found in corn have been associated with varying levels of phenolic compounds in plant tissues. Phenolics are phytochemicals found in fruits, vegetables, cereals, and herbs. They cause specific biological functions that can increase disease resistance, stress protection, and pest resistance. To characterize these compounds across a wide range of diversity in maize, phenolics were extracted from ground kernel tissue from the Wisconsin Diversity Panel inbred lines with a solution of methanol, formic acid, and an internal standard. After the extraction process, phenolic compounds were then quantified and profiled through liquid chromatography – mass spectrometry (LC-MS), creating unique phenolic profiles for the diverse maize kernels used. These profiles lead to a better understanding of natural diversity of maize phenolic compounds and the relationships among the phenolics and different maize genotypes. Future work in spectral profiling of ground tissue may lead to faster detection methods of phenolics in future studies, which would allow rapid phenotyping and ease of detecting desirable maize traits for breeding purposes.

Funding acknowledgement: United States Department of Agriculture (USDA), Michigan State University

P142

Public transcriptomic resources reveal variations in co-expression patterns of TFs and key diterpenoid metabolism genes across gene regulatory networks in *Zea mays*

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The combined pressure of shifting climate conditions and associated increases in pest and pathogen damage cause severe harvest losses in many crops, including globally important food crops such as maize. Recently, unique maize diterpenoid defense metabolites that share common precursors with primary gibberellin (GA) phytohormone metabolism have been discovered in maize and shown to impact plant fitness. The two diterpenoid groups, known as the Kauralexins (KAs) and Dolabralexins (DAs), show potent and distinct activities in pathogen defense and abiotic stress resistance. Using public genetic and genomic data, a list of 50 candidate TFs have been identified that co-express with key diterpenoid-biosynthetic genes. Candidates were selected based on co-expression with two or more key genes of interest, either within root tissue only, root and/or leaf tissue, or within leaf tissue only in a survey of over 45 publicly available RNAseq datasets. Furthermore, these TFs are predicted to bind to the promoters of several key genes involved in GA, KA, and DA metabolism as determined by 421 publicly available ChIPseq datasets. Interestingly, similarities in TF candidates varied across the root and leaf GRNs for each gene of interest, as seen with the ent-CPP enzymes AN1 and AN2, which are involved almost exclusively in GA and KA/DA metabolism, respectively. Given the critical roles diterpenoids play in plant development and defense, elucidation of the TF regulatory network governing maize diterpenoid metabolism can aid efforts in improving crop stress resiliency. Results from this approach will generate a comprehensive genome-wide atlas of TFs with probable roles in general and stress-inducible specialized diterpenoid metabolism, and provide high-priority TF candidates to pursue for functional characterization.

Gene / Gene Models described: *AN1, AN2*; Zm00001d032961, Zm00001d029648
Funding acknowledgement: National Science Foundation (NSF)

P143
QTL mapping for ear fasciation and kernel row number in two connected RIL populations in maize

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Numerous studies showed that the ear fasciation, namely an abnormal ear characterized by enlarged or multiple tips and disorganized kernel rows is under the control of several loci, many of which remain uncharacterized or unknown. In this study, we utilized QTL mapping in two connected recombinant inbred populations (RILs) from the crosses B73 x Lo1016 and Lo964 x L01016 to genetically dissect the ear fasciated phenotype present in the common parent line (Lo1016). Fasciation was analysed collecting four ear traits (fasciation index - FAS, ovality index - OVA, kernel disorder index - DIS and ear diameter ratio - DIA). Kernel row number (KRN) was also analyzed. Traits were collected in replicated field experiments over three years (2017 to 2019). Trait heritability values ranged 0.50 - 0.95 for fasciation-like traits and 0.59 - 0.85 for KRN. KRN and fasciation-related traits correlated (r = 0.32 - 0.49, P less than 0.01). Principal component analysis of the four fasciation-related traits showed that the first component (PC1) accounted for 80% of variation suggesting a single major cause shaping ear fasciation. QTL analysis by composite interval mapping on the combined datasets identified two major fasciated-ear QTL on chr. 2 and chr. 7. At both QTL, ear fasciation was contributed by Lo1016. For KRN, five QTL were mapped on chr. 2, 5, 7, 8 with positive and negative genetic effect assorted among parental lines. Correlation and QTL overlaps between fasciated-related traits and KRN as well as candidate genes at major QTL will be discussed.

P144
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: *RAMOSA3*; GRMZM2G014729, Zm00001d002781, Zm00001d025981
Funding acknowledgement: National Science Foundation (NSF)

P145
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. *Cochliobolus 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*; GRMZM2G042992
Funding acknowledgement: National Science Foundation (NSF)

P146 @JoeGage10
Rare variants play a key role in regulating the maize proteome

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Natural variation of the maize proteome is poorly characterized and mechanisms controlling it are not well understood, despite the fact that proteins are the cellular machinery by which gene action manifests. The higher energetic cost and longer half-lives of protein relative to mRNA mean that protein abundance often contains information that is non-redundant with mRNA abundance. In this study, we used dense SNP genotyping and shotgun proteomic data on diverse inbred maize lines to study the effects of rare variants on protein abundance. Because rare variants are associated with dysregulation of mRNA levels, we hypothesized that rare variants would have a similar effect on the proteome but potentially act through different mechanisms. We found that mass spectrometric quantifications of protein abundances are highly heritable, confirming their utility for quantitative studies. By contrasting protein abundance between alleles with and without rare SNP variants, we demonstrate that rare SNP alleles are associated with more extreme levels of protein abundance. This effect is particularly strong for SNPs located in the 5' UTR of genes. These findings implicate the 5' UTR as a sensitive and important region for regulation of gene expression and protein accumulation, reinforcing similar findings in yeast and validating model-based results in maize. However, mechanisms by which 5' UTR variants affect gene expression, including secondary structure, GC content, and modification of upstream open reading frames, remain poorly understood. This study begins to unveil how natural variation in sequence drives variation in protein abundance, which has practical implications for identifying deleterious mutations and purging load in maize.

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

P147

The effect of an ACT-like domain on the regulatory activity of the basic helix-loop-helix (bHLH) transcription factor R1

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In the maize aleurone, the basic helix-loop-helix (bHLH) transcription factor R interacts with the R2R3-MYB regulator C1 to activate the anthocyanin biosynthesis pathway. However, it is unclear how the coordinate regulation of all the pathway genes is accomplished, without any obvious conservation of the respective regulatory regions. Our previous studies showed that the monomer/dimer configuration of an ACT-like domain[1,2] at the C-terminus of R affects DNA-binding of the R bHLH motif. We proposed a model in which when the ACT-like domain forms a dimer, the bHLH is monomeric and R is tethered to DNA indirectly, through the interaction with C1[3]. When the dimerization of the ACT-like is impaired, then the bHLH motif dimerizes and recognizes a canonical G-box (CACGTG). To further illustrate the mechanism of how the ACT-like domain affects DNA-binding by the bHLH motif, we examined the *in vitro* DNA binding capacity of the bHLH motif on the promoter region of anthocyanin biosynthetic gene *A1* and *Bz1* in the absence/presence of the ACT-like domain. Using amplified luminescent proximity homogeneous assay (ALPHA), we identified multiple non-canonical DNA-binding sites of the bHLH motif in the *A1* and *Bz1* promoter and demonstrated that in the presence of the ACT-like domain, the bHLH binds better non-canonical DNA-binding sites. We will discuss these results in the context of what this may mean for the regulation of the anthocyanin pathway. Funding for this project was provided by MCB-1822343. References cited1. **Feller A, Hernandez JM, Grotewold E** (2006) An ACT-like domain participates in the dimerization of several plant bHLH transcription factors. J Biol Chem **281:** 28964-289742. **Feller A, Yuan L, Grotewold E** (2017) The BIF Domain in Plant bHLH Proteins Is an ACT-Like Domain. Plant Cell **29**: 1800-18023. **Kong Q, Pattanaik S, Feller A, Werkman JR, Chai C, Wang Y, Grotewold E, Yuan L** (2012) Regulatory switch enforced by basic helix-loop-helix and ACT-domain mediated dimerizations of the maize transcription factor R. Proc Natl Acad Sci U S A **109**: E2091-2097

Gene / Gene Models described: *R1, A1, Bz1*; Zm00001d026147, Zm00001d044122, Zm00001d045055
Funding acknowledgement: National Science Foundation (NSF)

P148 @edbertolini

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 cereals, inflorescence branching and leaf angle are important traits that contribute to planting density and yield potential. Several classical 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 branch number (TBN) and leaf angle (LA) in maize by defining transcriptional networks that function in lateral organ boundaries to promote development of these morphologically distinct organs. Using a set of nine mutants with specific developmental defects in one or both traits, we generated dynamic, context-specific gene regulatory maps that describe ligule and tassel branch development at the molecular level. Mutants introgressed into B73 and control 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 development of the ligular region, including the shoot apical meristem, were also collected from mutants with LA defects. RNA-seq was performed on 140 samples and integrated into gene regulatory and 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 help prioritize novel genes and regulatory elements underlying diversity in LA and tassel branching phenotypes. We also used these transcriptional networks to guide multi-trait genome-wide association studies (GWAS) based on three years of field phenotyping TBN and LA traits in over 500 diverse maize lines. Various network-assisted GWAS approaches were used to identify polymorphisms in candidate genes that associate with these architecture traits and the pleiotropy between them. Our data provide novel insight into regulatory mechanisms controlling architectural pleiotropy that can be used for targeted crop improvement.

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P149

The role of strigolactones in iron homeostasis in maize

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Gene discovery is a necessary step towards crop improvement. Our research aims to identify new players involved in iron homeostasis and understand their functional role in the uptake and translocation of iron to the different tissues. We are currently analyzing a novel mutant in maize that is leading us to new insights into the previously unknown role of strigolactone hormone signaling in iron homeostasis. The mutant was identified through a screening of Maize COOP stocks for iron-deficient phenotypes (yellow stripes). This mutant was analyzed genetically, and the underlying causative gene was identified as an enzyme involved in the production of strigolactone hormones. These hormones have been implicated in regulating a wide range of developmental features, including root growth and shoot branching. However, they have not been previously linked to iron homeostasis. We will present experiments aimed at uncovering the role of strigolactones in iron homeostasis. These experiments focus on answering two main questions; whether strigolactones are involved in the regulation of iron homeostasis or whether they affect iron uptake due to regulation of plant root architecture. These investigations are providing new information about the specialized ways that grasses perform and regulate iron uptake. This is of great importance, as most staple crops, including maize, have evolved an iron uptake strategy that is distinct from non-grasses. Increasing our understanding of these processes will be critical to developing biofortification strategies in staple crops like maize.
Funding acknowledgement: United States Department of Agriculture (USDA)

P150
The thermal adaptation profile of the maize proteome

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All organisms are affected by the temperature of their habitat and require a variety of adaptation strategies. Protein thermal adaptation is especially important; environmental temperature affects enzyme kinetics and reaction rates. Biochemical reactions would not occur fast enough to sustain life without enzyme catalysis. While there are numerous species- and environment-specific approaches to temperature adaptation, proteins are also governed by physical and chemical rules that remain consistent across Archaea, Bacteria, and Eukaryotic species. Furthermore, functional protein domain motifs, such as those identified in the Pfam database, can be identified and aligned across the tree of life. In this study we developed a method to identify temperature-sensitive amino acid residues using Pfam domains and a protein-independent estimation of prokaryote optimal growth temperatures. Nearly 20% of all tested amino acid residues are associated with temperature in prokaryotes, with consistent amino acid properties driving evolution at these sites. We applied a similar analysis to maize and created a thermal adaptation profile for the maize proteome. We are now testing the hypothesis that protein thermal stability is under selection in maize environmental adaptation. Future work will create thermal proteome profiles of maize accessions adapted to distinct temperature environments and use these to determine the extent to which plant proteomes are able to adapt to the temperature of their surroundings.

Funding acknowledgement: United States Department of Agriculture (USDA)

P151
Tuning maize auxin repressor degradation rate

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Auxin is a growth hormone that plays an important signaling role in developmental processes in plants. In Zea mays, ear and tassel development is highly dependent on auxin. The maize nuclear auxin signaling pathway includes receptor and repressor components that are crucial to perceiving and responding to the auxin signal. Manipulating sequences in both the repressors and receptors allows us to identify the key motifs that contribute to the functions of these proteins. Crucial parts of repressors that are more significant to fast auxin degradation rates are called “rate motifs”, and were originally identified in Arabidopsis. We have used a synthetic auxin signaling system in yeast to identify maize auxin repressor rate motifs. For both the ZmIAA25 and ZmIAA16 auxin repressors, we have identified sequences C-terminal of the degron region to be of high importance to fast degradation in presence of auxin. Ongoing work is focused on identifying rate motifs in other ZmIAA family members and characterizing what regions of maize auxin receptors also contribute to ZmIAA degradation dynamics. Identifying and characterizing auxin repressor rate motifs in Zea mays will allow for greater understanding of the full capacity of the auxin signaling pathway in maize, and point the way towards manipulation of certain growth processes to enhance the agricultural utility of corn.

Gene / Gene Models described: *ZmIAA25, ZmIAA16*; GRMZM2G115357, GRMZM2G121309
Funding acknowledgement: National Science Foundation (NSF)

P152

Two maize E3 ubiquitin ligases control hyper-sensitive response in maize

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The plant hypersensitive response (HR), a rapid cell death at the point of pathogenesis, is mediated by nucleotide-binding leucine rich repeat (NLR) resistance proteins (R-proteins) which recognize the presence of specific pathogen-derived proteins. Rp1-D21 is an autoactive maize NLR R-protein which triggers HR spontaneously. We previously mapped loci associated with variation in the strength of HR induced by Rp1-D21. Here we identify two E3 ligases, ZmCER9 and ZmMIEL1 which are causal genes at chromosomes 9 and 10 modifier locus, respectively. Transient expression of ZmCER9 in *N. benthamiana* reduced HR induced by Rp1-D21, while suppression of ZmCER9 expression in maize carrying Rp1-D21 increased HR. Similar results were seen with ZmMIEL1, though the earlier one found to suppress HR stronger. E3 ligases mediate protein degradation via the ubiquitin-mediated proteasome pathway. We demonstrated that both ZmCER9 and ZmMIEL1 are functional E3 ligases and that the effect of ZmCER9 and ZmMIEL1 was dependent on the proteasome, but only ZmCER9 reduced protein levels of Rp1-D21 and other autoactive NLRs when co-expressed in the *N. benthamiana* system. In contrast the ZmMIEL1 did not reduce protein levels of Rp1-D21 and RPM1D505V. ZmMIEL1 found to interact and regulate the protein levels of ZmMYB83, which is a positive regulator of VLCFA biosynthesis and HR. Similar mechanism was identified and proved in *Arabidopsis*.

Gene / Gene Models described: ; GRMZM2G145104, GRMZM2G056270
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P153

Understanding the role of a cysteine protease in regulating stay-green in maize

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Stay-green is an important trait that can improve plant productivity by extending the period of photosynthetic assimilation. The genetic regulation of this quantitative trait is highly complex. Through a comprehensive systems genetic analysis, we have identified 64 candidate genes that have a role in the regulation of senescence in maize (Sekhon et al., 2019; The Plant Cell). One of the candidate genes, *mir3*, encodes for a cysteine protease putatively involved in proteolysis and, therefore, N remobilization and signaling. We also reported delayed onset of cysteine protease activity in a stay-green inbred (PHG35) compared to the non-stay-green inbred (B73). These results suggest that differential activity of mir3 can be contributing to the stay-green phenotype. To confirm the role of *mir3* in the regulation of the stay-green trait, we are characterizing a diverse set of inbred lines with varying degrees of stay-green phenotype. Association of the distinct mir3 alleles with cysteine protease activity, Rubisco activity, N remobilization, and C/N status will be presented. Characterization of the maize *mir3* in Arabidopsis is underway using transient and stable transgenics. Together, these data will elucidate the role of mir3 in senescence and allow the manipulation of the stay-green trait in maize.

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P154 @r_benke12

Using metabolomics and association genetics to map lesion mimic mutants

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Plant mutants that form lesions and/or undergo spontaneous cell death in the absence of pathogens or stress have been isolated from a wide variety of genetic systems. These mutants form spontaneous lesions due to constitutively active disease signaling or defects in metabolism that result in cell death. Of the more than 50 maize lesion-forming mutants, the etiologies of only four of these are known. Using untargeted metabolite profiling we have explored the metabolic consequences of 24 maize lesion-forming mutants and compared them to mutants with constitutively activated hypersensitive-response (*Rp1-D21*) and defects in chlorophyll metabolism (e.g. *Oy1-N1989*). Clustering on metabolite abundances clearly indicates a metabolic syndrome similar to the HR-signaling mutant *Rp1-D21* in a subset of these 24 mutants. The dominant mutant *Les10* exhibits similar shifts in metabolite levels, including predicted defense metabolites (e.g. salicylic acid) and chlorophyll breakdown products, as observed in *Rp1-D21*. The *Les10* gene was previously mapped to chromosome 2L using translocation stocks and mutant linkage. We used genome-wide association to explore whether natural variation encoded suppressor or enhancer alleles affecting *Les10* phenotypic severity. An F1-hybrid population was generated by crossing an association panel by *Les10/+* pollen parents and phenotyped for lesion severity, chlorophyll content, and plant height. The polymorphisms with the strongest association with these traits mapped to the region encoding *Les10*, suggesting that a cis-QTL affecting the *Les10* locus itself may be responsible for the largest genetic effect on the mutant phenotype in this population. The top SNP is near an ankyrin-domain containing gene similar to Arabidopsis *ACCELERATED CELL DEATH6.* A dominant allele of *acd6* in Arabidopsis undergoes spontaneous cell death, suggesting this maize gene is a good gene candidate for *Les10*.

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P155 @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, flexible 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. Additional plants were sampled at 15 days after sowing and at anthesis for metabolic characterization using $^{13}CO_2$ labeling. 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.

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P156

A multiple genome alignment workflow shows the impact of masking and parameter tuning on alignment of functional regions

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Alignments of multiple genomes are a cornerstone of comparative genomics, but generating these alignments remains technically challenging and often impractical. We developed the msa_pipeline workflow (https://bitbucket.org/bucklerlab/msa_pipeline) to allow practical and sensitive multiple alignment of eukaryotic genomes with minimal user inputs. Our workflow only requires a set of genomes in FASTA format. The workflow outputs multiple alignments in MAF format, and optionally also outputs a phylogenetic tree inferred from k-mers and genome-wide conservation scores calculated using GERP++. In addition, we explored the impact of different masking approaches and alignment parameters using genome assemblies of 33 grass species. Compared to conventional masking with RepeatMasker, a k-mer masking approach increased the alignment rate of CDS and non-coding functional regions by 20.56% and 13.80% respectively. We further found that default alignment parameters generally perform well, but parameter tuning can increase the alignment rate particularly for non-coding functional regions by up to 20% compared to default settings.

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P157

Chromatin types and epigenomic features shape the differentiation of the maize subgenomes

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Whole genome duplication or polyploidy is a ubiquitous phenomenon in many eukaryotic species. While biased fractionation of the two subgenomes have been demonstrated in maize, relatively little is known about the evolutionary forces that shape the pattern of genome differentiation. Here, we demonstrate that the dominance of one subgenome is more significant in chromosomal arms than that in pericentromeric regions, which is reflected by a higher rate of gene loss and larger differences of evolutionary distances including both nonsynonymous substitution (Ka) and synonymous substitution (Ks) between the two subgenomes in chromosomal arms. Furthermore, the relaxed selection, more small interfering RNAs and higher levels of CHH (H = A, T, or C) DNA methylation level around genes of one subgenome are only observed in chromosomal arms, but not in pericentromeric regions. Interestingly, singleton genes of the dominant genome have been subject to stronger relaxed selection than duplicated genes only in chromosome arms. In addition, we investigated histone marks including H3K27me3, H3K36me3, H3K9ac and H3K27ac, and our results reveal that they distribute differently between subgenomes and between subgenomes in chromosome arms. Together our data suggest that chromatin environments and epigenomic features have significant impact on the maize genome architecture, and perhaps are important determinants that shape the biased fractionation of the two maize subgenomes.

P158

Genome-wide mediation analysis: bridging the divide between phenotype and genotype via gene expression

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Connecting genotype to phenotype (G2P) is an essential topic in genetics. As the genome-wide markers become increasingly available, genome-wide association study (GWAS) has been widely applied to establish the relationship of G2P. GWAS, however, usually hits peak spanning wide genomic regions with many candidate genes, making the downstream results interpretation or gene validation a challenging task. Under the context of genetics research, we hypothesized a causal chain from genotype to phenotype partially mediated by gene expression. To test this hypothesis, we applied the high dimensional mediation analysis, a class of causal inference method with an assumed causal chain from exposure to the mediator to outcome, to maize diversity panel (N=280 lines) with 40 publicly available agronomic traits, 66 newly generated metabolic traits, and published RNA-seq data from seven different tissues. After conducting the mediation analysis, N=736 unique mediator genes were detected, explaining a 12.7% proportion of the variance mediated (PVM) on average. Noticeably, 83/736 mediator genes were identified in more than one trait, suggesting the prevalence of pleiotropic mediating effects. Among those pleiotropic mediators, 2-oxoglutarate-dependent dioxygenase (BX13) which controls benzoxazinoids synthesis was identified in 40 different agronomic and metabolic traits consistent with the multi-functional roles of benzoxazinoids as reported previously. Our results suggested the genome-wide mediation analysis is a powerful tool to integrate multi-Omics data in providing promising candidate causal genes to connect G2P.

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P159 @spalalidelen

Identification of the yield-related traits associated loci under different nitrogen conditions in maize

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The growing need for corn in the human diet, as animal feed, and as an industrial feedstock require that maize yield continuously increase. At the same time, the need to decrease the energy usage and ecological impact of agriculture require developing varieties of maize which require less nitrogen input. Prior to the 1940s selection likely occurred in nitrogen-limited agricultural systems and since the 1960s selection likely occurred in systems where nitrogen was not the limiting constraint on productivity or yield. In order to identify genomics loci under the shifted nitrogen regimes, we carried out a large-scale field experiment using the maize diversity panel (N=231 lines) under two nitrogen treatments (standard and low N conditions). After harvesting, we collected yield-related phenotypic traits from the mature ears. We then performed a genome-wide association study to identify traits-associated SNPs. Additionally, we conducted genome-wide complex trait Bayesian (GCTB) analysis to estimate population genetics parameters using genome-wide SNPs, including putative deleterious ones. This study will help understand the purifying selection and their effects on yield-related traits.

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P160

Inferring the recombination history of European maize

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Since its domestication in Central Mexico maize has spread around the globe and adapted to a variety of local environments. The domestication and expansion of maize therefore provide an excellent model to understand the genomic basis of plant adaptation. Selection and demographic changes shaped the morphological and genetic diversity of modern maize populations. Two major introduction routes built the foundation of European maize, one from the Caribbean and one from North America. Since then, maize adapted to European growth conditions and local landraces evolved. Previous studies have identified adaptive loci within European landraces and breeding pools. However, the source and selection history of adaptive haplotypes that allowed maize to become one of the most important crops in Europe remain vastly unknown. Genetic trees have been used to describe the relationship between individuals. But in diploid outcrossing species like maize, recombination reshuffles parental genomes every generation which creates a large number of distinct trees along the genome. Our goal is to reconstruct the whole recombination history of maize using genetic trees to gain insight into the adaptation history of the crop. We use the introduction of maize to Europe as model for the recent adaptation of a crop to a new environment and employ a novel inference algorithm to reconstruct the recombination history of European maize. We infer the age and origin of adaptive haplotypes, using the recombination graph based on genotyping data from over 2000 European maize individuals. We display the genome wide diversity and relationship of our samples in hundreds of thousands of trees and reconstruct their ancestral haplotypes. Our analysis shows the utility of recombination graphs to elucidate the population history of maize landraces and breeding pools.

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P161

Knob diversity and conservation in the maize pangenome

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Knobs in maize are extra-long tandem repeats that are well-known for their neocentromeric role during meiotic drive in the presence of the abnormal chromosome 10 haplotype (Ab10). Due to their highly repetitive structure and large size (up to several Mb), knobs are very difficult to reconstruct in a genome assembly. Therefore, they are typically identified and studied as cytological elements in the genome, described by their rough chromosomal position and major repeat type—either knob180 or TR-1. In the recently-released maize pangenome, however, knobs are well annotated and many smaller knobs are fully assembled, which provides the first opportunity to do in-depth genomic studies of knob repeats across diverse genetic backgrounds. In this study, we describe and categorize knob repeat arrays based on size, repeat composition, and genomic position, revealing knob structural and positional variation not observable with cytological data. These results provide novel insights into the evolution of knobs in maize.

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P162

Maize domestication is a stable process during evolution

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Despite decades of genetic research on trait differences between crops and their progenitors, far less is known about how domestication was constrained by the genetic architecture of crop progenitors and how genetic architecture was altered in the crop species. Yang et al. (2019) drew multiple conclusions about how the genetic architecture influenced and was altered by maize domestication based on single teosinte and maize populations. To test the generality of these conclusions, we investigate the genetic architecture of 16 domestication traits in a diverse sample of four teosinte populations and four matching maize populations. Applying an evolutionary quantitative genetics approach, we assayed the structure of genetic variances, genetic correlations among traits, strength of selection during domestication, and diversity in genetic architecture within teosinte and maize. Our results confirm that additive genetic variance is decreased while dominance genetic variance is increased during maize domestication. The genetic correlations are moderately conserved among traits between teosinte and maize, while the genetic variance–covariance matrices (*G*-matrices) of teosinte and maize are quite different, primarily due to changes in the submatrix for reproductive traits. Selection intensities during domestication were generally weak, and reproductive and environmental response traits rejected neutral hypothesis across all populations, suggesting they were major targets of selection during domestication. The *G*-matrix in teosinte placed considerable constraint on selection during the early domestication process became greater along the domestication trajectory. Further, we assayed variation among populations showing that there is far less difference in genetic architecture among populations within teosinte or maize than that between teosinte and maize. While selection drove changes in essentially all traits between teosinte and maize, selection is far less important for explaining trait differences among populations within teosinte or maize.

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P163
Megabase-scale structure variation with *Tripsacum* origin contributes to maize adapation

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Structural variants (SVs) are significant contributors to the genome diversity and environmental plasticity for diverse species. In contrast to the prevalent small SVs in higher Eukaryotes, large size SVs rarely survived against the evolutionary purging effects, which can conceal the insights on speciation and adaptation. In this study, we discovered and characterized several megabase-scale presence-absence variations (PAVs) in maize genome. Surprisingly, a 3.2 megabase PAV fragment showed high integrity and presented as complete presence or absence in the natural diversity panel. This PAV is embedded within the nucleolus organizer region (NOR). The suppressed recombination within NOR was found to underpin the resistance to evolution purging. Interestingly, sequence analysis of this PAV not only revealed the domestication trace from teosinte to modern maize, but also the footprints of its origin from *Tripsacum*, shedding light on previously unknown contribution from *Tripsacum* to the speciation of *Zea* species. The functional consequence of the *Tripsacum* introgression was also investigated, and environmental fitness conferred by the PAV may explain the strong selection on the whole element during maize domestication and improvement. These findings provide a novel perspective that *Tripsacum* contributed to *Zea* speciation, and also instantiate the use of SVs to profile the genome evolution.

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P164 @mihai_miclaus
Probing the pristine maize germplasm of open pollinated varieties in SE Europe

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Maize Open Pollinated Varieties (OPV), untouched by modern agriculture, are still present in the hilly to mountainous regions of the Carpathian Mts. They have been passed-on "from father to son" ever since maize was introduced in Romania, four centuries ago. Part of these OPVs have served in the extraction of inbred lines. We have previously genotyped-by-sequence (GBS) more than 2,000 inbred lines from Romania and the Balkan region underling the unique genetic background of inbred lines originating from OPVs. Here, we complement our existing data by genotyping the first 475 of the Romanian 3,000 OPVs collection. SNP data confirms the already known pristine locations and also identifies new reservoirs of genetic diversity, within mountain depressions and arid planes. By corroborating GBS data on OPVs and inbred lines we shed new light on the existing genetic diversity in SE Europe and its potential for future breeding programs.

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P165

The molecular domestication syndrome: simulations reveal a rewiring of genetic architectures

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Domestication is associated with important genomic changes, some well-documented as the loss of genetic diversity and modifications of gene expression patterns. Here, we explored theoretically the effect of domestication at the genomic level by characterizing the impact of a domestication maize-like scenario on gene regulatory networks. We ran population genetics simulations in which individuals were featured by their genotype (an interaction matrix encoding a gene regulatory network) and their gene expressions. Our domestication scenario included a population bottleneck, and a selection switch (change in the optimal gene expression level) mimicking phenotypic canalization, i.e. genes within the network evolve towards more stable expression to parallel enhanced environmental stability in man-made habitat. We showed that domestication profoundly alters genetic architectures including (i) a deep rewiring of the gene regulatory networks, with a trend towards gain of regulatory interactions between genes, and (ii) a global increase in the genetic correlations among gene expressions, with a loss of modularity in the resulting coexpression patterns. Our model provides empirically testable predictions and contribute to define a molecular domestication syndrome.

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P166

Two teosintes made modern maize

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Maize arose from a single domestication from *Zea mays* ssp. *parviglumis* in southern Mexico. Post-domestication introgression from *Zea mays* ssp. *mexicana*, which is a wild relative to maize, help highland landraces to adapt to new environments. However, introgression from *mexicana* to maize is far more extensive than we had thought. Here, we show that the ~18% genome of each single line of 847 modern landrace and maize were introgressed from *mexicana* by f4 statistics and ancestry HMMs. The admixture graph reveals that the hybrid of maize and *mexicana* is the ancestor of all tested landraces and maize from Americas and China. And we estimated this hybridization was happened at ~6,000 generations ago. Furthermore, we prove that *mexicana* introgression impacts maize phenotype by quantifying the proportion of phenotypic variation and identified many candidate loci. As a case study, the knocked-out mutants by CRISPR/Cas9 of *ZmPRR37a* whose haplotype was derived from *mexicana* but fixed in maize exhibit significantly early flowering phenotype than wild type. This comprehensive analysis of *mexicana* introgression improve our understanding of maize evolution and highlight *mexicana* could be a great genetic resources for continued maize improvement.

Gene / Gene Models described: *ZmPRR37a*; Zm00001d022590
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P167

A genetic and molecular approach to identify transcription factors control maize root adaptive response to water deficit

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Water stress is recognized as the most severe abiotic stress for agricultural productivity. Root traits play a key role in tolerance to water stress but have largely been neglected in selection schemes. In order to identify the maize genetic bases of the root adaptive responses to water deficit, we used a MAGIC mapping population of 400 lines based on the intercrossing of 16 genotypes. The fine phenotyping of the different genotypes was performed under contrasting water supply on the French root phenotyping platform (www6.dijon.inrae.fr/plateforme4pmi_eng/4PMI-platform). On the founder genotypes of the mapping population, in addition of phenotyping, we sampled different root tissues daily over 7 days after irrigation arrest. On the basis of the 448 transcriptome samples, we built a workflow that identified 6945 differentially expressed genes between axial and lateral roots and in response to water deficit and inferred a regulatory gene network to identify transcription factors (TF). Using a hierarchical clustering, we split the network in 35 clusters homogeneous in their expression pattern. Fine analysis of individual cluster pointed out without prior knowledge already known FTs and identified new candidates. We selected 51 TF and to functionally validate them identified and phenotyped homozygous KO mutants for those genes in Arabidopsis. Many genotypes have an altered root developmental response to in vitro osmotic stress. In parallel, the phenotyping and a transcriptomic analysis by RNAseq of the genotypes of the mapping population under optimal conditions and water deficit enabled a GWAS and an eQTL analysis. Both approaches identified polymorphisms in genes of interest and notably identified SNPs colocating near transcription factors also identified by the gene network approach. Taken together all the data identified candidate genes and alleles potentially controlling adaptive root development that can be interesting target for breeding.

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P168

A maize male gametophyte-specific gene encodes ZmLARP6c1, a potential RNA-binding protein required for competitive pollen tube growth

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Members of the La-Related Protein family (LARPs) contain a conserved La module, which has been associated with RNA-binding activity. Expression of the maize gene GRMZM2G323499/Zm00001d018613, a member of the LARP family, is highly specific to pollen, based on both transcriptomic and proteomic assays. This suggests a pollen-specific RNA regulatory function for the protein, designated ZmLARP6c1 based on sequence similarity to the LARP6 subfamily in *Arabidopsis*. To begin testing this hypothesis, a *Ds-GFP* transposable element insertion in the *ZmLarp6c1* gene (*tdsgR82C05*) was obtained from the Dooner/Du mutant collection. Sequencing confirmed that the *Ds-GFP* insertion is in an exon, and thus likely interferes with ZmLARP6c1 function. Tracking inheritance of the insertion via its endosperm-expressed GFP indicated that the mutation was associated with reduced transmission from a heterozygous plant when crossed as a male (ranging from 0.5% to 26.5% transmission), but not as a female. Moreover, pollen from homozygous plants produced ears with full seed set. The transmission defect apparent in pollen from heterozygotes was significantly alleviated when less pollen was applied to the silk, reducing competition between mutant and wild-type pollen. Pollen grain diameter measurements and nuclei counts showed no significant differences between wild-type and mutant pollen. However, *in vitro*, mutant pollen tubes were significantly shorter than those from sibling wild-type plants, and also displayed altered germination dynamics. These results are consistent with the idea that ZmLARP6c1 provides an important regulatory function during the highly competitive progamic phase of male gametophyte development following arrival of the pollen grain on the silk. The conditional, competitive nature of the *Zmlarp6c1::Ds* male sterility phenotype (i.e., reduced ability to produce progeny seed) points toward new possibilities for genetic control of parentage in crop production. Preliminary data indicating significant differences between wild-type and mutant transcriptomes will be presented, further supporting a regulatory role for ZmLARP6c1.

Gene / Gene Models described: *ZmLARP6c1*; GRMZM2G323499/Zm00001d018613
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P169
Analysis of a receptor like kinase in grass asymmetric cell divisions

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Asymmetric cell divisions are critical for establishing cell fate in plants and other eukaryotes. In plants, such divisions are of paramount importance due to plant cells' inability to migrate after division. Grass stomatal complexes have a unique morphology consisting of two dumbbell shaped guard cells each flanked by a subsidiary cell. The guard mother cell is formed by an asymmetric division and each subsidiary cell also arises from an asymmetric division. Subsidiary cells polarize several proteins prior to division. Two of the known polarized proteins are catalytically inactive Leucine Rich Repeat – Receptor Like Kinases (LRR-RLKs).. This project aims to characterize BdPAN1, an LRR-RLK, in *Brachypodium distachyon*. *Bdpan1,* is particularly interesting due to its 9 base pair, in-frame deletion in the first leucine rich repeat of its extracellular domain, indicating the LRR domain is important for BdPAN1 function. BdPAN1's function is not known. BdPAN1 may detect a ligand or interact with another critical protein via its LRR or pseudokinase domain. This unusual mutation confers a stomatal phenotype like that observed in *Zea mays* (maize) null mutants of the homologous gene *ZmPan1*.. PAN1 is not only expressed in subsidiary mother cells, but in new cell plates of all dividing cells indicating a generalized or alternative role in cell division. This project aims to uncover the molecular function of BdPAN1 in subsidiary cells, as well as its role in diverse cell types. *Bdpan1* mutants are being transformed with a full-length *BdPAN1* gene fused to a YFP marker. A similar construct, with the coding region for the extracellular LRR domain deleted, and a third construct with the intracellular kinase domain deleted is also being transformed into *Bdpan1* mutant plants. These transgenic plants will allow me to determine the contributions of each protein domain to PAN1 polarization vs PAN1 function.

Gene / Gene Models described: *pan1*; GRMZM5G836190, BRADI_3g39910v3
Funding acknowledgement: National Science Foundation (NSF)

P170 @plantsdonttweet
Analysis of aberrant TANGLED1 localization in the preprophase band defective maize mutant discordia1

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The orientation of cell division in plants is critical for proper growth and development. Establishment of the division plane is mediated through the correct placement of the division site. In plants, the formation of a cortical ring of microtubules and actin called the preprophase band (PPB) sets up the division site and predicts the cell plate insertion site. A small number of proteins are known to colocalize with the PPB and remain at the division site after PPB disassembly in metaphase. One of these proteins is the microtubule binding protein TANGLED1. Deletion constructs and chemical treatments have shown there to be two distinct TAN regions required for early and late TAN localization to the division site. However, the mechanism by which TAN is recruited remains unknown. Here we show that TAN1 is recruited through a PPB-independent mechanism in late telophase using a mutant with defects in proper PPB formation. Additionally we are examining TAN1 localization in a mutant which forms defective PPBs to investigate how TAN1 is recruited to the cell cortex. Together with experiments that disrupt PPB formation or alter proper new cell wall formation, we provide insight into distinct mechanisms by which TAN1 is recruited to the cell cortex independent of the PPB.

Funding acknowledgement: National Science Foundation (NSF)

P171
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. In particular, BETL cell elongation and cell wall ingrowths are delayed or decreased in heterozygous mutant seeds. 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 and seed size in maize.

Gene / Gene Models described: *stt1, stt2, stt3*; none
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Wisconsin Lutheran College Faculty Mini-Grant Program

P172 @subbaiahchalive
Aspergillus flavus SrbA is required for conidial germination and survival under hypoxia, and colonization of intact maize seeds

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Although maize is a strict aerobe, its seeds develop under severe oxygen (O_2) deprivation. This remarkable adaptability provides a case study for the plasticity of seed development and further, has implications in the colonization of seed pathogens. *Aspergillus flavus* is a major ear rot mycotoxigenic pathogen that contaminates corn and other commodities with carcinogenic aflatoxin (AF) and neurotoxins. Our earlier studies show that *A. flavus* is an obligate aerobe (its growth and morphogenesis are stringently O_2-dependent in culture media). Yet, the fungus successfully invades severely hypoxic maize seeds and produces abundant AF, implying that the fungus is compensated for low O_2 by seed components or it may co-opt seed hypoxia-adaptive mechanisms. Understanding the basis of this cross-talk is important to identify new targets and limit the contamination of the food chain with mycotoxins. Using genetic and molecular approaches, we have been analyzing how *A. flavus* navigates the stressful seed microenvironment. Transcription factors belonging to SREBP (sterol regulatory element-binding proteins) family are critical for survival of human pathogenic fungi under hypoxia and for their pathogenicity in hypoxic host tissues. Here, we present the role of *A. flavus* SrbA (sterol regulatory element-binding protein A) in the hypoxic survival, virulence and aflatoxigenicity of *A. flavus* in maize. A deletion mutant, Δ*srbA*, shows divergent regulation of hypoxia tolerance and AF biosynthesis. *srbA* conidia fail to enter the polar growth stage of germination under *in vitro* hypoxia (3% O_2) and do not recover after reaeration. The mutant is severely impaired in its infectivity of intact maize seeds while showing robust colonization of injured seeds. At the same time, Δ*srbA* mutant is hypertoxigenic, producing more AF than the isogenic wild type under normoxia in synthetic media. Our data strongly supports the proposal that hypoxia fitness is critical for *A. flavus* infection of maize seeds.

Funding acknowledgement: National Corn Growers Association

P173

Autophagy is upregulated in response to high temperatures in maize

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Autophagy is a major degradation and recycling pathway that delivers cargo such as organelles and proteins to the vacuole. Autophagy is upregulated in plants by environmental stresses such as heat, drought, and salt to help plants survive these conditions. A better understanding of autophagy in crop species under field conditions may therefore lead to beneficial agricultural applications. We took advantage of a controlled environment facility called the Enviratron to simulate field conditions, using temperature as a varying parameter to study the effect of heat stress on autophagy. We found that autophagy in a W22 inbred line is upregulated in the virtual afternoon at a maximum daily temperature of 35℃ or 37℃. The upregulation of autophagy correlated with the induction of the unfolded protein response, a response that is known to activate autophagy under heat stress. We also confirmed the findings in the Enviratron by analyzing samples collected on a hot summer day from field-grown plants. In addition, RNA-seq analysis revealed that several autophagy-related genes were upregulated in response to heat. We are now investigating potential transcriptional regulation of autophagy under these conditions.

Funding acknowledgement: National Science Foundation (NSF)

P174

Characterization of *ramosa suppressor locus*12.2995,* a novel allele of *opaque1* that regulates plant architecture in maize

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Ideal plant architecture optimizes canopy structure and increases grain yield in maize. However, its underlying genetic mechanisms remain poorly characterized. We recently identified and characterized a recessive, EMS-induced maize mutant, *ramosa suppressor locus*2995.12 (rsl*-12.2995)* with aberrant plant architecture. *rsl*-12.2995* mutant is semi-dwarf with compressed internodes, reduced tassel branch number, and opaque kernels. It also has displays reduced leaf size, significantly narrower leaf midrib, reduced auricle, and ectopic ligule tissue displaced into the midrib. We also investigated the genetic interaction between *rsl*-12.2995* and *ramosa* mutants, *rsl*-12.2995* partially suppresses *ra1* and *ra2* tassel and ear phenotypes. Using map-based cloning and whole-genome sequencing, we localized *rsl*-12.2995* to a small region containing a missense mutation in a gene encoding a Myosin XI motor protein, the same gene as *opaque1 (o1)* in maize. Complementation tests also verified that *rsl*-12.2995* is allelic to *o1* (*o1-ref* and *o1-N1243*). Based on these results, we propose that *rsl*-12.2995*, a novel allele of *o1*, plays a role in leaf patterning, inflorescence development, and overall plant architecture.

Gene / Gene Models described: *o1*; GRMZM2G449909
Funding acknowledgement: National Science Foundation (NSF)

P175

Comparative analysis of DNA replication in maize and sorghum

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In eukaryotes, DNA replication is tightly choreographed in both space and time and integrates many aspects of genome function, including transcriptional activity, chromatin structure, epigenetic state, and 3-D architecture. This project seeks to compare DNA replication timing programs and their interplay with chromosome organization and function in two important cultivars of maize (B73 and Mo17) and their close relative, sorghum. The high level of genetic diversity in these lines will be exploited to learn more about replication timing program regulation. Understanding this process is important because chromosomal events that occur early in development, and are coupled to DNA replication timing, may impact gene expression programs throughout the plant life cycle. We have used 5-Ethynyl-2'-deoxyuridine (EdU) labeling and flow cytometry in combination with 3D microscopy and high throughput sequencing to characterize the spatiotemporal patterns of DNA replication across the genome in maize B73 roots. These experiments also included a comparison of cells in the mitotic cycle and those entering a developmentally programmed endocycle. We now plan to compare the DNA replication program as a phenotype using two maize inbred lines and sorghum to define and examine regions with genotype-specific altered replication timing. This will allow us to investigate how such alterations are related to replication initiation sites, transcription rates, TE composition, chromatin accessibility and cytological and molecular interactions of replicating DNA. Such a comparative analysis will provide clues to regulatory mechanisms and enable future investigation of genetic controls on DNA replication. To facilitate this and similar work, we have developed the *Repliscan* pipeline for analysis of replication timing data and made it available on the CyVerse cyberinfrastructure platform.

Funding acknowledgement: National Science Foundation (NSF)

P176

Daily temperature cycles promote alternative splicing of RNAs encoding SR45a, a splicing regulator in maize

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Elevated temperatures in maize enhance alternative RNA splicing with the potential to expand the repertoire of plant responses to heat stress. Alternative RNA splicing generates multiple RNA isoforms for many maize genes, and we have observed changes in the pattern of RNA isoforms with temperature changes. Increases in maximum daily temperature elevate the frequency of the major modes of alternative splices, in particular, retained introns and skipped exons. The genes most frequently targeted by increased alternative splicing with temperature encode factors involved in RNA processing and plant development. Genes encoding regulators of alternative RNA splicing are themselves among the principal alternative splicing targets in maize. We have observed under controlled environmental conditions in maize that daily changes in temperature comparable to field conditions alter the abundance of different RNA isoforms including the RNAs encoding the splicing regulator, SR45a, a member of a family of SR45 genes. We have established an "in protoplast" RNA splicing assay to show that during the afternoon on simulated hot summer days, SR45a RNA isoforms are produced with the potential to encode proteins that are efficient in splicing model substrates. With the RNA splicing assay, we have also been able to define the exonic splicing enhancers that the splicing efficient SR45a forms utilize to aid in the splicing of model substrates. Hence, with rising temperatures on hot summer days, SR45a RNA isoforms in maize are produced with the capability to encoded proteins with greater RNA splicing potential.

Gene / Gene Models described: *SR45a*; Zm00001d047847
Funding acknowledgement: National Science Foundation (NSF)

P177

Dissecting NARROWSHEATH function and leaf margin development in maize at the single-cell level

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The width of the maize leaf is a key parameter of shoot architecture; the wide and flat leaf blade is optimized for photosynthesis while the sheathing leaf base encircles the stem and provides structural support to the plant. The duplicate maize *WUSCHEL HOMEOBOX3-LIKE* (*WOX3*) transcription factor-encoding genes *NARROWSHEATH1/2* (*NS1/2*) are redundantly required for full mediolateral outgrowth of the maize leaf. We are using single-cell transcriptomic analysis to characterize the genetic signatures of maize leaf margins in wild-type as well as in the *ns* mutant, where margins cease growth prematurely. Indeed, we see that the normal repertoire of genes expressed at growing margins is shut down at an earlier plastochron stage in *ns* seedlings, relative to normal plants. This is associated with earlier activation of genes characteristic of more differentiated leaf cell types. While we detect that NS1/2 are strictly expressed in a subpopulation of cells with an epidermal cell-type identity, transcriptional effects of NS loss-of-function are present non-cell autonomously in other cell types, potentially shedding light on pleiotropic effects of *ns* on plant growth and flowering time.

Gene / Gene Models described: *ns1, ns2*; GRMZM2G069028, Zm00001d052598
Funding acknowledgement: National Science Foundation (NSF)

P178

Elaborating the role of a putative CLAVATA receptor-coreceptor pair in meristem signaling and seed development.

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Maize ears arise from a specialized stem cell niche, the inflorescence meristem (IM), which is regulated by the CLAVATA-WUSCHEL (CLV-WUS) signaling pathway. This pathway involves an interplay between CLV receptor proteins and their CLE peptide ligands, and the transcription factor WUSCHEL. Maize mutants that lack CLV receptors and ligands have fasciated ears, with flat tips and disordered kernel rows, due to increased meristem activity. Weak alleles of *CLV* genes can enhance yield traits, however, making this pathway an attractive target for yield enhancement.FASCIATED EAR 3 (FEA3*)* is a Leucine Rich Repeat (LRR) receptor-like protein first described in maize. *fea3* mutants interact synergistically with *thick tassel dwarf 1* (*td1*) and *fasciated ear 2* (*fea2*), the orthologs of *CLV1* and *CLV2*, which encode the canonical receptor proteins in CLV signaling. Furthermore, RFP-FEA3 localizes below the organizing center of the IM, and *WUS* expression expands downwards in *fea3* mutants, as opposed to upwards in canonical *clv* mutants. Together, these data indicate that FEA3 acts independently of TD1 and FEA2 to exert control on meristem size. Intriguingly, an ortholog of *TD1*, *ZmBARELY ANY MERISTEM 1D (ZmBAM1D*), is upregulated in *fea3* mutants. *ZmBAM1D* and *FEA3* expression overlaps in the center of spikelet meristems, and they interact in co-immunoprecipitation experiments when co-expressed in *N. benthamiana*. These observations suggest that ZmBAM1D and FEA3 form a receptor- co-receptor pair. Because receptor interactions are often transient, we will assess this putative interaction with a proximity labelling approach using the Turbo-ID biotin ligase system in maize IMs. Additionally, ZmBAM1D is a QTL for seed size, indicating that CLV signaling may play a role in seed development. We will determine how these genes impact seed development through analyzing *fea3 Zmbam1d* double mutants. These studies may provide clues about how to uncouple control of seed number and seed size in maize.

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

P179 @erinlpatt

Evolution and development of awns in the grass subfamily Pooideae

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Awns, projections from the outermost protective tissue of grass flowers, appear in almost every clade of grasses, with highly variable morphologies. They likely play complex roles for the plants that bear them, participating in photosynthesis as well as seed dispersal and establishment. While a handful of genes involved in awn development are known, awns remain relatively understudied. Here, we use the model species *Brachypodium distachyon*, a relative of wheat and barley from the Pooideae grass subfamily, to further examine awn development and genetics. In particular, we take advantage of an existing *B. distachyon* mutant, *awnless1* (*awl1*), which shows defects in lignin deposition, leaf midrib development, and gynoecia development in addition to awnlessness. We examine these phenotypes and find evidence that a 90kb deletion containing a conserved regulatory region is responsible for the *awl1* mutation.

P180

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. Endosperm cells from the A636 inbred can be grown in tissue culture as explants from early developmental stages prior to 10 DAP. Prior reports suggest that endosperm tissue culture is only possible for a few inbred genotypes. We tested endosperm proliferation in four field corn and five sweet corn inbred 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. 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. We screened 38 rough endosperm (rgh) mutants for endosperm proliferation at 13-14 DAP. Six mutant lines 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 rgh*-117 showed no evidence of RNA splicing defects. Fine mapping results located rgh*-117 on chromosome 4 and rgh*-414 on chromosome 10. Sequencing two candidate genes showed an in-frame insertion on the transcription factor FHA1 gene in rgh*-117. Histological sections of rgh*-117 and rgh*-414 kernels at 11 and 17 DAP demonstrated severe embryo development defects.

P181

Functional dissection of the REL2 corepressor family

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Transcriptional corepressors of the TOPLESS (TPL) family function as master regulators of plant development, encompassing all nine phytohormone pathways. In maize, the TPL family is comprised of four members, RAMOSA1 ENHANCER LOCUS2 (REL2), REL2-LIKE1 (RELK1), REL2-LIKE2 (RELK2), and REL2-LIKE3 (RELK3). Prior characterization of *rel2* mutants revealed pleiotropic vegetative and reproductive phenotypes such as defective axillary meristem initiation and inflorescence meristem (IM) maintenance in single recessive mutants (Liu et al, Plant Phys 179:348-363, 2019). Recently, the mutant *short upright* (*su*) was identified in an unbiased EMS mutagenesis screen for *rel2* enhancers. Bulked Segregant Whole Genome Sequence analysis was performed and identified *RELK1* as the causal gene in two independent alleles. Double *rel2;relk1* mutant plants appear shorter with very upright tassel branches and enhanced ear fasciation. Expression analysis in *rel2* mutant inflorescences indicates that *RELK1* is strongly upregulated, suggesting a buffering mechanism between the two family members in agreement with the genetic enhancement. Additionally, preliminary data from CRISPR knock-out lines of both *RELK2* and *RELK3*, two closely duplicated genes co-orthologous to Arabidopsis *TPL*, revealed a branchless phenotype in *rel2;relk* mutant tassels. This analysis suggests that functional diversification occurred in the maize TPL family of transcriptional corepressors. We are currently investigating the role of each family member in maize development, and in particular in the regulation of the size of ear inflorescence meristems as it directly impacts grain yield.We acknowledge funding from the National Science Foundation (IOS#2026561).

Gene / Gene Models described: *rel2, relk1, relk2, relk3*; GRMZM2G042992, GRMZM2G316967, GRMZM2G030422, GRMZM2G550865
Funding acknowledgement: National Science Foundation (NSF)

P182 @jefowlerjr

Green kernels: The Dooner/Du Ds-GFP population enables a variety of research and outreach applications

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The mutagenized population generated by the Dooner/Du Plant Genome project currently consists of >14,000 mapped insertions of the engineered *Ds-GFP* element spread throughout the maize genome (acdsinsertions.org). The *Ds-GFP* mutagen significantly enhances the value of the population, as it carries a Green Fluorescent Protein coding sequence driven by a strong endosperm promoter, thus providing an easily visible linked marker, a green fluorescent kernel. With >2000 genes interrupted by insertions in coding sequence, we have found this population to be particularly valuable for investigating genes expressed in the male gametophyte. To increase throughput for quantitative assessment of *Ds-GFP*-linked transmission defects, we developed the EarVision phenotyping pipeline, which combines a digital imaging platform for visualizing the entire maize ear with a deep learning model that automates kernel categorization. The kernel marker enhances the ease of assessing the influence of competition on male transmission, following controlled variation of pollen load. The distribution of the element throughout the genome also provides readily available linked kernel markers for virtually any locus. For example, we measured the male-specific transmission defect of a *Mu* insertion in the rop2 gene using a tightly linked *Ds-GFP* element placed in repulsion to the mutant. In addition, the dosage sensitivity of the green fluorescent phenotype facilitates genotyping, as selecting the brightest kernels dramatically enriches for homozygous plants. Finally, the fluorescent kernels from *Ds-GFP* lines have an undeniable "WOW" factor that makes these ears particularly useful for outreach. Inexpensive materials enable hands-on activities that demonstrate Mendel's Laws; introduce the ideas of genetic markers, transposable elements and mutations; and engage students in generating and analyzing kernel count data. To facilitate the use of these approaches, we are working with MaizeGDB to establish a webpage with information on validated *Ds-GFP* insertions and associated methods.

Funding acknowledgement: National Science Foundation (NSF)

P183

Human and maize RNA Binding Motif Protein48 (RBM48) have conserved functions in stem cell proliferation and cell differentiation

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Eukaryotic gene expression is reliant on precise identification and splicing of non-coding introns from precursor mRNA (pre-mRNA). In addition to a major group of U2-type introns, a vast majority of eukaryotes also contain a minor group of U12-type introns. Derived from the last eukaryotic common ancestor, U12-type introns constitute less that 0.5% of all introns and are spliced by a distinct minor spliceosome. Although the biological ramifications of U12 introns remains largely unknown, mutations that interfere with the proper splicing of these introns have been linked to developmental defects in both plants and animals. We recently reported a maize *rbm48* mutant that displayed severe defects in endosperm development and genome wide aberration in the spicing of primarily U12 introns. Genetic and biochemical analysis revealed *rbm48* suppresses differentiation and induces cellular proliferation upon tissue culture of the mutant endosperm. We also generated a CRISPR/Cas9-mediated orthologous RBM48 functional knockout(RBM48 FunKO) in human cancer derived K-562 cells to demonstrate the essential role of RBM48 in U12 splicing is conserved between maize and humans. Using comparative RNASeq analysis between the RBM48 mutants of maize and humans we identified candidate Minor Intron Containing Genes (MIGs) with perturbed expression potentially underlying the mutant phenotype. In this report, we used the same guide RNA to create a functional knockout of RBM48 in human embryonic stem cells (hESCs) to investigate if the RBM48 FunKO impacts the cellular differentiation of hESCs. Our preliminary data indicates the mutant hESCs display significant increase in cell proliferation with marked reduction in cell differentiation compared to the hESCs infected with vector control. Our data points to a deeply conserved role of RBM48 and U12 introns in growth and development between maize and humans.

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

P184

Inflorescence development in Cenchrus americanus (pearl millet)

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Cenchrus americanus (pearl millet), a maize relative in the Panicoideae, is a grain consumed in many parts of the world. Pearl millet is drought and heat tolerant compared to other grains. These abiotic stressors weaken global food security and have direct influences on reduced crop yield. Pearl millet's drought tolerance makes it an attractive crop for further improvement and for discovery. Here, we analysed pearl millet inflorescence development using Scanning Electron Microscopy (SEM), and compared pearl millet inflorescence development to inflorescence development in other panicoid grasses. We also describe our progress in establishing Agrobacterium-mediated transformation of pearl millet in the lab.

Gene / Gene Models described: *N/A*; N/A
Funding acknowledgement: National Science Foundation (NSF)

P185 @Dr_AnnisR

Insights into the evolution of the maize leaf

(submitted by Annis Richardson <annis.richardson@ed.ac.uk>)
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There are over 10,000 species of grass (Poaceae), which show diversity in floral structures but share a common leaf shape. In contrast to eudicots, which have a narrow proximal petiole and broad distal lamina, the grass leaf has an ensheathing base, a middle hinge region and an upper lamina which bends away from the main axis of the plant. The specialised shape of the grass leaf enables vertical growth during the vegetative phase without stem extension, protecting the shoot apical meristem by keep it at, or below, ground level until the transition to flowering. This evolutionary innovation is thought to have contributed to the world-wide ecological success of the grasses, yet its origin and relationship with the eudicot leaf has been a subject of longstanding debate. By combining developmental maize genetics and computational modelling we have found that the grass leaf likely arose through *WOX*-gene-dependent extension of a primordial zone straddling concentric domains around the shoot apex. Gene expression patterns within this zone underlie growth patterns, and can account for wild-type and mutant maize leaf development. By contracting the primordial zone, but maintaining gene expression patterns, we found that we can regenerate a eudicot like leaf shape. Differences in grass and eudicot leaf shape most likely arose through modification of the downstream effects of these conserved patterning genes on growth rate patterns. By comparing our grass leaf model and eudicot model we find that that the grass leaf sheath likely derives from petiole, whereas the blade derives from the rest of the eudicot leaf, consistent with homologies proposed in the 19th century.

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P186

Interactions between Auxin and the *tassel-less4* mutant in maize

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tassel-less4 (tls4) is a mutant in *Zea mays* (maize) which is characterized by its deficiencies in inflorescence development resulting in a smaller tassel, the male reproductive structure in maize. The *tls4* mutant is also characterized by reduced plant height and narrow leaves. This phenotype is characteristic of mutants with defects related to the plant growth hormone, auxin. One of the primary functions of auxin is the control of organ formation in meristems, such as the tassel meristem. To explore the relationship between the *tls4* mutant and auxin, several double mutant analyses were performed between *tls4* and mutants with known functions related to auxin. The auxin biosynthesis mutants *vanishing tassel2 (vt2)* and *sparse inflorescence1 (spi1)* were crossed with *tls4* to determine how the gene responsible for the *tls4* mutant interacts with auxin. Both *vt2* and *spi1* had significant interactions with *tls4* indicating that *tls4* function is related to auxin. Additional double mutant analyses were performed between *tls4* and the auxin transport mutant *barren inflorescence2 (bif2)* and the auxin signaling mutants *Barren inflorescence1 (Bif1)* and *Barren inflorescence4 (Bif4)*. These also had significant interactions with *tls4* strengthening the hypothesis that *tls4* functions in the auxin pathway. Further characterization and cloning of *tls4* will provide a more complete picture of how auxin functions in plants.

Funding acknowledgement: Cherng Foundation, Amercian Society of Plant Biology

P187
Maize Trehalose-6-Phosphate synthases (ZmTPSs) interact with RAMOSA3 and function in embryo and inflorescence development

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In plants, trehalose, a non-reducing disaccharide, is associated with developmental and stress signaling, however, the underlying mechanisms are unclear. Trehalose is synthesized in a two-step process. First, trehalose-6-phosphate synthases (TPSs) catalyze trehalose-6-phosphate (T6P) from glucose-6-phosphate and UDP-glucose, then trehalose-6-phosphate phosphatases (TPPs) dephosphorylate T6P to trehalose. Most plants, including maize, encode multiple TPSs and TPPs, and many of the TPS proteins lack enzymatic activity, though the significance of this finding is not understood. The classical 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 and a yeast-2- hybrid experiment found that RA3 interacts with ZmTPS1 and ZmTPS12, two non-catalytic TPSs. The *zmtps1* and *zmtps12* mutants enhanced *ra3* phenotypes, suggesting the interaction between ZmTPS1, ZmTPS12, and RA3 is biologically significant. Interestingly, the yeast-2-hybrid experiment found that ZmTPS1 also interacts with the two maize catalytic active TPSs, ZmTPS11 and ZmTPS14. We knocked out these genes using CRISPR-Cas9 and found that *zmtps11zmtps14* double mutants fail to complete embryogenesis, indicating that the active TPSs are important for normal embryo development, as in Arabidopsis. Our working model is that the synthesis of trehalose in maize is catalyzed by a TPP-TPS complex in which ZmTPS11, ZmTPS14, and ZmTPPs carry out the catalytic activity, while the non-catalytic TPSs might have regulatory functions. In addition, non-catalytic TPSs appear to modulate RA3 non-catalytic functions in regulating inflorescence branching. We are expressing these proteins to test their ability to form the complex, investigate their subcellular and cellular localization, and characterizing these triple mutants to investigate RA3 interactions with catalytic and non-catalytic TPS proteins, to further understand the enigmatic role of trehalose signaling in plants.

Funding acknowledgement: National Science Foundation (NSF)

P188 @LindZeamays
Maize brace root biomechanics are determined by geometry within a genotype and material properties between genotypes

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Crop plants are susceptible to yield loss by mechanical failure, which is called lodging. In maize (Zea mays), aerial nodal brace roots impart mechanical stability to plants, with the lowest whorl of brace roots contributing the most. The features of brace roots that determine their contribution to mechanical stability are poorly defined. Here we tested the hypothesis that brace root mechanical properties vary between whorls, which may influence their contribution to mechanical stability. 3-point bending tests were used to determine that brace roots from the lowest whorl have the highest structural mechanical properties regardless of growth stage, and that these differences are largely due to brace root geometry within a genotype. Analysis of the brace root bending modulus determined that differences between genotypes are attributable to both geometry and material properties. These results show that brace root biomechanics may be important to determine the brace root contribution to mechanical stability.

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P189
Maize *unstable factor for orange1* has a critical function in endosperm cell differentiation

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The *ZmUfo1-1* is a spontaneous dominant mutation of *unstable factor for orange1* (*ufo1*) that has been associated with epigenetic modifications at *pericarp color1* (*p1*), causing ectopic and overaccumulation of *p1*-induced phlobaphenes. The *Zmufo1* orthologs are present only in sorghum, rice, Setaria, and Panicum. Characterization of dominant *ZmUfo1-1* and loss of function *Zmufo1-Dsg* mutant alleles have shown aberrant carbohydrate and hormone accumulation in developing endosperm. Specifically, defects were observed in the transfer cells of the basal endosperm transfer layer (BETL) and BETL-placentochalaza interface regions of both the mutant alleles. Moreover, a substantial remnant of nucellus at ten days after pollination and reduced starch and protein accumulation in endosperm indicated developmental delay. Leaf defects, including abnormal mesophyll cell organization and stomata subsidiary cell defects caused by *ZmUfo1-1* ectopic overexpression, suggests a role of *Zmufo1* in maintaining fundamental cell differentiation mechanism. The transcriptome of *ZmUfo1-1* revealed significant modifications of the OPAQUE11 and OPAQUE2 gene regulatory axis and the expression of crucial genes for BETL and ESR development. Moreover, these transcriptomes show downregulation of DNA replication and ribosome biogenesis. Our results revealed that ZmUFO1 localizes in the nucleus and nucleolus. Further, alternate splicing and protein-protein interaction assays are allowing us to explore *Zmufo1*'s interaction with chromatin remodeling factors during endosperm development.

Gene / Gene Models described: *ufo1*; GRMZM2G053177, Zm00001d000009
Funding acknowledgement: National Science Foundation (NSF), Indian Council of Agricultural Research (ICAR), Hatch PEN04613 project funding PSU, Department of Plant Science, PSU

P190 @maryjgalli
Mining transcriptional cis-regulatory modules in the maize genome

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Transcription factors (TFs) recognize short DNA sequence motifs in regulatory regions of their target genes and thus control the gene expression changes responsible for plant developmental programs and environmental responses. In crop species, variation in TFs and their regulatory regions have been frequent drivers of productivity gains during domestication and modern breeding, and continue to offer great potential for further trait engineering. A significant percentage of trait-associated variants lie within non-coding regions and could affect TF binding. Currently, genome-wide TF binding events in maize remain largely uncharacterized, limiting our view of functional non-coding spaces and the molecular nature of many useful traits. While potential TF binding sites are naturally abundant within a genome, only a very small fraction of sites are actually bound by TFs and able to affect expression of nearby genes; it therefore remains largely unclear how functional TF binding selectivity is achieved. This includes a lack of knowledge in how TF protein complexes cooperate to affect DNA binding. Using DAP-seq, we are carrying out a large-scale analysis of TF binding in maize inbreds, aiming to understand how TF-DNA binding and its variability in different genetic backgrounds affects phenotypic outcomes in maize.

Funding acknowledgement: National Science Foundation (NSF)

P191 @malehrer49

Morphological indicators and predictors of drought-responsive grain yield maintenance in *Sorghum bicolor*

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Climate change-induced variations in temperature and precipitation are inevitable and imminent and are expected to severely impede plant growth and development. Therefore, a critical need exists to identify the mechanisms of drought tolerance that maintain grain yield in order to ensure future food quality and availability. My research aims to identify the morphological underpinnings of drought-responsive grain yield maintenance in two accessions of *Sorghum bicolor* that differ in their pre- and post-flowering responses to drought. Plants were germinated, transplanted at the three-leaf stage, and given one week to recover. After recovery, drought stress was applied as follows: plants were watered to 100% soil moisture content (SMC), and allowed to dry to 0% SMC. Once dry, plants remained at this level for two days; controls were well-watered. After two days, plants were watered back to 100% SMC and allowed to dry again; this was repeated cyclically, two, four, and six times, to mimic periods of drought with intermittent rainfall. In the pre-flowering drought tolerant accession, consistent decreases in the width of the newest fully expanded leaf and alive leaf biomass were identified and are likely contributors to the observed grain yield and seed weight reductions. Although grain-related data was not collected for the pre-flowering drought sensitive accession in this study, early reductions in the greenness of the newest fully expanded leaf and stem diameter, and consistent reductions in stem biomass likely influence photosynthetic capacity and water uptake, and, in combination, are predicted to significantly impact grain yield and seed weight.

P192

Narrow odd dwarf and its partners, a crosstalk between immunity and development

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narrow odd dwarf (*nod*) is a maize mutant with striking developmental phenotypes affecting most vegetative and reproductive organs. NOD is a plasma membrane-localized protein that participates in calcium import, and in maize the defective mutant shows more severe phenotype than in other species. To investigate how NOD coordinates development, interacting partners were identified by immunoprecipitation and a yeast two hybrid screen. Two plasma membrane-localized receptor-like kinases were among the outstanding candidates. One is the Liguleless narrow protein (LGN), which was previously characterized in a screen for leaf developmental mutants. The *Lgn* mutant shows a pleiotropic developmental phenotype, severity depends on genetic background and temperature, and presents autoimmunity signatures, overall similar to *nod*. The other one is a kinase called WYNKEN (WNK), which is related to BRASSINOSTEROID SIGNALING KINASES. In Y2H, WNK was second in apparent interaction strength only to the homooligomerizing NOD protein itself. Both interactions were confirmed by BiFC. LGN and WNK are primarily composed of a transmembrane helix anchoring them to the plasma membrane and a cytoplasmic Ser/Thr protein kinase domain, and they are both grass-specific. We found that LGN can phosphorylate NOD *in vitro* and WNK phosphorylation is being tested. To determine genetic interaction, we generated a double *Lgn-R*; *nod* mutant, this one is more severe than the single ones. Transcriptomics analysis of these mutants suggest they are autoimmune mutants, however, they affect distinct pathways. *Lgn-R* shows signs of PTI while *nod* shows signs of ETI. We are investigating the molecular mechanisms by which crop plants respond to pathogen molecular patterns and what roles temperature and genetic background are playing. Our results will help to explain how NOD and its interactors function in immunity, will expand information gained to important cereal crops and will generate tools that can be applied to interrogate immune signaling and pathogen responses in plants.

Gene / Gene Models described: *Lgn, nod, wnk*; GRMZM2G134382, GRMZM2G027821, GRMZM2G164224
Funding acknowledgement: National Institutes of Health (NIH), National Science Foundation (NSF), United States Department of Agriculture (USDA), University of California Institute for Mexico and United States (UC MEXUS-CONACYT)

P193
This abstract has been pulled from the program at the author's request

P194 @Xiaosa_Xu
New insights into CLAVATA-WUSCHEL signaling in the maize inflorescence meristem using single-cell RNA sequencing (scRNA-seq)

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CLAVATA-WUSCHEL (CLV-WUS) feedback pathway plays a crucial role in regulating stem cell maintenance. However, the molecular signatures associated with this pathway remain to be fully revealed in maize. Genetic studies of *fasciated ear* mutants have identified critical CLV-WUS signaling components that control ear inflorescence meristem proliferation. Yet, understanding the underlying molecular mechanism requires cellular resolution insights. Our recent scRNA-seq profiling of the developing B73 ear (Xu et al., *Developmental Cell*, 2021) shows this technology's power in identifying markers of diverse cell types and how scRNA-seq data may facilitate maize genetic studies. However, we did not detect *CLV3* and *WUS* orthologs (*ZmCLE7* and *ZmWUS1*) in that study due to their low representation and expression. Thus, we finely dissected developing B73 ear tips (~ 600 µm), including inflorescence and initiating spikelet pair meristems, and further leveraged scRNA-seq to profile ~10,000 single cells. The resulting scRNA-seq atlas identified a distinct *ZmCLE7* marked stem-cell cluster and cell populations expressing *ZmWUS1*. Using scRNA-seq gene co-expression networks, we identified ~ 200 *ZmCLE7*- and ~ 60 *ZmWUS1*- co-expressed markers. These included many known maize meristem regulators, such as *ZmLONELY GUY7* (*ZmLOG7*) and *BARREN INFLORESCENCE4* (*BIF4*), and novel markers, such as a homolog of *Arabidopsis AINTEGUMENTA-LIKE*7 (*AIL7*). We also profiled ~9,000 ear tip cells from a *fasciated ear* double mutant, *fasciated ear3;Zmcle7* (*fea3*;*Zmcle7*), that has extreme meristem proliferation. We detected mis-expression of *ZmWUS1* in the epidermis cell cluster in these *fea3*;*Zmcle7* mutants. The expression of *ZmCLE7* was also significantly expanded in *fea3*;*Zmcle7* clusters compared to wild type B73. We validated these results by mRNA *in situ* hybridization. Together, scRNA-seq of maize ear tips identified candidate new regulators and provided cellular resolution insights into CLV-WUS signaling to maintain ear meristem. The datasets will be a valuable resource for maize community to inform maize genetics at a fundamentally new level.

Gene / Gene Models described: *ZmCLE7, ZmWUS1, ZmLOG7, BIF4, FEA3*; GRMZM2G372364, GRMZM2G047448, GRMZM2G059392, GRMZM5G864847, GRMZM2G166524
Funding acknowledgement: National Science Foundation (NSF). We thank Dr. Edgar Demesa-Arevalo for crossing fea3 with Zmcle7 to generate segregating population.

P195

Patterns of transcriptional response to overproliferating inflorescence meristems in *fasciated ear* mutants

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Several maize yield-related traits, such as kernel number, are determined during development when the inflorescence meristems (IMs) proliferate to generate a stereotypical series of spikelet and floral meristems that form kernels after fertilization. The IM activity is positively correlated with kernel numbers and maintained by a *CLAVATA* (*CLV*)-*WUSCHEL* (*WUS*) feedback signaling pathway. Null mutants of *CLV* genes (*td1*, *fea2*, *fea3*, *Zmcle7* and *Zmfcp1*) cause meristem over-proliferation, enlarged inflorescence stems and *fasciated ears*, which develop many more disorganized kernel rows and shorter ears with low grain yield. However, our recent studies suggest that weak alleles of *fea* genes, including CRISPR-Cas9 promoter edited weak alleles of *ZmCLE7* and *ZmFCP1* can significantly enhance grain yield by increasing the kernel numbers on the ear. These weak alleles maintain meristem organization and ear length, highlighting the potential to quantitatively manipulate fea genes for yield enhancement. To further understand the transcriptional changes in developing ears upon genetic disruption of the *CLV-WUS* signaling pathway, we conducted RNAseq of these *fea* mutants. By comparing differentially expressed genes (DEGs), we found ~5000 DEGs explained ~80% of the transcriptional changes underlying the *fea* phenotype variation, from mild to very strong fasciation, and these were defined as *fea-responders*. Several key biological processes were enriched in *fea-responders*, including response to metal ion, amino acid metabolic process, saccharometabolism, reproductive developmental process, chromatin assembly and translation. The cytokinins, brassinosteroid, jasmonic acid and ethylene biosynthesis related *fea-responders* were mostly positive responses to the *fea* phenotype variation, suggesting these hormones are affected by *CLV-WUS* signaling. In addition, some transcription factors and known inflorescence development-related genes, such as *WUS1*, *GRF interacting factor1*, *Tasselsheath4*, *Unbranched2* and *Unbranched3* were also among the *fea-responders*. The unveiling of transcriptional changes of these *fea-responders* in *fea* mutants could help us understand the response of key genes to meristem over-proliferation, and guide high-yield maize breeding by manipulating meristem activity.

Gene / Gene Models described: *TD1, FEA2, FEA3, ZmCLE7, ZmFCP11, WUS1, GRF interacting factor1, Tasselsheath4, Unbranched2, Unbranched3*; GRMZM2G300133, GRMZM2G104925, GRMZM2G166524, GRMZM2G372364, GRMZM2G165836, GRMZM2G047448, GRMZM2G180246, GRMZM2G307588, GRMZM2G160917, GRMZM2G460544
Funding acknowledgement: National Science Foundation (NSF)

P196

Spatial transcriptomics of the maize SAM

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PLASTOCHRON1 (PLA1) determines the rate of leaf initiation and stimulates organ growth by functioning as a timekeeper of cell division. PLA1 has a specific expression pattern in the SAM where molecular cues determine primordia initiation, outgrowth and patterning. To finetune the role of PLA1, we applied a spatial transcriptomics approach to profile the expression of ninety genes on longitudinal and transversal sections through the maize SAM. The simultaneous visualization of those ninety gene expression profiles position the PLA1 expression relative to marker genes and showed it to be highly reminiscent of that of ROUGH SHEATH1. Conversely, we use the spatial transcriptomics together with genetic interactions to study how PLA1 relates to genes functioning in leaf initiation and growth.

Gene / Gene Models described: *PLA1*; GRMZM2G167986
Funding acknowledgement: VIB Tech Watch

P197

Tale of the nucleus: A protein in the nuclear membrane is required for correct division plane orientation

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Both plants and animals rely on asymmetric cell division to generate new cell types, which is a core characteristic of multicellular organisms. Prior to asymmetric cell division, cell polarity is established. Cell polarity establishment and asymmetric cell division are universally important, although proteins important for polarity differ in plants and animals. *Zea mays* stomatal development serves as an excellent plant model system for asymmetric cell division. In this process, the nucleus migrates to the future division site after polarity establishment and before cytokinesis. In this study, we examined a mutant of the outer nuclear membrane protein, which is part of the LINC (linker of nucleuoskeleton and cytoskeleton) complex. Previously, plants harboring mutations in *Mlks2* (Maize LINC KASH AtSINE-like) were observed to have abnormal stomata (Gumber et al. 2019 Nucleus). We confirmed stomatal defects such as abnormal subsidiary cell size and shape, aborted guard mother cell, and extra inter-stomatal cells. We used cell markers to pinpoint the precise defects that lead to abnormal asymmetric divisions. Early markers of cell polarization are normal in *mlks2* mutants. Nuclear polarization is impaired. Notably, our preliminary data indicate that even in cells with abnormal nuclear positioning, the preprophase band forms in the correct spot; however, spindle orientation is more variable in *mlks2* mutants. Misoriented phragmoplasts are observed, consistent with abnormal division planes. Future studies using time-lapse imaging will clarify if cells with abnormal nuclear positioning is directly linked variable spindle angles. We will also determine how nuclear migration and spindle angle ultimately relate to division plane maintenance and phragmoplast guidance. Altogether, our study will help to dissect the role of nuclear movements during different steps of asymmetric cell division including polarization, division plane establishment, division plane maintenance, mitosis, and cytokinesis.

Gene / Gene Models described: *Mlks2*; Zm00001d052955

P198

Temporal regulation of cell division and expansion by auxin and gibberellins underlies medio-lateral growth and vein proliferation in maize leaf

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Leaf growth and development require modulation of cell division and cell expansion in both spatial and temporal dimensions. Auxin and gibberellins (GAs) play key roles in the spatial control of cell division and cell expansion in the developmental gradient at the base of the emerging leaf. Since medio-lateral division of cells and their transition to expansion determines the blade width and the number of veins in the maize leaf, we investigated the pre-emergence phase of leaf development. Using auxin and GA biosynthesis mutants, *vanishing tassel2* (*vt2*) and *dwarf3* (*d3*), which have broader and shorter leaves, we gleaned information from both fully expanded and developing leaves to investigate how auxin and GAs modulate cell division and cell expansion and to test whether these hormones interact to regulate these processes. The data suggests that the broad-leaf phenotype of *vt2* and *d3* does not result from a similar pattern of cell division and cell expansion, indicating independent roles of auxin and GAs in controlling medio-lateral leaf growth. Further, cell length profile and vein number and distribution in *vt2* indicate an earlier onset of the rapid medio-lateral growth in the developing leaf, suggesting a role of auxin in the temporal control of leaf expansion. We hypothesize that the rate of cell proliferation and the timing of cell expansion at a critical period during leaf development underly leaf growth and size delimitation, and auxin and GAs have separate but overlapping roles in these processes. We further test this hypothesis using cell cycle and auxin and GA response markers, as well as hormone quantification on key stages of leaf development. Our research provides insight into the developmental basis for medio-lateral growth and vein proliferation in the maize leaf.

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P199

The development and evolution of unisexual floral specification in Poaceae

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Grasses display a range of floral sexualities. Brachypodium distachyon and Avena sativa (oats) have hermaphroditic flowers with both female and male organs. Some Sorghum bicolor accessions have male and female flowers on a single inflorescence, while Zea mays (maize) has a male inflorescence at the top (the tassel) and female inflorescences at lower axillary buds (the ears). It is unknown exactly how unisexual flowers evolved from bisexual ones, and it is possible that unisexual flowers evolved several times in the grasses through different mechanisms. To understand these patterns, I will do a phylogenetic analysis of floral traits across Poaceae and look at the evolution of proteins involved in the development of unisexual flowers. One particular group of proteins I will focus on are involved in sugar signaling. Sugar signaling is one important aspect of floral specification, and the evolution of sugar signal responses could play a role in directing flower sexuality. Understanding the evolution and development of unisexual flowers has important implications for plant breeding, as plants with unisexual flowers allow humans to cross them more easily in crop improvement programs.

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P200

The genetics of leaf orientation and its impact on the interception of solar radiation

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The efficiency of canopy interception of solar radiation can greatly affect the photosynthetic efficiency of crop plants. Plants can increase light interception via the shade-avoidance syndrome, which results in more acute leaf angles. Light interception can also be affected by phyllotaxy: the arrangement of leaves along the stem. Here, we report on the ability of some maize genotypes to alter the azimuthal orientations of their leaves during development in coordination with adjacent plants. Although these genotypes retain the typical alternate-distichous phyllotaxy of maize, their leaves grow parallel to those of adjacent plants. A genome-wide association study (GWAS) conducted on whether plants do or do not exhibit this parallel phenotype identified candidate genes, many of which have been reported to be associated with the shade-avoidance syndrome, including ***phytochromeC2*** (***phyC2***). In addition, GWAS on the percent of photosynthetically active radiation (PAR) intercepted by the canopies of the diversity panel identified genes known to regulate leaf development, including ***liguleless1*** (***lg1***). Compared with wild type, ***phyC2*** and ***lg1*** mutants exhibited altered canopy architectures. Specifically, in both of these mutants, the numbers of leaves growing into the inter-row space is greatly reduced. This results in dramatically decreased light interception by the ***lg1*** mutant canopies. A similar phenotype was also observed in ***lg2*** and ***Lg3*** mutants. We hypothesize that the ability to adjust the azimuthal distribution of leaves, represents another pathway by which maize can maximize light interception by the canopy, possibly downstream of the shade-avoidance syndrome.

Funding acknowledgement: National Science Foundation (NSF)

P201

The secret lives of veins: single cell genomics and quantitative genetics of maize leaf vascular development

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Maize tissues that engage in efficient C4 photosynthesis have extremely high vein density. Vascular density varies across the sheath, auricle, and blade compartments of the leaf. Although mature leaf veins are anatomically similar, in maize and other C4 grasses there are several different vein types occurring in a stereotypical developmental sequence and spatial configuration throughout these leaf compartments. To characterize the gene expression and regulatory environments that distinguish these diverse vascular cell types within the maize leaf primordia, we used single cell RNA and ATAC sequencing (scRNA-seq and scATAC-seq) to obtain transcription and chromatin state profiles for individual nuclei from primordial tissue at different stages of development. With this high-resolution transcriptomic data, we will isolate the expression patterns of different types of maize vascular tissue and characterize the genetic signals and developmental trajectories of intermediate cell types transitioning into vascular tissue. To screen for natural variants of vascular development programs, we are conducting a GWAS correlating vein density with markers from the 942 Wisconsin Diversity Panel (WiDiv). To facilitate this study, we have devised a deep-learning based automated phenotyping system for estimating type-specific vein density in cleared leaf images. This system employs a convolutional neural network (CNN) trained on images of cleared leaves labeled with vein counts to estimate the number of veins present within a given sample.

P202

Transcriptomic signatures associated with maize defense against corn leaf aphid.

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Maize (*Zea mays* L.) is one of the major cereal crops cultivated throughout the world, with the United States producing over 40 percent of the crop annually. Corn leaf aphid [CLA*; Rhopalosiphum maidis* (Fitch)] is an economically important pest of several monocot crops, including maize. In addition to extensive crop damage, CLA also acts as a vector for viruses that cause devastating diseases in maize. Previously, we showed that the maize inbred line Mp708, which was developed by classical plant breeding, provides enhanced resistance to CLA. Feeding by CLA on Mp708 triggers the rapid accumulation of the *maize insect resistance1* (*mir1*) transcripts, which encodes a cysteine protease. In this study, transcript profiling of CLA susceptible (Tx601) and resistant (Mp708) shoots and roots after foliar release of CLA for 24 hours identified several sets of genes that are differentially regulated, including genes involved in phytohormone pathways, and may have important functions in *mir1*-dependent defenses against CLA. The underlying mechanisms of local and systemic CLA-induced signaling networks will be discussed.

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P203 @AHostetlerPhD

Utilizing high-throughput brace root phenotyping data to predict lodging susceptibility across 53 maize genotypes

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To meet the demands of a growing global population, food production must increase 50% by 2050. One factor threatening production is that the quality and quantity of crop yield is negatively impacted by lodging (the vertical displacement of stalks). Recent studies in maize (Zea mays) have shown that the presence of aerial nodal roots (termed brace roots) contribute to increased anchorage and lodging-resistance. Using a panel of 53 agronomically important maize genotypes with variable root lodging-susceptibility, we aimed to identify brace root traits that can predict the brace root contribution to anchorage. To determine if lodging was environmentally or genetically controlled and determine phenotypes associated with greater lodging resistance, we paired lodging data with high-throughput brace root phenotyping data that was collected immediately before the storm event. Through this analysis, we identified genotypes that are more susceptible to lodging due to underlying genetic controls. We hypothesized that these differences were in part due to variation in brace root phenotypes and thus anchorage. To link brace root phenotypes with anchorage, we used a field-based mechanical testing device (DARLING) to qualify the overall plant anchorage before and after removing whorls of brace roots. We paired these measures of anchorage mechanics with high-throughput brace root phenotyping data. From this analysis, we identified brace root traits that can predict plant biomechanics with greater than 70% accuracy. Together these data lay the foundation for engineering brace roots to improve plant anchorage and limit crop loss due to lodging.

P204

WPRs acting as a downstream of PAN2 receptor is involved in the polarity establishment in maize stomata

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Cell polarity is an essential step for asymmetric division, enabling the daughter cells to acquire a different cell fate. Polarity establishment which is usually triggered by a series of polarized proteins is a prerequisite for asymmetric cell division. In maize stomatal development, previous studies have shown the BRK-PAN2/PAN1-actin pathway promotes subsidiary mother cell (SMC) polarity establishment, in which two receptor-like kinases, PANGLOSS2 (PAN2) and PAN1 are recruited by BRK (Brick) to regulate actin accumulation in SMC. However, How BRK-PAN2/PAN1 connects with actin is poorly understood. Here, we identified a novel gene family, WPRs (WEB1-PMI2 RELATED), which play an important role in SMC polarity establishment. WPRs physically interact with PAN2/PAN1, and localize polarly in SMC at sites of guard mother cell (GMC) contact. The polarized localization depends on PAN2 but not PAN1. Additionally, we prove that WPRs also interact with F-actin through their N terminal regions. Disruption of F-actin in maize does not affect the polarization of WPRs. Together, these results implicate the WPRs act downstream of PAN2, potentially functioning as a scaffold for recruiting F-actin at SMC site.

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P205 @PlantsOverPants

Wavy Auricle in Blade2 (Wab2) is a semidominant ectopic auricle mutant suppressed by one copy of wavy auricle in blade1 (wab1) loss-of-function

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The maize leaf is separated into three domains: a blade that is optimized for photosynthesis, a sheath that tightly wraps around the stem, and a ligule/auricle that acts as a hinge bridging blade and sheath. The distinct roles and cellular activities of these domains involve several genetic regulatory mechanisms. We have been characterizing, Wavy Auricle in Blade2 (Wab2) that causes the formation of ectopic auricle-like clusters of tissue in the blade. Analysis of true breeding, hybrid, and segregating mutant families shows that Wab2 exhibits incomplete dominance and variable expressivity as a heterozygote. Histology of the Wab2 shoot apex shows severe mutant leaf blades have multiple bands of actively dividing cells as early as the fifth leaf primordium from the meristem, P5. These structures strongly resemble incomplete versions of the wild-type blade/sheath boundary where the auricle normally forms. Bulk segregant sequencing pools of 40+ BC2 mutants and 60+ wild-type siblings identified an unexpectedly-large 170 Mbp interval on chromosome 4, suggesting that recombination is suppressed in this region, supported by a predicted inversion overlapping the region in low coverage nanopore long-read sequencing. Genetic analysis of double mutants between Wab2 and the wab1-rev loss-of-function allele (reversion of the dominant Wavy Auricle in Blade1 [Wab1-R], Zm00001eb099370) shows that wab1-rev is epistatic to Wab2. Unexpectedly, plants heterozygous for wab1-rev also suppressed the Wab2 phenotype. Ongoing work aims to narrow down a mapping interval by mapping within the putative inverted background, reverting the Wab2 dominant allele by EMS mutagenesis, and characterizing gene expression in developing leaves. We hypothesize that physical interaction between Wab2 and DNA-binding defective wab1-rev may explain heterozygous suppression. Future work will examine potential interactions between these proteins as well as other genetic loci. There are still many questions left to be answered as we press forward in our study of this mutant.

Gene / Gene Models described: *wab1/bad1*; Zm00001eb099370

P206 @SongtaoGui

ZEAMAP, a comprehensive database adapted to the maize multi-omics era

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As one of the most extensively cultivated crops, maize (*Zea mays* L.) has been extensively studied by researchers and breeders for over a century. With advances in high-throughput detection of various omics data, a wealth of multi-dimensional and multi-omics information has been accumulated for maize and its wild relative, teosinte. Integration of this information has the potential to accelerate genetic research and generate improvements in maize agronomic traits. To this end, we constructed ZEAMAP (http://www.zeamap.com), a comprehensive database incorporating multiple reference genomes, annotations, comparative genomics, transcriptomes, open chromatin regions, chromatin interactions, high-quality genetic variants, phenotypes, metabolomics, genetic maps, genetic mapping loci, population structures, and populational DNA methylation signals within maize inbred lines. ZEAMAP is user-friendly, with the ability to interactively integrate, visualize and cross-reference multiple different omics datasets.

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P207

Comparing cytokinin oxidase/dehydrogenase gene expression in sorghum inflorescence and maize tassel development

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Many studies in rice have found that increased cytokinin abundance during inflorescence development results in increased branching of the inflorescence. In this study, the transcriptomic fluctuations of cytokinin oxidase/dehydrogenase genes during tassel development in maize and their syntenic ortholog expression during sorghum panicle development are compared using the COGs Gene Expression Browser. This study will inform future exogenous cytokinin application experiments in maize and sorghum.

Funding acknowledgement: Leiboff Lab, Oregon State University

P208

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 the prolamin accretions in the ER of aleurone cells come in close contact with the vacuolar membrane and 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 largely unknown but a few studies show that it can be either ATG8-dependent or ATG8-independent. To identify proteins mediating the microautophagy of prolamin accretions in maize aleurone cells, we conducted RNA-sequencing studies of aleurone and starchy endosperm tissues at 18 and 22 days-after-pollination and performed mass spectrometric analyses on vacuolar membrane-enriched fractions of aleurone cells. After analyzing the RNA-sequencing and proteomic data, we identified ten candidate proteins with potential function in autophagy and/or membrane modification. By transiently expressing mCherry-tagged candidate proteins in developing aleurone cells, we identified a hydroxyproline-rich glycoprotein family protein and a phospholipase that localize to the vacuolar membrane domain surrounding the prolamin accretions during microautophagy, supporting their potential roles in the microautophagy process. Our study has provided not only valuable data and insights for the plant microautophagy process but also research methods that can be adapted to other research studying the molecular mechanisms of plant biological processes.

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

P209 @carolynplants

Cell cortex microtubules contribute to division plane positioning during telophase in maize

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The phragmoplast is a plant-specific microtubule and microfilament structure that forms during telophase to direct new cell wall formation. The phragmoplast expands towards a specific location at the cell cortex called the division site. How the phragmoplast accurately reaches the division site is currently unknown. We show that a previously uncharacterized microtubule arrays accumulated at the cell cortex. These microtubules were organized by transient interactions with division-site localized proteins and were then incorporated into the phragmoplast to guide it towards the division site. A phragmoplast-guidance defective mutant, *tangled1*, had aberrant cortical-telophase microtubule accumulation that correlated with phragmoplast positioning defects. Division-site localized proteins may promote proper division plane positioning by organizing the cortical-telophase microtubule array to guide the phragmoplast to the division site during plant cell division.

Gene / Gene Models described: *tan1*; GRMZM2G03911
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P210 @Yan_Geneticist

Identification of genetic determinants of trait measurement 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, the differences between automated measurements and ground truth measurements, 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 these errors 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. Further, identification of TASs from GWASs conducted on the differences between the two values, indicates that a fraction of measurement error is under genetic control. Therefore, our study suggests that phenotyping errors cannot always be controlled simply by increasing population size and/or replication number.

Funding acknowledgement: National Science Foundation (NSF)

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Year	Annual	Location	Dates	Chair
2021	63	Online	March 8-12	Marna Yandeau-Nelson
2020	62	Online	June 25-26	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

Year	Annual	Location	Dates	Chair
1977	19	Allerton Park, IL	March 12-13	Earl Patterson
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

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