64th Annual Maize Genetics Meeting

Program and Abstracts

March 31 – April 3, 2022

MGC

Maize Genetics Cooperation, Inc.

Facilitated in partnership with

ConferenceDirect®

This conference received financial support from:

National Science Foundation
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We thank these sponsors for their generosity!

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

USDA ARS
Agricultural Research Service

Table of Contents

Cover image description

A drawing of a branched maize plant from the random-mating Shoepeg population, which was grown in Germany.

Cover art by

Mila Tost
University of Göttingen
Germany

General Information

Meeting Registration

Thursday: 3:00 PM to 9:30 PM: Depot Registration Office
Friday: 7:00 AM to 12:30 PM: Depot Registration Office

Meals

All meals will be served in the Midway, with physical distancing at the dining tables. Attendees have the option of taking their meals to go; serving hours as listed in the Program. Coffee, tea, and soft drinks are available at no charge during the beverage breaks.

Talks and Posters

All Talks will be presented in the Grand Ballroom.

Posters will be presented in the Midway, adjacent to where the meals will be held. Posters should be hung Thursday starting at 3 PM and stay up until Sunday morning, but must be removed by 9 AM on Sunday. During poster sessions, presenters of odd number posters are asked to stand by their posters 1:30-3:00 PM on Friday and 3:15-5:00 PM on Saturday. Presenters of even numbered posters should stand by their posters 3:00-4:30 PM on Friday and 1:30-3:15 PM on Saturday.

The maize meeting is a forum for presentation and discussion of unpublished material.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

COVID Safety Protocols

We have worked with the Union Station Hotel to ensure the safety of all in-person attendees. Things you can expect to see are increased spacing between chairs in meal and meeting areas.

All in-person attendees have attested that they are fully vaccinated per CDC guidelines. All meeting and event space has been set to allow for physical distancing and masks are required when not eating.

Masks are required at all times during the meeting when in common spaces with the following exceptions: masks may be removed while eating or drinking, but we ask that you try to maintain distance from the next person; speakers will be allowed to remove their masks during their podium presentations. All presentation materials and AV equipment will be sanitized between speakers to limit the spread of disease.
Recommendation: All attendees should wear the most protective mask possible such as N95, KN95, or at a minimum surgical masks, while at the MGM to limit the spread of disease.

You **should not** attend the in-person meeting sessions (even if you have already arrived at the meeting) if: you are currently or within the past 10 days experiencing symptoms of COVID-19 that cannot be attributed to known conditions; have been exposed to a confirmed or suspected case of COVID-19 within the past 10 days; have tested positive for COVID-19 and have not been cleared as non-contagious. We are unable to offer refunds, but you will be able to attend the virtual component of the meeting with your previously purchased in-person meeting registration. If you knowingly attend the meeting and have experienced any of the above, you will forfeit your membership to the Maize Genetics Cooperation and be expelled from the meeting with no refunds.
Recommendation: Before boarding your flight or getting in your car to come to the MGM, take a COVID-19 test to ensure that you are not infected. If possible, this should be a PCR-based test, but rapid tests are also acceptable. Bring one or more COVID-19 rapid tests with you to the MGM and test yourself upon arrival and throughout the meeting.

If you develop COVID-19 symptoms at the Maize Meeting, please stay in your hotel room, follow CDC guidance and if you need assistance, please contact the hotel front desk or contact Tricia Simmons at 720-250-7033.

Hospitality

After the evening sessions on Thursday and Friday there will be informal socializing and poster gazing in the Midway, with refreshments and games provided from 9 PM - 11 PM and a cash bar until 1 AM. On Saturday evening there will be informal socializing in the Midway, with refreshments from 9 PM - Midnight, trivia from 10 PM - 11 PM, and a cash bar from midnight - 2 AM.

Virtual Maize Genetics Meeting platform

Our 2022 virtual meeting is being hosted on the SwapCard platform. Each meeting registrant will receive a personalized invitation email sent by SwapCard (noreply@swapcard.com) on Monday, April 4, 2022. If you do not receive the email by close of business on April 4th, please check your junk/spam folder. If you still haven't received it, or you are having issues with 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 Wednesday, April 6th, during either of the two conference blocks that day. If you are still experiencing issues, please email virtual@conferencedirect.com.

Steering Committee

Please share your suggestions and comments about the meeting with the 2022 Steering Committee

Erin Sparks, Chair .....(esparks@udel.edu)
Matthew Hufford, co-Chair .....(mhufford@iastate.edu)
Marna Yandeau-Nelson .....(myn@iastate.edu)
Madelaine Bartlett .....(mbartlett@umass.edu)
Joe Gage .....(jlg374@cornell.edu)
Mei Guo.....(guomei@kenfeng.com)
Frank Hochholdinger .....(hochhold@uni-bonn.de)
Todd Jones .....(todd.j.jones@corteva.com)
Ruben Rellan-Alvarez.....(rrellan@ncsu.edu)
Samantha Snodgrass .....(snodgras@iastate.edu)
Maud Tenaillon .....(maud.tenaillon@inra.fr)
Petra Wolters .....(petra.wolters@corteva.com)

Ex officio:
Carson Andorf - MaizeGDB
David Braun - Treasurer
Darwin Campbell – Planning / Audio Visual
Marty Sachs - Local Host
John Portwood - Logistics Coordinator

Meeting planning:
Tricia Simmons – Conference Direct

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 Tricia Simmons 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. Special thanks are also extended to the Union Station staff for their help in organizing this conference. Thanks go to Mei Guo, Todd Jones, and Petra Wolters for their efforts in securing funding to offset meetings costs. Finally, many, many thanks go to the Steering Committee for organizing the 64th Maize Genetics Meeting.

From the Maize Genetics Cooperation Board of Directors

Maize Genetics Awards:


The 2022 MGC Cooperator Awardees

Candice Gardner, NCRPIS, USDA-ARS
Mary Schaeffer, MaizeGDB, USDA-ARS

The 2022 MGC Leadership Awardee

Thelma Madzima, University of Washington-Bothell

The 2022 M. Rhoades Early Career Awardee

Madelaine Bartlett, U Mass, Amherst

The 2022 L. Stadler Mid-Career Awardee

Jianbing Yan, Huazhong Agricultural University


The 2022 R. Emerson Lifetime Awardees

Sarah Hake, USDA ARS and U C Berkeley
Major Goodman, North Carolina State University

The Barbara McClintock Prize for Plant Genetics and Genome Studies

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

The **2022** Barbara McClintock Prize for Plant Genetics and Genome Studies has been awarded to Dr. Robin Buell who will present a McClintock Prize Address on Friday, April 2, 8:10pm CDT (See Page 22).

(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 2018 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 was held in October 2020 and focused on public-private partnerships and career development. The fourth mid-year virtual conference was held in January 2022 and focused on strategies for a healthy and inclusive research community. 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; Industry Interface; and diversity, equity, and inclusion within the maize community. 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 MGC Membership Committee:

The Membership Committee was established in 2021. The Membership Committee is responsible for recruitment, retention, enrollment, and engagement of Maize Genetics Cooperation, Inc. (MGC) members and maintenance of a secure membership database. The Membership Committee was formed within the MGC and works with the Board of Directors to contribute to annual budget development as it relates to membership dues rates, and to implement contracts and legal obligations related to management of the membership database. The activities assumed by the Membership Committee include:

1. Membership signup and membership database maintenance. Maintain the membership website, secure membership database, and secure transaction service.

2. Membership expansion. Recommend and implement programs for increasing the membership base, especially among groups historically underrepresented among MGC members.

3. Membership benefits. Identify the needs of members and recommend the development of services to meet those needs. Make prospective and current members aware of the resources, services, and membership benefits.

4. Membership engagement. Recommend and implement ways to acknowledge members and to encourage participation in MGC activities.

More information about the Membership Committee can be found at the Membership Committee website (https://www.maizegdb.org/mgc/membership/) and information on becoming a member can be found at the MGC Members website (https://mgcmembership.org). If you are interested in learning more about the Membership Committee or becoming a member of the Membership Committee, please contact Dr. Wojtek Pawlowski (wp45@cornell.edu) or Dr. Candice Hirsch (cnhirsch@umn.edu).

The MaGNET Program and 2022 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 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; or employed at a US-based institution, 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.

2022 MaGNET Awardees

Undergraduate
Anadaisy Aguirre, University of Florida ..... Poster #90
Huda Ansaf, University of Missouri
Lea Barros, Hamilton University ..... Poster #169
Danielle Davis, University of Missouri ..... Poster #38
Luis Garcia-Lamas, Oregon State University ..... Poster #68
Leslie Harris, Oregon State University ..... Poster #183
Medelyn Hernandez, University of Washington Bothell ..... Poster #3
Sarah Joe, University of Florida

Postbac
Shannon Schrope, Michigan State University ..... Poster #128

Graduate Student
Olufunke Ayegbidun, Southern Illinois University ..... Poster #25
Eric Butoto, North Carolina State University ..... Talk #24
Martin Costa, University of Wisconsin ..... Poster #55
Juan Gonzalez, University of Florida ..... Poster #13
Gabriela Madrid, University of Florida ..... Poster #122
Stephanie Martinez, University of California Riverside ..... Poster #180
Nadia Mourad-Silva, University of Florida ..... Poster #89
Christopher Mujjabi, University of Illinois ..... Poster #19
Ann Murithi, Iowa State University ..... Talk #26
Jessi Noel, Florida A&M University ..... Poster #15
Xue Pan, University of Arizona
Jada Smith, University of Missouri

Postdoc
Dayane Cristina Lima, University of Wisconsin
Maria-Angelica Sanclemente, Utrecht University ..... Poster #124

Mentor Accompanying Student
Ramesh Katam, Florida A&M University ..... Poster #136
Thelma Madzima, University of Washington
Natalie Nannas, Hamilton University ..... Poster #215

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

Primarily Undergraduate Institutions and Disciplinary Breadth Awards

Primarily Undergraduate Institutions (PUI) and Disciplinary Breadth (DB) are two 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 badges, 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; or employed at a US-based institution. 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.

2022 PUI Awardees

Student

Shelby McVey, Hamilton University.....Poster #169

Faculty

Irina Makarevitch, Hamline University.....Poster #2
Allison Phillips, Wisconsin Lutheran College.....Poster #147

2022 DB Awardees

Student

Heather Chamberlain, Iowa State University
Leila Fattel, Iowa State University.....Poster #123
Igor Ferreira Coelho, University of Florida .....Poster #18
Lauren Jenkins, University of Missouri.....Poster #117
Matthew Szarzanowicz, UC Berkeley
Colleen Yanarella, Iowa State University .....Poster #56

Postdoc

Anuradha Singh, Michigan State University.....Poster #22
Brian St. Aubin, Michigan State University.....Poster #192

The 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 2022 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 2022 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. BIP awardees receive waived registration to the virtual meetings platform.

Faculty
Zahoor Ahmed Dar, India

Research Scientists
Sneha Adhikari, India
Mohammad Ismail, Egypt
Anjali Joshi, Arid Forest Research Institute, India
Mpeguzi Masunga Hinile
Allen Oppong, Ghana

Graduate Students
Showkath Babu BM, India
Isabela Figueiredo de Oliveira, UFSJ - Universidade Federal de São João del Rei
Raquel Gomes de Oliveira, Brazil
Irene Ibiwari Ikiriko, University of Port Harcourt Nigeria
Mariana Lourenço Campolino, UFSJ, Brazil
K Thanduanlung, India

FAIR Data Management

...A Reminder from the MaizeGDB team

MaizeGDB integrates large amounts of published data so our community can find and use it easily. Your efforts to make your data Findable, Accessible, Interoperable and Reusable (FAIR; go-fair.org) allow MaizeGDB and others to integrate even more data. Here are basic guidelines for FAIR DATA management for you to apply to data you have generated, and to data in papers and grants that you review.

An example of an outstanding FAIR data paper is:
Savadel SD, Hartwig T, Turpin ZM, Vera DL, Lung PY, Sui X, Blank M, Frommer WB, Dennis JH, Zhang J, Bass HW. **The native cistrome and sequence motif families of the maize ear.** PLoS Genet. 2021 Aug 12;17(8):e1009689. doi: 10.1371/journal.pgen.1009689. PMID: 34383745; PMCID: PMC8360572.

Note the Supporting Information has even BED and bigwig files! These were easy to directly incorporate into the MaizeGDB Genome Browser. We encourage authors to take this much care with their data- to help consolidate information we have on each gene, each protein, each genome, etc.!

- **Put your Data in the right Database.** For example, DNA/RNA/Protein Sequences, genome assemblies and annotations should go to the long term repositories like NCBI. Report the accession numbers in your paper. All maize SNPs should be submitted to EVA at EBI. (https://www.ebi.ac.uk/eva/?Submit-Data). See more repositories at maizegdb.org/FAIRpractices and journal websites
- **Publish the data with the paper.** If your journal article refers to big data NOT published with your article, please make sure to obtain, from the data repository where you put your data, a persistent identifier like a DOI number, and add that to your article. You can publish datasets alone in journals like https://www.micropublication.org, just make sure to link data with the paper describing it. When reviewing papers, please ensure reported data is actually present and FAIR.
- **Use established unique identifiers for genes, gene models, genomes, etc.** Don't rename genes that already have names. For gene names, please look up your gene name symbol at MaizeGDB. If reporting on a gene sequence, please use the **exact gene model ID** (which will also ID the genome from which it came). If there is no gene model for your gene, please deposit your sequence at NCBI, and report the NCBI identifier in your paper. If reporting on a protein, please use the correct ID from NCBI or UniProt. If it's not there, please submit your protein sequence to NCBI or Uniprot.
- **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 quality of the research.
- **Ensure data sets are "machine readable".** When describing 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.
- **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.
- **Familiarize yourself with the FAIR data sharing standards.** Here are some resources: https://www.go-fair.org, https://doi.org/10.1093/database/bay088.

We are always happy to answer your questions on these issues! https://www.maizegdb.org/contact
For more information on FAIR principles visit: https://www.maizegdb.org/FAIRpractices

What's NEW at MaizeGDB!

In 2021, MaizeGDB expanded its pan-genomic resources* for the representative B73 genome, all NAM founder lines, and other historically important lines. These resources now include:

- 50 genomes
- Over 1 million gene model annotations
- 206 downloadable files
- 134 target databases in BLAST
- Genome browsers for each genome with over 1,000 total tracks
- Expression data for B73 v5 and the NAM founder lines on qTeller
- 400+ high-throughput sequencing data for over 80 tissues/conditions
- 300+ traits linked to over 40,000 positions in the genome
- 80+ million SNPs from EVA and Ensembl Plants
- Over 1 million predicted GO terms across 31 genomes
- Resources for 4 insertion mutation collections
- AlphaFold protein structures on the browser and gene model pages
- Transposable elements, structural variation, regulatory sites, and more…

If you have questions on how to access/use these resources, contact us https://www.maizegdb.org/contact
*Woodhouse MR et al. (2021) A pan-genomic approach to genome databases using maize as a model system. BMC Plant Biology. doi: https://doi.org/10.1186/s12870-021-03173-5.

Thank you to the 2021 MaizeGDB Editorial Board Members!
Mohammad Arif Ashraf (TWO YEARS!), PostDoc, UMass Amherst, MA
Zhaobin Dong, Faculty, China Agricultural University, Beijing, China
Yanfang Du, Faculty, Huazhong Agricultural University, China
Brianna Griffin, Graduate Student, Iowa State University, Ames, IA
Samantha Snodgrass (TWO YEARS!), Graduate Student, Iowa State University, Ames, IA
Meixia Zhao, Faculty, Miami University, Oxford, OH

Welcome to the 2022 MaizeGDB Editorial Board Members!
Mohammad Arif Ashraf (Year 3!!), PostDoc, UMass Amherst, MA
Kaitlin Higgins, Graduate Student, Iowa State University, Ames, IA
Beibei Liu, Graduate Student, Miami University, Oxford, OH
Hai Wang, Faculty, China Agricultural University, China
Lei Liu, Faculty, Huazhong Agricultural University, China
Lander Geadelmann, Graduate Student, Iowa State University, Ames, IA
Anuradha Singh, Postdoc, Michigan State University (April 2022)

New in 2022 – Editorial Board DEI Papers recommended by CODIE Editors!
Andrew Egesa, Graduate Student, University of Florida, Gainesville, FL
Tessa Durham Brooks, Faculty, Doane University, Crete, NE

New in 2022: MaizeGDB has partnered with the journal microPublication Biology (Caltech) to encourage publication of data that would otherwise go unpublished! This is an exciting development, as data from these papers will go directly into MaizeGDB. See the next page for more.

MaizeGDB has partnered with *microPublication Biology!*

***microPublication Biology* (Caltech Publishers) is a new peer-reviewed, open-access journal that publishes single experiment results, which are incorporated directly into community knowledgebases like TAIR, FlyBase, WormBase, PomBase, and now MaizeGDB**! Thus, *microPublication Biology* gets your individual research findings, that might otherwise remain unpublished, out to the scientific community while providing credit to those who did the work. Articles are small (one figure, few pages), peer reviewed, assigned a DOI and are discoverable on PMC, PubMed, EuropePMC, and Google Scholar.

How this works: Each maize *microPublication Biology* submission will be vetted by MaizeGDB curators at the time of peer-review to ensure data meets FAIR data standards (see https://www.maizegdb.org/FAIRpractices). Upon acceptance, your article is curated into MaizeGDB: coupling publication with curation and discoverability in MaizeGDB. The cost to publish is only $250.

Here are some example publications:

New Finding: Oh S, Kong Q, Montgomery BL. Guard-cell phytochromes impact seedling photomorphogenesis and rosette leaf morphology. MicroPubl Biol. 2022 Jan 31;2022. doi: 10.17912/micropub.biology.000521. PMID: 35128344; PMCID: PMC8808294.

Materials and Reagents: Marques J, Matiolli CC, Abreu IA. Visualization of a curated *Oryza sativa* L. CDPKs Protein-Protein Interaction Network (CDPK-OsPPIN). MicroPubl Biol. 2022 Jan 26;2022. doi: 10.17912/micropub.biology.000513. PMID: 35098050; PMCID: PMC8792674.

Negative Results: Martineau CN, Maynard CA, Pujol N. ATFS-1 plays no repressive role in the regulation of epidermal immune response. MicroPubl Biol. 2022 Feb 22;2022. doi:10.17912/micropub.biology.000525. PMID: 35224461; PMCID: PMC8864481.

For more information:

Visit the journal: https://www.micropublication.org

Read this article: Raciti D, Yook K, Harris TW, Schedl T, Sternberg PW. Micropublication: incentivizing community curation and placing unpublished data into the public domain. *Database (Oxford)*. 2018;2018:bay013. doi:10.1093/database/bay013

Or talk to Lisa Harper or Carson Andorf at MaizeGDB, https://www.maizegdb.org/contact

SCHEDULE OF EVENTS

Talks will be held in the Grand Ballroom.
Posters will be displayed in the Midway.

Thursday, March 31, 2022

9:00 AM – 6:00 PM	**OPTIONAL PRE-CONFERENCE WORKSHOPS**	
	All workshops will be located on the 1st floor in the Midway Suites 1-4	
9:00 AM – 4:00 PM	**Maize Development Genetics Workshop** Pre-registration is required.	Midway Suites 1-4
3:00 PM – 9:30 PM	**REGISTRATION** (Depot Registration Office)	
3:00 PM – 6:00 PM	**POSTER HANGING** (Midway)	
5:00 PM – 5:45 PM	**MaGNET Awardees and Mentors Introductions**	Midway Suites 1-4
6:00 PM – 7:00 PM	**DINNER** (Midway)	
7:00 PM – 9:00 PM	**SESSION 1 – WELCOME / THE GENES THAT MAKE MAIZE** Chair: Erin Sparks	Talks 1-4. Pages 24-27.
7:00 PM	**WELCOME AND ANNOUNCEMENTS**	(Grand Ballroom)
7:15 PM	**Maruti Nandan Rai, University of Illinois Urbana-Champaign** [T1] *Glossy15 overexpression alters Carbon partitioning and Nitrogen remobilization in maize stem*	
7:35 PM	**Singha Dhungana, University of Missouri-Columbia** [T2] *A novel DNAJ-thioredoxin-like protein is required for carbohydrate partitioning in maize*	
7:55 PM	**Rajdeep Khangura, Purdue University** [T3] *The maize semi-dominant lesion mutant Bella fleck1 provides resistance to common rust and Physoderma brown spot*	
8:15 PM	**Jiahn-Chou Guan, University of Florida** [T4] *Strigolactones regulate domestication phenotypes of cupule architecture, kernel size, and their coordination via Tga1-dependent and -independent networks.*	
8:35 PM	**Poster Lightning Talks**	
9:00 PM – 1:00 AM	**INFORMAL POSTER VIEWING & HOSPITALITY**	(Midway)

Friday, April 1, 2022

7:00 AM – 8:00 AM	**BREAKFAST** (Midway)	
7:30 AM – 12:30 PM	**REGISTRATION** (Depot Registration Office)	
8:00 AM – 10:10 AM	**SESSION 2 – GENOME BIOLOGY** Chair: Joe Gage	Talks 5-9. Pages 28-32.
8:00 AM	**ANNOUNCEMENTS**	(Grand Ballroom)
8:15 AM	**Mila Tost, University of Goettingen** *Experimental evolution in maize with replicated divergent selection identifies plant-height associated SNPs*	[T5]
8:35 AM	**Jonathan Cahn, Cold Spring Harbor Laboratory** *Maizecode: DNA regulatory elements in maize and teosinte inbreds provide insight into maize domestication*	[T6]
8:55 AM	**Andrea Eveland, Donald Danforth Plant Science Center** *Gene networks underlying architectural pleiotropy guide genome-wide association studies to highly connected regulators of tassel and leaf development*	[T7]
9:15 AM	**Mateus Mondin, CYNGELA** *A knob variant sequence and its relatednesses with maize evolutionary history*	[T8]
9:35 AM	**Shujun Ou, Iowa State University** *Differences in activity and stability drive transposable element variation in tropical and temperate maize*	[T9]
9:55 AM	**Poster Lightning Talks**	
10:10 AM – 10:40 AM	**BREAK**	**Foyer A**
10:40 AM – 12:30 PM	**SESSION 3 – INVITED SPEAKERS** Chair: Marna Yandeau-Nelson	Pages 18 & 19.
10:40 AM	Introduction	
10:50 AM	**Kirsten Bomblies, ETH Zürich** *Learning to tango with four - problems and solutions to autopolyploid meiosis*	[IS1]
11:40 AM	**José Dinneny, Stanford University** *The divining root: exploring adaptive responses to water using the grass root system*	[IS2]

Friday, April 1, 2022 (continued)

12:30 PM – 1:30 PM **LUNCH** (Midway)
PUI Networking Lunch (Midway Suites 1&2)
MGC BoD and MGAC Lunch (Midway Suites 3&4)

1:30 PM – 4:30 PM **POSTER SESSION 1** (Midway)

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

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

Beverages will be available from 2:30 to 4:00 PM in Midway

4:40 PM – 6:00 PM **SESSION 4 – REGULATING GENES & GENOMES**
Chair: Sam Snodgrass Talks 10-12. Pages 33-35.

4:40 PM **Amanpreet Kaur, Purdue University** [T10]
Hypothesis-driven identification of alleles affecting known pathways using a transcript accumulation index

5:00 PM **Bridget Hua Bai, UC San Diego** [T11]
Unbiased RNA and protein co-expression networks highlight important regulatory role of coordinated organelle protein homeostasis in maize heterosis

5:20 PM **Benjamin Oakes, The Ohio State University** [T12]
Heritable regulatory states dependent on parental RNA polymerase IV contribute to hybrid vigor

5:40 PM **Poster Lightning Talks**

6:00 PM – 7:00 PM **DINNER** (Midway)

7:00 PM –9:00 PM **SESSION 5 – AWARDS & McCLINTOCK PRIZE PRESENTATION**

7:00 PM **Hank Bass, Florida State University**
Presenting: Leadership and Cooperator Awards

7:15 PM **Paula McSteen, University of Missouri**
Presenting: M. Rhoades Early-Career, L. Stadler Mid-Career

7:30 PM **Candice Hirsch, University of Minnesota**
Presenting: R. Emerson Lifetime Awards

8:00 PM **Candice Hirsch, University of Minnesota**
McClintock Prize Presentation Introduction

8:10 PM **Robin Buell, University of Georgia**
Plant natural product biosynthesis: An enigma no longer

9:00 PM – 1:00 AM **INFORMAL POSTER VIEWING & HOSPITALITY** (Midway)

Saturday, April 2, 2022

7:00 AM – 8:00 AM	**BREAKFAST** (Midway)
8:00 AM – 12:00 PM	**REGISTRATION** (Depot Registration Office)

8:00 AM – 10:00 AM — SESSION 6 – EMERGING TOOLS & APPLIED RESEARCH
Chair: Rubén Rellán-Álvarez — Talks 13-18. Pages 36-41.

8:00 AM — **Minjeong Kang, Iowa State University** [T13]
A rapid and simplified transformation and genome editing method for maize inbred B104 using Agrobacterium ternary vector system and immature embryos

8:20 AM — **Baoxing Song, Cornell University** [T14]
AnchorWave: sensitive alignment of genomes with high diversity, structural polymorphism and whole-genome duplication variation

8:40 AM — **Hannes Claeys, Inari Agriculture** [T15]
Coordinated gene upregulation in maize through CRISPR/Cas-mediated enhancer insertion to improve nitrogen use efficiency

9:00 AM — **Michael Tross, University of Nebraska-Lincoln** [T16]
Data driven trait quantification across a maize diversity panel using hyperspectral leaf reflectance

9:20 AM — **Devin O'Connor, Pairwise** [T17]
A tunable increase in maize kernel row number using DNA base editing

9:40 AM — **Marc Albertsen, Corteva Agriscience Retired** [T18]
From discovery to smallholder farmers: Ms44 and its 45-year journey to Africa

10:00 AM – 10:40 AM — BREAK — Foyer A

10:40 AM – 12:30 PM — SESSION 7 – INVITED SPEAKERS
Chair: Petra Wolters — Pages 20 & 21.

10:40 AM — Introduction

10:50 AM — **Cranos Williams, North Carolina State University** [IS3]
Harnessing the Ag data revolution for modeling plan and agronomic systems across scale

11:40 AM — **Neelima Sinha, University of California Davis** [IS4]
Interactions between the parasitic plants and their hosts

Saturday, April 2, 2022 (continued)

12:30 PM – 1:30 PM **LUNCH** (Midway)
MGMSC Meeting (Midway Suites 3&4)
MaGNET Lunch (Midway Suites 1&2)

1:30 PM – 5:00 PM **POSTER SESSION 2** (Midway)

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

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

Beverages will be available from 2:30 to 4:00 PM in Midway

5:00 PM – 6:00 PM **COMMUNITY SESSION - Maize Genetics Cooperative**
MGAC Chair: Ruth Wagner (Grand Ballroom)

6:00 PM – 7:00 PM **DINNER** (Midway)

7:00 PM – 8:20 PM **SESSION 8 – CELLULAR PROCESSES**
Chair: Todd Jones Talks 19-22. Pages 42-45.

7:00 PM **Aimee Uyehara, University of California, Riverside** [T19]
Cell cortex microtubules contribute to division plane positioning during telophase in maize

7:20 PM **Le Liu, UMass, Amherst** [T20]
A Receptor-Like Proteins PAN2 is required for ABA and dark-mediated grass stomatal closure

7:40 PM **Xiaosa Xu, Cold Spring Harbor Laboratory** [T21]
A comprehensive single-cell atlas of plant shoot meristems facilitates functional analysis

8:00 PM **Sanzhen Liu, Kansas State University** [T22]
Revealing Gene Regulation of Cuticular Wax Biosynthesis Using Artificial Transcription Factors

8:20 PM – 8:40 PM **BREAK** **Foyer A**

8:40 PM – 9:30 PM **SESSION 9 – FOSTERING DIVERSITY IN THE MAIZE COMMUNITY**
Chair: Madelaine Bartlett Page 22.

8:40 PM **Banu Subramaniam, University of Massachusetts Amherst** [IS5]
Decolonizing Botany

9:30 PM – 2:00 AM **INFORMAL POSTER VIEWING, & HOSPITALITY** (Midway)
10:00 PM – 11:00 PM **TRIVIA** (Midway)

Sunday, April 3, 2022

7:00 AM – 8:20 AM **BREAKFAST** (Midway)

Posters should be taken down by 9 AM!

8:20 AM – 10:00 AM **SESSION 10 – BIOTIC FRIENDS & FOES**
Chair: Erin Sparks Talks 23-27. Pages 46-50.

8:20 AM **Alonso Favela, University of Illinois Urbana-Champaign** [T23]
Ancient Roots: Disruption and rewilding of the Zea rhizosphere microbiome

8:40 AM **Eric Butoto, North Carolina State University** [T24]
A tale of two selection methods for resistance to Fusarium ear rot and fumonisin in maize

9:00 AM **Mara Sgroi, University of Cambridge** [T25]
Invisible to Arbuscular Mycorrhizal Fungi: positional cloning and characterisation of the ina arbuscular mycorrhizal mutant in Zea mays

9:20 AM **Ann Murithi, CIMMYT** [T26]
Discovery and validation of a recessively inherited major effect QTL conferring resistance to maize lethal necrosis (MLN) disease

9:40 AM **Ksenia Krasileva, University of California, Berkeley** [T27]
Evolution of plant immune receptors

10:00 AM – 10:30 AM **BREAK** **Foyer A**

10:30 AM – 11:40 AM **SESSION 11 – MAIZE & THE ENVIRONMENT**
Chair: Matt Hufford Talks 28-30. Pages 51-53.

10:30 AM **Jialu Wei, Iowa State University** [T28]
Investigating Dynamic Heat Stress Responses with Diverse Maize Inbreds through Transcriptome Analyses

10:50 AM **Parisa Sarzaeim, University of Nebraska-Lincoln** [T29]
Consolidating OMICS and Environmental Database for Maize Yield Predictions in a Changing Climate

11:10 AM **Diego Jarquin, University of Florida** [T30]
Unleashing The Potential Of Weather Data For Improving Yield Forecasting In G2F Maize Hybrids

11:30 AM **CLOSING REMARKS**

11:40 AM **ADJOURNMENT**

Poster List

Education & Outreach

P1	**Kathryn Parsley** <KParsley@danforthcenter.org>	*Genotype to Phenotype: An authentic maize research experience for high school students*
P2	**Irina Makarevitch** <imakarevitch01@hamline.edu>	*Community and Cooperation: The MGC Committee on Outreach, Diversity, Inclusion, and Education (CODIE) 2021-22 update*
P3	**Medelyn Hernandez** <mh0106@uw.edu>	*A course-based undergraduate research experience (CURE) to investigate DNA methylation in maize.*
P4	**John Gray** <john.gray5@utoledo.edu>	*Employing the maize TFome for expanding GRNs while fostering the Integration of Research with Undergraduate Education (F.I.R.E.)*
P5	**Addie Thompson** <thom1718@msu.edu>	*Seeding public-private partnerships for agricultural genome-to-phenome training*
P6	**Hank Bass** <bass@bio.fsu.edu>	*The Maize-10-Maze Project, an educational public chromosome map garden featuring the mutants of maize.*

Quantitative Genetics & Breeding

P7	**Yu-Ru Chen** <yuruchen@iastate.edu>	*Advances in maternal haploid inducer development in maize*
P8	**Ravi Mural** <rmural2@unl.edu>	*Assembling and curating multi-environment, multi-trait datasets from community association panels as a prelude to phenotypic yield prediction*
P9	**Matthew Murphy** <mdm10@illinois.edu>	*Assessment of two statistical approaches for variance genome-wide association studies in plants*
P10	**Cleopatra Babor** <cleopatrababor@gmail.com>	*Breeding colored and high lysine prime eating stage sweetcorn*
P11	**Jonathan Niyorukundo** <jonathan.niyorukundo@huskers.unl.edu>	*Breeding of diversely colored quality protein popcorn*
P12	**John Searl** <jsearl@wisc.edu>	*Characterization of inbreds with expired plant variety protection – 2022 update*
P13	**Juan Gonzalez** <juangonzalez@ufl.edu>	*Computer vision for high-throughput quantitative genetics for disease resistance in sweet corn*
P14	**Hongyu Jin** <hjin5@huskers.unl.edu>	*Cross environment yield prediction using genomic and phenotypic data in maize*
P15	**Jessi Noel** <jessi1.noel@famu.edu>	*DNA fingerprinting, and evaluation of genetic diversity of indigenous bread and durum wheat using ISSR marker system*
P16	**Mahule Elyse Boris Alladassi** <aboris@iastate.edu>	*Dynamics of genetic control of sorghum plant height across developmental stages*
P17	**Laura Tibbs-Cortes** <ltibbs@iastate.edu>	*Environmental and genetic basis of phenotypic plasticity in the maize NAM population*
P18	**Igor Ferreira Coelho** <ferreiracoelho.i@ufl.edu>	*Evaluating the implementation of double haploid technology and genomic selection into a sweet corn breeding pipeline*
P19	**Christopher Mujjabi** <mujjabi2@illinois.edu>	*Evaluation of the usefulness of Ex-PVP maize germplasm in hybrid development for organic maize systems using participatory variety testing*

P20	**Anuradha Singh** <annusingh1206@gmail.com>	*Genetic analysis of multiple biomass related traits in sorghum*
P21	**Marjorie Hanneman** <marjih29@gmail.com>	*Genetic analysis of extreme trigonelline levels in maize grain*
P22	**Dylan Schoemaker** <schoemaker@wisc.edu>	*Genetic analysis of natural variation for pericarp color in dent maize*
P23	**Jennifer Wilker** <jlwilker@wisc.edu>	*Genetic control of maize aerial node root number and diameter*
P24	**Emily Mikulski** <mikuls14@msu.edu>	*Genetic variation for photosynthetic efficiency and leaf hyperspectral imaging in Sorghum*
P25	**Olufunke Ayegbidun** <oayegbi@siue.edu>	*Genome Wide Association Study of selected commercial maize germ plasms under environmental stress*
P26	**Melissa Draves** <madraves@iastate.edu>	*Genome wide association studies of auxin responses in maize seedlings using the Wisconsin Diversity panel*
P27	**Brandi Sigmon** <bsigmon2@unl.edu>	*Genome-wide association study of maize tassel architecture phenotypes under nitrogen deficit stress*
P28	**Zhikai Yang** <zyang35@huskers.unl.edu>	*Genome-wide mediation analysis: an empirical study to connect phenotype with genotype via intermediate transcriptomic data in maize*
P29	**Brandon Webster** <webst250@msu.edu>	*Hyperspectral based prediction of nutrient content in maize leaves*
P30	**Sebastian Urzinger** <sebastian.urzinger@tum.de>	*Identification of a genomic region influencing early development and cold tolerance in an adapted maize landrace*
P31	**Baris Alaca** <bar.alaca@yahoo.com>	*Identification of epistasis by environment interaction in maize*
P32	**Semra Palali Delen** <semrapalali.sp@gmail.com>	*Identification of the genetic locus to decouple the genetic linkage between harmful heavy metals and essential minerals in maize*
P33	**Robert Twohey III** <twohey2@illinois.edu>	*Identifying the genetic regulators of leaf δ13C and stomatal traits in Zea mays*
P34	**Alden Perkins** <acperkins3@wisc.edu>	*Impact of exotic introgressions on maize hybrid performance in multi-environment trials*
P35	**Shane Spence** <spences4@msu.edu>	*Investigating rapid progeny screening approaches using hyperspectral imaging*
P36	**Miriam Nancy Salazar-Vidal** <msalazarvidal@ucdavis.edu>	*Is local adaptation of highland maize from Mexico and South America associated with different genomic regions?*
P37	**Mariana Vianna** <viannam@ufl.edu>	*K-mers based GWAS in sweet corn*
P38	**Danielle Davis** <dtdqkd@umsystem.edu>	*Kernel color variation in heirloom varieties and their properties in food.*
P39	**Malachy Campbell** <mcampbell@inari.com>	*Leveraging variant functional annotations to explore the role of allelic heterogeneity in genomic prediction*
P40	**Corey Schultz** <crs68219@uga.edu>	*Maize genetic diversity and microbial interactions*
P41	**Hallie Longest** <13hallie13@gmail.com>	*Mapping QTLs contributing to germination and seed vigor in Sorghum Bicolor*
P42	**Mohamed El-Walid** <mze3@cornell.edu>	*Mapping freezing tolerance in Tripsacum by bulk segregate analysis*

P43	**Garret Hall** <gmhzpy@umsystem.edu>	*Missouri heritage corn: A flavorful source for distillation & food products*
P44	**John Hodge** <jghodge@okstate.edu>	*Nested function-value traits as a framework to explore quantitative traits*
P45	**Savanah Dale** <smd346@cornell.edu>	*Optimization of sweet corn speed breeding for advancement of fresh kernel nutritional quality traits*
P46	**Sean A McLaughlin** <seanmclaughlin123@gmail.com>	*Phenotyping a new maize shoot mutant in inbred and F1 hybrid genetic backgrounds*
P47	**Linda Dao** <ldao@ufl.edu>	*Phenotyping for cold tolerance in sweet corn seedling emergence*
P48	**Kyle Linders** <klinders@huskers.unl.edu>	*Plasticity of sorghum panicle architecture in response to nitrogen deficit stress*
P49	**Michael Burns** <burns756@umn.edu>	*Predicting moisture content during maize nixtamalization using machine learning with NIR spectroscopy*
P50	**Robert Shrote** <shrotero@msu.edu>	*PyBrOpS – Python Breeding Optimizer and Simulator*
P51	**Irina Makarevitch** <imakarevitch01@hamline.edu>	*QTL mapping of seedling tolerance to exposure to low temperature in the maize IBM RIL population*
P52	**Sarah Oliver** <sloxmd@umsystem.edu>	*Reducing free asparagine content in maize to minimize acrylamide formation potential*
P53	**Carolina Freitas** <freitas4@msu.edu>	*Relating leaf area index to sorghum canopy traits*
P54	**Balázs Szabó** <balazs.szabo@gabonakutato.hu>	*Resistance of maize genotypes against Fusarium verticillioides isolates with different pathogenicity in artificial inoculation experiments*
P55	**Martin Costa** <MCCOSTA@wisc.edu>	*Selection signatures underlying genotype by environment interaction during modern hybrid maize breeding*
P56	**Colleen Yanarella** <cfy@iastate.edu>	*Speech-based phenotyping methods for field studies*
P57	**Thomas Lubberstedt** <thomasl@iastate.edu>	*Spontaneous haploid genome doubling (SHGD) mechanism to accelerate crop breeding*
P58	**Anna Giulini** <annapiamaria.giulini@crea.gov.it>	*Study of Aspergillus flavus resistance in maize Italian breeding varieties*
P59	**Julian Cooper** <coop0409@umn.edu>	*Temporal analysis of maize canopy cover using aerial high-throughput phenotyping*
P60	**Michael Busche** <busche.michael@gmail.com>	*Terminal ear1 and PhytochromeB1; PhytochromeB2 act independently to regulate leaf initiation in Zea mays*
P61	**Zhongjie Ji** <jizhongj@msu.edu>	*The exploration of UAS-based multispectral imaging in field phenotyping*
P62	**Sharon Liese** <sharonf2@illinois.edu>	*The impact of structural variation on heterosis and combining ability in maize*
P63	**Carl Branch** <cbranch2@wisc.edu>	*The sugary enhancer1 (se1) allele is associated with significant decreases in the amount of lutein, zeaxanthin, and tocotrienols in yellow (Y1) sugary1 (su1) kernels*
P64	**Fabio Guffanti** <fabio.guffanti@tum.de>	*Towards the understanding of the genetic control of lateral root development in adult maize plants*
P65	**Michael Meier** <michael.meier@huskers.unl.edu>	*Uncovering links between maize genetics and root-colonizing microbes under nitrogen stress*
P66	**Nikee Shrestha** <nshrest@okstate.edu>	*Understanding the genetic basis of shattering in pearl millet*

P67 **Rafael Della Coletta** <della028@umn.edu>
Understanding the relationship between genetic architecture and genetic marker selection for improved genomic prediction accuracy

P68 **Luis Garcia-Lamas** <garciall@oregonstate.edu>
Using Generalized Linear Model (GLM) and Power analysis to investigate mutations associated with aberrant sexual reproduction in maize

P69 **Sidney Sitar** <sitarsid@msu.edu>
Validating genetic resistance to maize tar spot in a stiff-stalk MAGIC population

Transposons & Epigenetics

P70 **Mowei Li** <li.10425@buckeyemail.osu.edu>
Cytosine methylation patterns associated with pl1 paramutation are reestablished and trans-chromosomally adopted during embryogeny

P71 **Jonathan Gent** <gent@uga.edu>
DNA demethylation in endosperm

P72 **Michelle Stitzer** <mcs368@cornell.edu>
Elevated transposable element copy number is associated with reduced fitness in maize

P73 **Kaitlin Higgins** <km.higgins26@gmail.com>
Exploring endosperm regulation through the DNA glycosylase maternal de-repression of R1 (mdr1)

P74 **Yibing Zeng** <yz77862@uga.edu>
Genic DNA methylation in maize

P75 **Beibei Liu** <liub32@miamioh.edu>
Genome-wide analysis of maize hybrids and their progeny to understand the initiation and maintenance of epigenetic silencing in maize

P76 **Daniel Laspisa** <dlaspisa@hawaii.edu>
H2A.Z and transcription influence neocentromere positioning in CEN5 of Zea mays.

P77 **Rocco Giarratano** <rocky.giarratano@uga.edu>
Helitron sequence dynamics across the maize genome

P78 **Mohammad Mahmood Hasan** <hasanm6@miamioh.edu>
High-resolution crossover maps to understand the role of DNA methylation in meiotic recombination

P79 **William Clore** <whclore@iastate.edu>
Identifying putative LTR retrotransposons in maize insertions using LTR Predictor

P80 **Grace Campidilli** <gec83@cornell.edu>
Mutator transposable element behavior across maize genotypes

P81 **Erika Magnusson** <magnu513@umn.edu>
Mutator transposon insertions within genes often provide a novel outward reading promoter

P82 **Hua Yang** <yanghu@missouri.edu>
Predominantly inverse modulation of transposon element expression in haploid and diploid aneuploidies in maize

P83 **Justin Scherer** <jts34805@uga.edu>
Quantifying the insertion frequency of the mutator transposon across tissue types and over developmental timing

P84 **Benjamin Berube** <bberube@cshl.edu>
RNAi mediates drive in teosinte-maize hybrids

P85 **Claire Menard** <menar060@umn.edu>
TIPs and tricks for identifying transposable element insertion polymorphisms in large genomes at the population-level

P86 **Stephanie Klein** <spklein@iastate.edu>
Transposable element-derived genotypic variation is likely associated with diverse root responses to nitrogen stress

P87 **Yinjie Qiu** <qiuxx221@gmail.com>
Whole-genome variation of transposable element insertions in a maize diversity panel

P88 **Oliver Marchus** <marchus.3@buckeyemail.osu.edu>
pl1 paramutation requires an SNF2-type ATPase encoded by the rmr13 locus

Biochemical and Molecular Genetics

P89	**Nadia Mourad Silva** <nmourad@ufl.edu>	*A new sorbitol dehydrogenase mutant in maize*
P90	**Anadaisy Aguirre** <anadaisy.aguirre@ufl.edu>	*Adapting corn to heat stress via carbohydrate metabolism modification*
P91	**Austin Chiles** <chilesac@whitman.edu>	*Altering the binding site of a plant hormone in corn proteins affects how fast they breakdown*
P92	**Ankita Abnave** <Ankita.Abnave@rockets.utoledo.edu>	*An expanded gene regulatory network governing the phenylpropanoid pathway in maize*
P93	**Dirk Winkelman** <dwink@iastate.edu>	*An integrated biochemical and genetic approach to assess the roles of Glossy2 and Glossy2-like in maize cuticle formation*
P94	**Laurie Smith** <lgsmith@ucsd.edu>	*Analysis of functional and compositional diversity of the adult maize leaf cuticle reveals a key role for wax esters in water barrier function*
P95	**China Lunde** <lundec@berkeley.edu>	*Autoactive and pathogen-dependent enhanced disease resistance mutants of 'Kronos' durum wheat*
P96	**Nate Korth** <nate.korth@gmail.com>	*Breeding food for health: Identification of substrates from quality protein popcorn that promote growth of specific beneficial bacteria in the human gut microbiome*
P97	**Sarah Hamade** <shamade@oakland.edu>	*Conserved function of RNA Binding Motif Protein 48 (RBM48) Armadillo Repeat Containing 7 (ARMC7) in maize and human U12 splicing*
P98	**Emma Anderson** <andersen@whitman.edu>	*Cutting up a maize auxin repressor determines the regions most important for degradation*
P99	**Bharath Kunduru** <bkundur@clemson.edu>	*Deciphering the morphological, geometric, and metabolic components of stalk lodging resistance in maize (Zea mays L.)*
P100	**Ryan Benke** <rbenke@purdue.edu>	*Disruption of Zea mays isochorismate synthase1 suppresses PHENYLALANINE AMMONIA LYASE activity and hypersensitive response induced metabolism*
P101	**Rohit Kumar** <mohank@clemson.edu>	*Dissecting the genetic architecture of source-sink regulated senescence in maize*
P102	**Hui Liu** <liu.hui@ufl.edu>	*Distinct roles of plastid and cytosolic pathways for aromatic amino acid biosynthesis in kernel and plant development*
P103	**Katie Murphy** <katiemurphy61@gmail.com>	*Dolabralexin-deficient maize mutant provides insight to maize defense and root architecture*
P104	**Hope Hersh** <hopehersh@ufl.edu>	*Engineered 6-phosphogluconate dehydrogenase in amyloplasts assessed in field corn hybrids for mitigation of grain yield loss under heat stress*
P105	**Jonathan Saunders** <jonosaun@ufl.edu>	*Enhancing transposon resources: Probing Robertson's originals and other unexplored Mu-active lines*
P106	**Alex Ferris** <acferris@stanford.edu>	*Establishment of Ustilago maydis infection in maize anthers*
P107	**Manwinder Singh Brar** <mbrar@clemson.edu>	*Exploring the metabolome diversity for staygreen in maize*
P108	**Jen Jaqueth** <jennifer.jaqueth@corteva.com>	*Fertility restoration of maize CMS-C altered by a single amino acid substitution within the Rf4 bHLH transcription factor*

P109 **Usha Bhatta**
<usha.bhatta@uga.edu>
Identification and comparative analysis of resistance against Ustilago maydis in maize, teosinte, and near-isogenic lines

P110 **Lina Gomez-Cano**
<gomezca5@msu.edu>
Identification of genomic regions associated with phenolic metabolism

P111 **Matthew Runyon**
<matthewrunyon98@gmail.com>
Investigating the contribution of the endogenous Zea mays aquaporin ZmPIP1;5 in regulating water-use efficiency

P112 **Mike Kolomiets**
<kolomiets@tamu.edu>
Ketols produced by a 9-Lipoxygenase play a role in defense against insect herbivores.

P113 **Fangyi Li**
<fangyishukers@gmail.com>
Low-cost photosynthesis phenotypic solutions for GWAS

P114 **Jeffrey Simpson**
<jsimpso1@purdue.edu>
Maize accessions exhibit considerable variation in phenylalanine-derived metabolites

P115 **Heidi Kaeppler**
<hfkaeppl@wisc.edu>
Maize genetic engineering and gene editing research and services at the Wisconsin Crop Innovation Center (WCIC)

P116 **Alex Austin**
<aaustin1@iastate.edu>
Metabolomic analysis of maize pollen during storage

P117 **Lauren Jenkins**
<lmjxy6@umsystem.edu>
Opaque-2 mutant development provides insight into protein rebalancing mechanism

P118 **Dawei Dai**
<daweidai@ufl.edu>
Paternal imprinting of dosage-effect defective1 contributes to seed weight xenia in maize

P119 **Jae-Hyung Lee**
<jalee@cshl.edu>
RAMOSA3 determines inflorescence branching and undergoes liquid-liquid phase transition

P120 **Brianna Griffin**
<bdg@iastate.edu>
REL2 mediated plant-pathogen response

P121 **Mark Lubkowitz**
<mlubkowitz@smcvt.edu>
SWEETs and SUTs are regulated by distinct and overlapping transcription factors suggesting functional redundancy as well as gene specific functions in carbohydrate partitioning

P122 **Gabriela Madrid**
<gabriela.madridc@ufl.edu>
Single-cell RNA sequencing reveals tissue localization of the trehalose-6-phosphate pathway in maize leaves

P123 **Leila Fattel**
<lfattel@iastate.edu>
Standardized genome-wide function prediction enables comparative functional genomics: a new application area for Gene Ontologies in plants

P124 **Maria-Angelica Sanclemente**
<sanangelma@gmail.com>
Sugar and oxygen responses are modulated by maize NDPK1

P125 **Tomasz Paciorek**
<tomasz.paciorek@bayer.com>
Targeted suppression of gibberellin biosynthetic genes ZmGA20ox3 and ZmGA20ox5 produces a short stature maize ideotype

P126 **Olga Zimina**
<oz32@cornell.edu>
Targeting meiotic recombination in maize

P127 **Maxwell McReynolds**
<maxwellm@iastate.edu>
The auxin response factor ZmARF27 is required for maize root morphogenesis

P128 **Shannon Schrope**
<schrope2@msu.edu>
The effect of the ACT-like domain on the C-terminal region of B in Zea mays

P129 **Tianxiao Yang**
<tianxiao.yang@ufl.edu>
The maize rough endosperm6 (rgh6) mutant encodes a predicted Dead-box RNA helicase

P130 **Gregorio Hueros**
<gregorio.hueros@uah.es>
The making of a transfer cell. Insights from the transcriptome of CRISPR-generated ZmMRP mutants.

P131 **Nathaniel Schleif**
<nschleif2@wisc.edu>
Tissue-specific promoters in maize: Identification, cloning, and characterization

P132 **Lyudmila Sidorenko** <lyudmila.v.sidorenko@gmail.com> — *Transcriptomic signatures of asymptomatic stress in maize*

P133 **Jason Karl** <jrk36@cornell.edu> — *Unlimited maize height*

P134 **Leannah Hicks** <lhicks@umass.edu> — *Using iron transporter genes to enhance the nutritional quality of maize grain*

P135 **Junxi Chen** <chenj@whitman.edu> — *What parts of receptor proteins affect the speed of hormone response in corn cells?*

P136 **Ramesh Katam** <ramesh.katam@famu.edu> — *Wheat proteomic analysis of salt tolerance in response to Bacillus safensis (ST17) and Bacillus tequilensis (ST25)*

Evolution and Population Genetics

P137 **Jinliang Yang** <jinliang.yang@unl.edu> — *A historically balanced locus under recent directional selection in responding to changed nitrogen conditions during modern maize breeding*

P138 **Meghan Brady** <meghan.brady@uga.edu> — *Detection of Abnormal Chromosome 10 in genotype by sequencing data*

P139 **Charles Hale** <coh22@cornell.edu> — *Exploring Cis-Regulatory Evolution across Andropogoneae*

P140 **Samantha Snodgrass** <snodgras@iastate.edu> — *Identifying fractionation events across the Tripsacinae subtribe*

P141 **Sierra Raglin** <sraglin2@illinois.edu> — *Lost Phenotypes: Assessing the effect of maize breeding on Rhizosphere Nitrification Suppression*

P142 **Zachary Loschinskey** <zfl9d4@umsystem.edu> — *PIF1 helicase phylogeny and function on the maize B chromosome*

P143 **Evandro Novaes** <evandro.novaes@ufla.br> — *Population genomics identifies candidate genes within selective sweeps in sweet corn*

P144 **Catherine Li** <chli6@illinois.edu> — *Using near-isogenic lines to dissect the genetic architecture of evolutionary change following long-term selection for grain protein concentration*

Cell and Developmental Biology

P145 **Chong Teng** <CTeng@danforthcenter.org> — *24-nt phasiRNA biogenesis and regulation in maize anther*

P146 **George Chuck** <georgechuck@berkeley.edu> — *A disordered protein mediates sex determination and auricle development in maize*

P147 **Allison Phillips** <allison.phillips@wlc.edu> — *Analysis of stunter2 and stunter3, maize maternal effect mutants with reduced kernel size*

P148 **Edoardo Bertolini** <ebertolini@danforthcenter.org> — *Architectural pleiotropy between tassel branching and leaf angle decoded through gene regulatory network rewiring*

P149 **Jason Gregory** <Jason.gregory@rutgers.edu> — *Boom or Bust: Regulation of meristem size by the REL2 corepressor family*

P150 **Yuguo Xiao** <yxiao@danforthcenter.org> — *Boundary domain genes were recruited to suppress bract growth and promote branching in maize*

P151 **Amina Chaudhry** <amina.chaudhry@waksman.rutgers.edu> — *Characterization and positional cloning of the prolific mutant eary-517**

P152 **Lander Geadelmann** <Landerg@iastate.edu> — *Characterization of maize genes that regulate leaf initiation and phyllotaxy*

P153 **Leo Koenigsfeld** <lgk778@mail.missouri.edu> — *Characterization of the tassel-less4 mutant and its interactions with auxin*

P154 **Madison Lane** <mlane@iastate.edu>	*Characterizing the role of the FDL1 transcription factor in cuticular wax deposition on maize silks*
P155 **Penelope Lindsay** <lindsay@cshl.edu>	*Control of maize ear development by a putative receptor-coreceptor pair in a CLAVATA-related signaling pathway*
P156 **Kyle Swentowsky** <swentow@cshl.edu>	*Developmental genetics and genomics of perennial regrowth in Zea diploperennis*
P157 **Brian Cox** <bjcox21@byu.edu>	*Disruption of an mRNA processing gene results in bract growth in maize tassel sheath 5 (tsh5) mutants*
P158 **George Chuck** <georgechuck@berkeley.edu>	*Distinctive features of maize vascular development*
P159 **Michael Scanlon** <mjs298@cornell.edu>	*Evidence for the developmental homology of the grass ligule*
P160 **Xinxin Ding** <xding4@wisc.edu>	*Exploring the roles of two putative maize BAG (Bcl-2 associated athanogene) family proteins in modulating plant growth, autophagy, and senescence*
P161 **Brad Nelms** <nelms@uga.edu>	*Gametophyte genome activation occurs at pollen mitosis I in maize*
P162 **Jacob Zobrist** <jzobrist@iastate.edu>	*Genetic transformation of maize progenitor teosinte (Zea mays ssp. parviglumis)*
P163 **Xingli Li** <xingli.li@ur.de>	*Impact of heat stress during maize pollen development*
P164 **Eddie Ross** <ehross3@illinois.edu>	*In vitro kernel culturing of hybrids: Kernel development and fl2-RFP accumulation in response to variable nitrogen*
P165 **Daniel Marchant** <dbmarchant@gmail.com>	*Maize anther development with fixed single-cell RNA-seq*
P166 **Steve Moose** <smoose@illinois.edu>	*Multiplex genome editing of maize inbred lines via biolistics delivery of CRISPR/Cas9 to Type I embryogenic calli*
P167 **Cynthia Waite** <waitecy@oregonstate.edu>	*Mutation of a predicted arabinogalactan protein gene (famp1) in maize is associated with a significant male-specific transmission defect*
P168 **Chris Larson** <christopher.d.larson@und.edu>	*Non-Mendelian segregation for maize embryo-specific mutations*
P169 **Shelby McVey / Lea Barros** <smcvey@hamilton.edu; lbarros@hamilton.edu>	*Rebuilding Zea mays Ab10 meiotic drive system in budding yeast using kindr and trkin kinesins*
P170 **Craig Cowling** <ccowling@iastate.edu>	*Roles of putative auxin transporters in root growth and development*
P171 **Hao Wu** <hw388@cornell.edu>	*Single cell analysis of maize embryo development*
P172 **Diana Ruggiero** <ruggiedi@oregonstate.edu>	*Single cell genomics and high-throughput phenotyping for determining the quantitative genetics of maize leaf vascular development.*
P173 **Prameela Awale** <pa96f@umsystem.edu>	*The enhancer of spi1 (eos1) gene affects inflorescence development in maize.*
P174 **Prameela Awale** <pa96f@umsystem.edu>	*The tls5 gene functions in vegetative and reproductive development in maize*
P175 **Madelaine Bartlett** <mbartlett@umass.edu>	*The transcription factor gene GRASSY TILLERS1 (GT1) and the trehalose-6-phosphate phosphatase gene RAMOSA3 (RA3) interact to regulate carpel suppression in maize flowers*

P176 **Johannes Scharwies** <joscha@stanford.edu>
Uncovering the genetic basis for diversity of moisture regulated root branching in maize

P177 **Xiaosa Xu** <xxu@cshl.edu>
Uncovering the non-enzymatic function of RAMOSA3 in maize inflorescence branching

P178 **Sabrina Chin** <schin7@wisc.edu>
Unraveling the maze of gravity-sensing cells in maize with RNA-Seq to discover gravity- specific transcriptional networks

P179 **Yuguo Xiao** <yxiao@danforthcenter.org>
Using molecular genetics and precision phenotyping to map gene function contributing to drought resilience in sorghum

P180 **Stephanie Martinez** <smart046@ucr.edu>
Using an allelic series of katanin mutants in maize to determine the role of KATANIN in plant growth

P181 **Willian Goudinho Viana** <viana@stanford.edu>
Using the crown root defective mutant to understand crown root development under well-watered and drought-stressed conditions

P182 **Maike Stam** <m.e.stam@uva.nl>
Vgt1: a cis-regulatory element regulating flowering time and growth speed

P183 **Leslie Harris** <harriles@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

P184 **Brian Zebosi** <bzebosi@iastate.edu>
bds1 and bds2 function redundantly to regulate inflorescence and shoot architecture in maize via brassinosteroid biosynthesis

Computational and Large-Scale Biology

P185 **Aimee Schulz** <ajs692@cornell.edu>
101 Evolutions: Evaluating maize gene annotations with genome sequences across the Andropogoneae

P186 **Allen Hubbard** <ahubbard@danforthcenter.org>
A novel paradigm for optimal mass-feature peak picking in large scale LC-MS datasets using the 'isopair': isoLock, autocredential and anovAlign; and application to plant metabolite genome wide association studies (GWAS)

P187 **Daniel Hickey** <hickeyda@oregonstate.edu>
Bioinformatic identification and analysis of microRNAs associated with maize male gametophyte development

P188 **Hua Yang** <yanghu@missouri.edu>
Cell size and RNA transcriptome size measurements in hybrids and inbreds in diploid and tetraploid maize

P189 **Keting Chen** <kchen@iastate.edu>
Characterization of the gene networks underlying cuticle production in maize via systems' biology approaches

P190 **Nolan Bornowski** <bornowsk@msu.edu>
Comparative genomics of expired Plant Variety Protected maize lines in the Stiff Stalk heterotic germplasm pool

P191 **Zhikai Liang** <liang795@umn.edu>
Deciphering the contribution of 3D genome organization to gene regulation in maize inbreds and hybrids

P192 **Brian St. Aubin** <staubinb@gmail.com>
Desiccation tolerance in grasses: two methods evolved to limit damage and survive

P193 **Jordan Manchego** <manchego@msu.edu>
Detection and quantification of tar spot foliar infection in maize using machine learning, object detection, and application development framework

P194 **Clay Christenson** <christensonclay@gmail.com>
Development of an instance segmentation pipeline for comprehensive high-throughput phenotyping of maize ears

P195 **Alexandria Tran** <tran30@illinois.edu>
Dynamic maize metabolic gene regulatory network construction by regression analysis

P196 **Jay Hollick** <hollick.3@osu.edu>
Global Run-On sequencing identifies nascent transcription in the B73 genome

P197 **Colin Finnegan** <cpf@iastate.edu>
Identification of potential haploid expressed genes in maize

P198 **Jack Gardiner** <jack.m.gardiner@gmail.com>
MaizeGDB's MaizeMine: New genome and community data sets

P199 **Andrea Gallavotti** <agallavotti@waksman.rutgers.edu>
Mapping and functional characterization of cis-regulatory variation in maize

P200 **Travis Wrightsman** <tw493@cornell.edu>
Modeling chromatin state from sequence across angiosperms using recurrent convolutional neural networks

P201 **Joseph Gage** <jlgage@ncsu.edu>
North American maize yield prediction contest

P202 **Janeen Braynen** <braynen@cshl.edu>
One-stop pan-genome browser for exploring the rich genetic diversity in maize

P203 **Yan Zhou** <yzhou86@iastate.edu>
Phenotyping and dissecting genetic variations of maize self-organized azimuthal canopy orientation and its impacts on light interception

P204 **Janeen Braynen** <braynen@cshl.edu>
Regulatory networks governing nitrogen use efficiency in maize and sorghum

P205 **Ruth Epstein** <rke27@cornell.edu>
Simulating breeding effect of recombination landscapes in maize.

P206 **Amanda Gilbert** <agilber@umn.edu>
Structural and functional properties of core and dispensable genes in maize

P207 **Taylor Strayhorn** <taylor.strayhorn@uga.edu>
Systematic exploration of transcription factor function in maize

P208 **Dior Kelley** <dkelley@iastate.edu>
Temporal and spatial auxin responsive networks in maize primary roots

P209 **Dhineshkumar Thiruppathi** <dthiruppathi@danforthcenter.org>
The classic maize mutant Rootless1 impairs in shoot-borne root formation and affects the root system architecture

P210 **Hanxia Li** <hl46161@uga.edu>
The effect of maize genotypes, enviroments, and GXE interactions on maize endophytes

P211 **Joseph Gage** <jlgage@ncsu.edu>
Variation in upstream open reading frames contributes to allelic diversity in protein abundance

Cytogenetics

P212 **James A. Birchler** <BirchlerJ@missouri.edu>
B chromosome induced ploidy variation in high loss lines

P213 **Liz Dominguez** <lizethd@illinois.edu>
Can sorghum ethanol production be increased with colchicine induced autotetraploid lines?

P214 **Hua Yang** <yanghu@missouri.edu>
Copy number variation analysis of the cis factor (region) required for the B chromosome non-disjunction in maize

P215 **Natalie Nannas** <njnannas@hamilton.edu>
Frequent spindle assembly errors require structural rearrangement to complete meiosis in Zea mays

P216 **Claire Milsted** <claire.milsted@gmail.com>
Further genomic and cytological investigation of RAD51 and BRCA2 genes in maize

P217 **Mateus Mondin** <mmondin@usp.br>
Satellites DNA evolution might be affected by their genomic position suggest analysis of the K180 and Cent-C sequences

P218 **Mingyu Wang** <mw36149@uga.edu>
Smd13 is a candidate adaptor protein that mediates the interaction between KINDR and knob180 repeats, facilitating meiotic drive of abnormal chromosome 10

Late Posters

P219 **Jialu Wei**
<jlwei@iastate.edu>

Investigating dynamic heat stress responses with diverse maize inbreds through transcriptome analyses

Plenary Speaker Abstracts

Invited Speaker 1 (@KBomblies) **Friday, April 1 10:50 AMCDT**

Learning to tango with four – problems and solutions to autopolyploid meiosis

(submitted by Kirsten Bomblies <kirsten.bomblies@biol.ethz.ch>)
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Many plant species, including maize, have polyploid ancestry. Polyploidy arises from genome duplication, either within species (autopolyploid) or coupled with hybridization between species or diverged populations (allopolyploidy). In all cases, polyploids face challenges early in their evolution, including the pairing and segregation of the now-multiple copies of each chromosome. Allo- and auto-polyploids solve this in apparently distinct ways, with allopolyploids generally evolving pairing preferences among the more similar chromosomes, while autopolyploids achieve diploid-like pairing even when they lack any pairing preference among homologs. While the mechanistic basis of allopolyploid meiotic adaptation is to some extent known, neither the actual problem, nor the molecular basis of the evolution of diploid-like pairing and segregation in autopolyploids, is well understood. We have undertaken genome scans for selection followed by cytological characterization and functional follow-up studies in the natural autotetraploid *Arabidopsis arenosa* and its diploid progenitor. We investigated what the problems actually are in neopolyploid meiosis, and how the natural tetraploids evolved a solution. We find evidence that adaptation to polyploid meiosis (and polyploidy more generally) is multigenic. At least eight core meiotic proteins, including components of cohesin, two axis proteins, and the central element of the synaptonemal complex, show strong evidence of selection in the tetraploid lineage. From functional follow-up of three genes to date, we show that different genes contribute both additively and non-additively, even at times perhaps antagonistically, to the evolution of polyploid meiosis. The road to adaptation was, apparently, neither straight nor smooth, and has interesting implications for better understanding the functional genetic architecture of multigenic adaptations.

Funding acknowledgement: Horizon2020-ERC, SNSF

Invited Speaker 2 **Friday, April 1 11:40 AM CDT**

The divining root: exploring adaptive responses to water using the grass root system.

(submitted by Jose Dinneny <dinneny@stanford.edu>)
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Water represents the most limiting resource for plant growth on earth and plant root systems have evolved mechanisms to search for and capture this resource from their soil environments. Through our exploration of the developmental responses of roots to the environment, we have identified several mechanisms by which the architecture of plant roots is shaped by the spatial distribution of water. In my talk I will illustrate two of these mechanisms that impact the architecture of grass roots, which are dominated by shoot borne roots, termed crown roots, and the secondary branches of roots, termed lateral roots. Crown root development occurs near the soil surface and is highly sensitive to the local availability of water in this region. In response to a watering event, crown root initiation is rapidly induced and leads to a flush of new root growth. When water becomes limiting, crown root growth is suppressed at the post-emergence stage, which allows plants to preserve water in the soil. We have recently identified a novel locus termed *CROWN ROOT DEFECTIVE* (*CRD*), which promotes crown root development under well-watered conditions in *Setaria viridis*. *CRD* homologues are present in many plants and in Arabidopsis also functions to promote root branching, suggesting that the pathway is conserved across flowering plants.Moisture can also pattern root branching at the micron scale through a process we termed hydropatterning, which allows roots to detect the spatial pattern of water across the circumferential axis of the root. In maize inbreds, extensive phenotypic variation exists for hydropatterning and we have used GWAS and TWAS to identify genetic loci contributing to this variation. Phenotypic variation in hydropatterning is a significant predictor of root system depth in field grown maize suggesting that characterizing the genetic basis for this variation will facilitate the breeding of root system traits.

Funding acknowledgement: Department of Energy (DOE)

Invited Speaker 3 **Saturday, April 2 10:50 AM CDT**

Harnessing the Ag data revolution for modeling plant and agronomic systems across scale

(submitted by Cranos Williams <cmwilli5@ncsu.edu>)
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The next revolution in precision agriculture solutions will require an improved understanding of the complex regulatory mechanisms that are instrumental in plant growth, development, and adaptation. Key in these efforts is the ability to acquire and analyze data across biological scales (from molecular to phenotypic scales). High-throughput data that have been collected across biological scales include molecular data such as gene expression profiles and confocal imaging to data capturing plant physiology such as hyperspectral imaging and remote sensing. The diversity of these datasets (in combination with the complexity of plant systems) has created opportunities to develop novel computational intelligence and machine learning approaches that are capable of modeling plant systems within and across biological scales. In this presentation, we provide a brief overview of approaches for analyzing various types of high-throughput biological data. These approaches address the many challenges associated with analyzing biological data, including the need to mitigate high variation and/or uncertainty in data, the need for novel segmentation and feature extraction, and the integration of disparate datasets for making causal inferences across scale. The application of these approaches has led to scientific contributions such as the modeling of key gene regulatory mechanisms involved in plant stress response, the identification of emergent properties that link molecular activity to phenotypic outcomes, and the development of automated high-throughput phenotyping approaches for early detection of plant diseases. The continued acquisition of high-throughput data across scale and the continued development of novel machine learning and modeling tools will provide opportunities to further push the boundaries of our understanding of plant systems and will be key to a better understanding of how plants respond to complex environments.

Funding acknowledgement: National Science Foundation (NSF)

Invited Speaker 4 (@PlantDevo) **Saturday, April 2 11:40 AM CDT**

Interactions between the parasitic plants and their hosts

(submitted by Neelima Sinha <nrsinha@ucdavis.edu>)
Full Author List: Sinha, Neelima R[1]; Jhu, Min-Yao[1]; Farhi, Moran[1]; Wang, Li[1]; Zumstein, Kristina[1]
[1] University of California, Davis

Parasitic angiosperms directly attach to host plants using specialized organs known as haustoria, which function as physiological bridges to extract nutrients and water from their hosts. While Cuscuta species (dodders) are common and agriculturally destructive flowering stem parasitic plants, others like *Striga* (witchweed) and *Orobanche* (broomrapes) attach to the roots of their hosts. Because of the intimate physiological connection between host plants and parasites, traditional herbicides and control methods are ineffective in controlling these parasites. Analyses of host parasite interactions at the level of developmental morphology and gene expression provide insights into the process of parasitism. We have explored techniques to module expression of key parasite and host upregulated genes and monitored their effects on parasitism. The mechanisms by which certain hosts mount parasite resistance has also been explored. Such studies may help develop a parasite-resistant system in crops to reduce economic losses in agriculture.

Funding acknowledgement: United States Department of Agriculture (USDA)

Invited Speaker 5 **Saturday, April 2 8:40 PM CDT**

Decolonizing Botany

(submitted by Banu Subramaniam <banu@wost.umass.edu>)
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[1] University of Massachusetts Amherst

What does it mean to be a feminist botanist? Drawing on recent interdisciplinary scholarship, I show how gender, race, class, sexuality, and nation shape the foundational language, terminology, and theories of modern botany, and how botany remains grounded in the violence of its colonial pasts. Decolonizing Botany reckons with these difficult origins and lays a roadmap to reimagine the practices of experimental biology.

McClintock Prize Abstract

McClintock Prize (M1) **Friday, April 1 8:10 PM CDT**

Plant natural product biosynthesis: An enigma no longer

(submitted by Robin Buell <Robin.Buell@uga.edu>)
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Plants and their natural products have been harnessed by humans for millennia for their health promoting activities, insecticidal and anti-microbial activities, as well as fragrance and food additive properties. These natural products, or specialized metabolites, have diverse functions in plants as pollinator attractants, defense and signaling molecules, and components of cell walls. The sheer diversity of specialized metabolites in plants is attributable to the dynamics of gene and genome evolution which are catalysts of species diversification. The advent of genomics technologies has revolutionized the discovery of the genes encoding specialized metabolites revealing a myriad of genome features and evolutionary mechanisms that underlie extant chemodiversity. For example, the ability to produce nepetalactone in catnip (*Nepeta* species), the chemical that induces euphoria in felines, arose via parallel evolution. Subsequently, at the population level, duplication and neo-functionalization of genes within the nepetalactone biosynthetic gene cluster yielded chemotypes of catnip with distinct profiles of nepetalactone stereoisomers. Interestingly, specialized metabolites are not only taxon-restricted but also can be frequently restricted to specific cell types such as trichomes. Recent access to single cell -omics technologies has revealed exquisite cell type specificity in the biosynthesis of monoterpene indole alkaloids in Madagascar perwinkle, a species still sourced for the key anti-cancer drug vinblastine. When coupled with other omics datasets, we are accelerating discovery of the regulatory and structural sequences involved in the biosynthesis, transport and storage of this complex specialized metabolite, a feat not imagined prior to the genomics era.

Funding acknowledgement: National Science Foundation (NSF)

Short Talk Abstracts

SESSION 1 – THE GENES THAT MAKE MAIZE
Chair: Erin Sparks Thursday, March 31. 7:00 PM – 9:00 PM CDT

T1 @_Nandan_Rai

Glossy15 overexpression alters Carbon partitioning and Nitrogen remobilization in maize stem

(submitted by Maruti Nandan Rai <mnrai@illinois.edu>)
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Glossy15, an AP2 type transcription factor, is crucial in controlling juvenile to adult phase transition in Maize. Transgenic overexpression of Glossy15 (Gl15-TG) promotes juvenile traits, delays the onset of flowering and increases number of nodes in the stem leading to increased stem biomass, and lower grain yield. We hypothesized that less remobilization of Carbon and Nitrogen from maize stem to ear shoot is the underlying reason of strong stem phenotype and lower grain yield in Gl15-TG lines. To address this, we employed a two-pronged approach and performed metabolomic and RNA-seq profiling of wild type and Gl15-TG maize lines across different developmental stages. Our work demonstrates that prolonged expression of Glossy15 not only delays vegetative phase change but also modulates Carbon partitioning and Nitrogen remobilization during stem development. Gl15-TG maize displayed prolonged anthesis-silking interval, higher amounts of simple sugars (glucose & fructose) and lower levels of cell wall polymers (lignin & xylan) compared to wild type maize. Amino acid profiling revealed increased accumulation of most amino acids in transgenic Gl15-TG stem internodes compared to wildtype maize. Finally, transcriptional profiling of stem internodes across developmental stages corroborated the observed modulation in metabolome of Gl15-TG line as well as revealed the gene regulatory networks associated with stem functions in maize. Overall, current work illustrates that the shoot maturation pathway influences the competence of remobilization of Carbon and Nitrogen metabolites in maize stem.

Funding acknowledgement: Department of Energy (DOE)

T2

A novel DNAJ-thioredoxin-like protein is required for carbohydrate partitioning in maize

(submitted by Singha Dhungana <srdm93@mail.missouri.edu>)
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[2] Division of Plant Science and Technology, Interdisciplinary Plant Group, and Missouri Maize Center, University of Missouri-Columbia, Columbia, MO 65211

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 exhibit reduced plant growth, chlorotic leaves, and hyperaccumulation of soluble sugars and starch in mature leaves. Intriguingly, the mutant leaves exhibit a unique crossbanding pattern of alternating chlorotic and green regions. We recapitulated the chlorotic leaf phenotype by growing mutant plants under high daytime temperature. By using polymorphic DNA 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 domains 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. Expressing a translational fusion of CPD13 to a red fluorescent protein in tobacco leaves localized the protein to the endoplasmic reticulum, consistent with a potential chaperone function. We hypothesize that the carbohydrate hyperaccumulation in mutant leaves is due to the inability of the defective CPD13 protein to properly interact with or process target proteins. Ongoing studies to examine the subcellular localization of fluorescent reporter proteins, and their aggregation, in response to heat and other stresses are currently testing this hypothesis and will help elucidate the function of this novel protein in carbohydrate partitioning. Funding provided by a grant from NSF PGRP to DMB (IOS-1025976).

Funding acknowledgement: National Science Foundation (NSF)

T3 @KhanguraRajdeep

The maize semi-dominant lesion mutant *Bella fleck1* provides resistance to common rust and Physoderma brown spot

(submitted by Rajdeep Khangura <rkhangur@purdue.edu>)
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Lesion-forming (*les*) mutants of maize produce spontaneous lesions or speckles on the leaf blade and sheath in the absence of pathogens. These mutants are distinguishable by their unique lesion morphology, color, and lesion progression. Over 30 *les* mutant complementation groups have been recovered but only a small number have been molecularly identified. Known molecular causes of lesion formation in maize include disruption of porphyrin metabolism and chlorophyll degradation, or auto-active NBS-LRR proteins. In all *les* mutants that have been characterized, lesion severity is positively correlated with leaf age. The novel semi-dominant lesion mutant *Bella fleck1* (*Bfl1*), on the other hand, forms white flecks in newly emerging leaves, but lacks the progressive increase of lesion severity with leaf age that characterizes all other *les* mutants of maize. In addition to leaf flecking, *Bfl1* mutants are stunted, exhibit suppressed nodal root growth, and show high lodging incidence. This mutant is resistant to two fungal diseases: common rust (caused by *Puccinia sorghi*) and Physoderma brown spot (caused by *Physoderma maydis*). Consistent with these observations, we found that both gene expression and metabolite accumulation in *Bfl1* mutants are similar to those of *Rp1-D21*, an autoimmune lesion mutant of maize that encodes an NBS-LRR protein. These similarities include the high accumulation of defense responsive metabolites and transcripts, indicating that *Bfl1* may function in plant immunity. We mapped *Bfl1* to the long arm of chromosome 2. Association mapping of modifiers of *Bfl1* using F1 hybrids between a maize association panel and *Bfl1* identified a likely cis-acting modifier allele in the region encoding *Bfl1*. The molecular identity of the gene underlying *Bfl1* and its natural modifier is currently being investigated.

Gene / Gene Models described: *rp1*; Zm00001d023325
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

T4

Strigolactones regulate domestication phenotypes of cupule architecture, kernel size, and their coordination via *Tga1*-dependent and -independent networks.

(submitted by Jiahn-Chou Guan <guanjc@ufl.edu>)
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[4] Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, The Netherlands
[5] United States Department of Agriculture - Agricultural Research Service, Columbia, MO, U.S.A.

The remarkable transition from ancestral grass to modern maize required the pivotal formation of a cob, which arose from restructuring tissues that otherwise enveloped each kernel with a hard fruitcase. The fruitcase-to-cob conversion is shown here to involve previously unrecognized input from strigolactones (SLs) that act in part by mediating known roles of a domestication gene for *Teosinte glume architecture 1* (*Tga1*). Potential impacts of SLs were first evident in the striking, primitive-ear phenotype of an SL-deficient *carotenoid cleavage dioxygenase 8* (*ccd8*) maize mutant and its commonalities with that of the recessive pre-domestication allele (*tga1*) of *Tga1*. Both showed distinctive, partial encasements of kernels by protective maternal tissues. The SL-specific phenotype was confirmed by i) transposon reversion of a *ccd8* mutant, ii) phenotypic rescue using a synthetic SL analog, and iii) recapitulation with SL-signaling mutants. Genetic analysis uncovered an increasingly primitive ear morphology in combinations of recessive *tga1* and *ccd8*. The SL-deficient *ccd8* markedly enhanced reversion of tissues imbedded deep in ears of modern maize to cupules partially encasing kernels (a primitive, teosinte-like architecture). Shared modes of action for *tga1* and *ccd8* were apparent in single-allele transcriptomes, where overlap included over 50% of genes differentially expressed in ears. Underlying mechanisms of SL action were revealed by a combination of yeast two-hybrid and *in-planta* assays for binding by TGA1 to both the SL receptor (D14) and D53 components in the SL-signaling network. Impacts of SLs extended still further to seed size in maize, as well as the coordination of growth between kernels and protective maternal tissues. Collective data show that SLs operate through both *Tga1*-dependent and -independent networks. Together these SL-responses release kernels from protective maternal encasement, coordinate increases in kernel size, and mediate a distinctive conversion of cupule architecture to that of modern maize.

Gene / Gene Models described: *CCD8, D14a, D14b, D53, Tga1*; Zm00001d043442, Zm00001d028094, Zm00001d048146, Zm00001d023208, GRMZM2G101511
Funding acknowledgement: National Science Foundation (NSF-IOS-PGRP-1748105)

T5 @mila_leonie

Experimental evolution in maize with replicated divergent selection identifies plant-height associated SNPs

(submitted by Mila Tost <mila.tost@agr.uni-goettingen.de>)
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Experimental evolution studies are common in agricultural research, where they are often deemed "long term selection". A challenge of previous experimental evolution studies in agriculture has been the specification of robust significance thresholds, since long term selection studies are rarely replicated. Usually significance thresholds in long term selection experiments are based on the empiric distribution or drift simulations. These significance thresholds are prone to either miss true positives or include false positives. Under laboratory conditions, replicated selection has been shown to allow accurate significance threshold specification in species such as Drosophila melanogaster, and yeast. In this study, we conducted divergent replicated selection for short and tall plant-height in a random mating maize population under real field conditions. Selection persisted for three generations, providing a total of 4 temporal subpopulations. Selected sites were identified based on F_{ST} between the subpopulations selected in opposite directions. Significance thresholds were specified using the false discovery rate (FDR) for selection, which leverages our replicated experimental evolution design. When compared to thresholds based on drift simulations or the empiric distribution, we found that the FDR for selection identifies selected sites with much higher reliability. Overall, we found 41 significant markers putatively influencing plant height, including candidates extremely close to or within the coding region of the cloned plant-height genes *Dwarf1* and *iAA8*.

T6 @jon_cahn

Maizecode: DNA regulatory elements in maize and teosinte inbreds provide insight into maize domestication

(submitted by Jonathan Cahn <jcahn@cshl.edu>)
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Early maize lines were domesticated from *Teosinte parviglumis (Z.mays parviglumis)*, with subsequent introgressions from neighboring *Teosinte mexicana*. Domestication traits in modern maize include increased kernel row number, loss of the hard fruit case and dissociation from the cob upon maturity, as well as fewer tillers. Molecular approaches have identified several transcription factors involved in the development of these traits. However, these studies have also shown that a more complex regulatory network is responsible for these strong morphological differences than originally hypothesized, and our understanding of the tissue-specific regulation as well as of its variability across inbred lines is still lacking. In this study, we investigate the transcriptional regulation that resulted from the domestication process, focusing on conservation and variability across multiple tissues and inbred lines. We generated histone-modification and transcription factor ChIP-seq in parallel with transcriptomics datasets in up to 5 different tissues of 3 inbred lines which span the phenotypic diversity of maize inbreds, as well as the teosinte inbred TIL11. We developed an automatized computational pipeline to integrate these datasets as well as publicly available data. This pipeline generates metrics and outputs for both quality control and functional analyses and it can also be applied to other species. We identified regulatory regions that emerged during the domestication process and are responsible for the tissue-specific expression of developmental genes. We show that, even though pollen grains are the most differentiated tissue on a transcriptomic level, and especially with respect to the regulation of transposable elements, ears show the least conservation, corroborating the very distinct morphological and physiological differences between maize and teosinte. With this study, we hope to provide the maize community with a framework for a collaborative effort that follows the footsteps of the ENCODE project in order to better understand and potentially improve the regulatory landscape of the maize genome.

Funding acknowledgement: National Science Foundation (NSF)

T7

Gene networks underlying architectural pleiotropy guide genome-wide association studies to highly connected regulators of tassel and leaf development

(submitted by Andrea Eveland <aeveland@danforthcenter.org>)
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An early event in organogenesis is establishment of a boundary between the meristem and differentiating lateral organ. Several lines of evidence suggest a common gene network functions at boundaries of distinct lateral organs and contributes to pleiotropy between leaf angle (LA) and tassel branch number (TBN), two important agronomic traits. Here, we leverage a panel of classical maize mutants with developmental defects in one or both traits to investigate network plasticity around pleiotropic loci (e.g., *liguleless2*), and identify new factors contributing to regulation of LA and TBN. RNA-seq profiles were generated from precisely-staged tassel primordia that capture developmental transitions during branch initiation and outgrowth for nine mutants and B73 controls, and from sequential immature shoot sections enriched for developing ligule. We integrated transcriptome data into tissue-specific co-expression and gene regulatory networks based on transcription factor (TF)-target predictions and identified conserved and divergent network modules, including rewiring around key developmental regulators. By subsetting SNP markers based on gene sets derived from co-expression modules and network motif analyses, we can explain a substantial portion of the heritability for LA and TBN in association studies. Using a genomics selection approach, we selected ~1200 inbred lines from the Ames and Goodman-Buckler association panels that maximized diversity in LA and TBN, and phenotyped these traits in the field. Multi-trait GWAS using LA and TBN phenotype values with SNP subsetting based on network analyses identified high-confidence SNP-trait associations, including in uncharacterized TFs that were highly connected in the networks and within motifs involving known developmental homeobox genes. Further using the maize networks to subset SNPs in sorghum, we observed SNP-trait associations for LA in orthologs of highly connected maize TFs. Our analyses support utility of high-resolution, context-specific networks for guiding GWAS, and provide insights into re-use of pleiotropic TFs in different developmental contexts through network rewiring.

Funding acknowledgement: National Science Foundation (NSF)

T8 @citogenetica

A knob variant sequence and its relatednesses with maize evolutionary history

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Maize evolution was developed by gene analysis, and little attention was given to satellite DNA sequences. A K180 knob variant showed important sequence features allowing connection to the ancestors throughout the evolutionary history. The central question that drove the study was the origin of this knob variant motif departing from the B73 inbred line to the most remote wild species related to maize. A sequence mining was carried out in B73, Mo17, Z. mays spp. mexicana, Zea luxurians, Z. diploperennis, Tripsacum dactyloides, Coix aquatica, and C. lacryma-jobi genomes to verify the existence of the K180_2. The K180_2 motifs were individually analyzed, comparing their structure and nucleotide composition to the original K180. They differ mainly in the second half of the motif sequence, being the first half well-conserved. The K180_2 appeared in all Zea species and Tripsacum but not in Coix. At the same time, the original K180 is present in all species analyzed, and it is well conserved. In Tripsacum, it was possible to map the origin of the K180_2 by a series of motif rearrangements. The results suggest that K180_2 arose during the K180 motif blast in T. dactyloides and was maintained in the Zea species. The origin point of the K180_2 showed that beyond the genes, satellite DNA sequences are also important markers to understanding the genome behavior during species divergence and evolution, bringing interesting insights about maize history.

T9 @SigmaFacto

Differences in activity and stability drive transposable element variation in tropical and temperate maize

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Eukaryotic genome size varies extensively within and between species. Much of the interspecific variation has been attributed to transposable elements (TEs). The extent of TE variation within species is less known. Here we analyzed the TE content of 26 high-quality maize genomes and revealed an excess of 42.4 Mb of TE sequences per genome in tropical maize relative to temperate maize. A small number of TE families, mainly LTR retrotransposons, drive these differences. Evidence from the methylome, transcriptome, LTR age distribution, and LTR insertional polymorphism revealed that 64.7% of the variability was contributed by LTR families that were young, less methylated, and more expressed in tropical maize, and 18.5% was driven by LTR families with removal or loss in temperate maize. This study demonstrates the important role of TEs in driving genomic variation within species via amplification and purging of TEs.

Funding acknowledgement: National Science Foundation (NSF)

T10 @AmanArora_7

Hypothesis-driven identification of alleles affecting known pathways using a transcript accumulation index

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The assignment of molecular identity and causality for a quantitative trait from observations of phenotypic variation remains a major challenge in biology. Often loci are formally identified in a chromosomal region, but the molecular identity(ies) of the causal loci are unclear. Prior knowledge of gene functions and interactions can facilitate the identification of candidates and causal variants responsible for a phenotype. Expression-based genome-wide association studies (eGWAS) bypass some of this complexity by identifying SNPs linked to natural variation in the accumulation of a single transcript. Cis-acting polymorphisms at the gene itself are straightforward to identify, for example. But these studies provide limited insight into phenotypic consequences or pathway impacts. Here we demonstrate an index-based GWAS approach that turns gene expression patterns into a hypothesis test of SNP-to-pathway association. A pathway-level phenotype, a coordinated change in a group of genes, should help us interpret expression level changes and their phenotypic impacts in a biologically meaningful way. We captured the gene expression signatures associated with a series of biological processes and used these to construct unique quantitative trait values called an index. The index provides a measure of activation state for each pathway. By incorporating prior knowledge about gene function or response, an index turns a GWAS result into a physiologically-anchored 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 indices from gene expression studies of brassinosteroid (BR) treated maize seedlings. GWAS identified alleles in genes responsible for BR 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.

Funding acknowledgement: Department of Energy (DOE)

T11

Unbiased RNA and protein co-expression networks highlight important regulatory role of coordinated organelle protein homeostasis in maize heterosis

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Heterosis, or hybrid vigor, is the difference in vigor between a hybrid and the average of its parents. Crops and livestock have been bred as hybrids for decades for increased vigor from hybridity, yet its underlying molecular mechanism remains elusive, and the ability to control heterosis will have great impact in agriculture. Our recent published work showed that the abundance of photosynthesis related protein complexes are elevated in the hybrids relative to mid-parent levels, suggesting greater photosynthetic capacity. Yet no connections were made with other pathways that could explain this observation. To identify key drivers of heterosis, we constructed RNA and protein co-expression networks from transcriptomics and proteomics data of maize hybrids by Weighted Gene Co-expression Network Analysis (WGCNA). Both networks revealed a set of organelle protein homeostasis (proteostasis) candidates with high positive correlations to a key heterotic trait, plant height heterosis (hybrid/mid-parent plant height). Proteostasis candidates included proteases, chaperones, chloroplast translocons (TOC/TIC) components, proteasome subunits and more, many of which are upstream and indispensable for photosynthesis. Increased abundance of photosynthesis protein complexes may be explained by higher protein folding and degradation efficiency from elevated expression of proteostasis machinery subunits in the hybrids. We found that bzip60, an unfolded protein response (UPR) hallmark in plants, was negatively correlated with heterosis. Comparison of transcript levels showed that most hybrids had below mid-parent levels of bzip60, and highly heterotic hybrids expressed bzip60 below low-parent levels. This suggested that lower levels of ER and cytosolic proteostasis complexes may render inbreds to protein aggregation and growth defects. Our results suggest a role for proteostasis in hybrid vigor.

Gene / Gene Models described: *bzip60*; Zm00001d046718
Funding acknowledgement: National Science Foundation (NSF)

T12

Heritable regulatory states dependent on parental RNA polymerase IV contribute to hybrid vigor

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Paramutation is a behavior in which one parental allele facilitates a meiotically heritable regulatory change at the other. This behavior occurs at specific alleles of multiple maize loci - including *purple plant1* (*pl1*) - encoding transcriptional activators of flavonoid biosynthesis. The *Pl1-Rhoades* (*Pl1-Rh*) allele can exist in a highly expressed reference state (*Pl-Rh*) or a repressed paramutant state (denoted *Pl'*). *Pl'* states often revert to *Pl-Rh* in *rmr6-1* mutants deficient for 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 (24nt) RNAs and conditions the heritable regulatory status of *Pl1-Rh*, we hypothesize that RNAP IV, and potentially small RNAs (sRNAs), define the heritable regulatory status of other genes. To test this idea, seedling RNA-seq and corresponding sRNA-seq profiles of heterozygous BC_5 progeny from sibling *rmr6-1* mutant and *Rmr6-B73* / *rmr6-1* fathers were compared to identify heritable RNAP IV-dependent effects. We found the absence of paternal RNAP IV significantly altered the RNA abundances of 140 genes. A significant overlap was seen with differentially expressed genes identified in a separate *rmr6-1* mutant RNA-seq analysis, further implicating RNAP IV in intergenerational regulation. Additionally, normalized read abundances of 711 24nt sRNA clusters were significantly distinct. We discovered that a significant number of upregulated genes overlap with downregulated 24nt sRNA clusters within 100, 50, and 10 kilobases upstream or downstream. These overlaps point to other epialleles, like *Pl1-Rh*, whose regulatory reprogramming in the absence of RNAP IV persists through meiosis. Extending our comparisons to B73/Mo17 and Mo17/B73 hybrids, field trial results show that absence of RNAP IV function in B73 parents diminishes heterotic traits. Because parental RNAP IV positively contributes to heterosis, we infer that the intergenerational regulatory variation defined by RNAP IV is of significant agronomic importance.

Gene / Gene Models described: *pl1, rmr6*; Zm00001d037118, Zm00001d031457 / Zm00001d031459
Funding acknowledgement: National Science Foundation (NSF)

T13

A rapid and simplified transformation and genome editing method for maize inbred B104 using *Agrobacterium* ternary vector system and immature embryos

(submitted by Minjeong Kang <mjkang@iastate.edu>)
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Maize genetic transformation is a critical technology for maize genomic studies as well as trait improvement. As a recalcitrant plant species, only a limited number of maize genotypes such as Hi Type II, A188 and B104 are amenable for genetic transformation. B104 is an attractive public inbred for transformation because it shares a high genetic similarity with B73, an important public inbred serving as the reference genome. Although the transformation of maize inbred B104 has been developed, it is not broadly applicable in academic lab settings due to labor-intensive and time-consuming procedure (Frame et al., 2006; Raji et al., 2018). Here we described an improved B104 transformation and genome editing protocol using *Agrobacterium* ternary vector system and CRISPR/Cas9. The original B104 transformation protocol requires about 120 to 160 days to produce rooted transgenic plants from the starting day of transformation experiment. The improved protocol described here reduces the transformation process to about 50 days, which saves about 70 to 110 days. Using this protocol, the T0 plants can be obtained with an average of 6.6% transformation frequencies (the number of transgenic T0 plants per 100 infected embryos). Transgenic T0 events containing Cas9 cassette targeting *Glossy2* gene showed over 66% of indel frequency at the cleavage site. We expect that this simplified and improved B104 transformation protocol can be readily transferrable to many academic groups that desire to set up the maize genetic transformation and CRISPR/Cas-mediated genome editing for fundamental and applied research. Frame et al. (2006) Improved Agrobacterium-mediated transformation of three maize inbred lines using MS salts. Plant Cell Rep., 25, 1024-1034. doi.org/10.1007/s00299-006-0145-2 Raji et al. (2018) "Agrobacterium-and biolistic-mediated transformation of maize B104 inbred," in Maize: Methods and Protocols, ed. L. M. Lagrimini (New York, NY: Springer, USA), 15-40. doi: 10.1007/978-1-4939-7315-6_2

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), State of Iowa funds, Crop Bioengineering Center of Iowa State of University, Plant Transformation Facility at the Donald Danforth Plant Science Center

T14 @baoxing_song

AnchorWave: sensitive alignment of genomes with high diversity, structural polymorphism and whole-genome duplication variation

(submitted by Baoxing Song <bs674@cornell.edu>)
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Millions of species are currently being sequenced, and their genomes are being compared. Many of them have more complex genomes than model systems and raise novel challenges for genome alignment. Widely used local alignment strategies often produce limited or incongruous results when applied to genomes with dispersed repeats, long indels, and highly diverse sequences. Moreover, alignment using many-to-many or reciprocal best hit approaches conflicts with well-studied patterns between species with different rounds of whole-genome duplication. Here, we introduce Anchored Wavefront alignment (AnchorWave), which performs whole-genome duplication–informed collinear anchor identification between genomes and performs base pair–resolved global alignment for collinear blocks using a two-piece affine gap cost strategy. This strategy enables AnchorWave to precisely identify multikilobase indels generated by transposable element (TE) presence/absence variants (PAVs). When aligning two maize genomes, AnchorWave successfully recalled 87% of previously reported TE PAVs. By contrast, other genome alignment tools showed low power for TE PAV recall. AnchorWave precisely aligns up to three times more of the genome as position matches or indels than the closest competitive approach when comparing diverse genomes. Moreover, AnchorWave recalls transcription factor–binding sites at a rate of 1.05- to 74.85-fold higher than other tools with significantly lower false-positive alignments. AnchorWave complements available genome alignment tools by showing obvious improvement when applied to genomes with dispersed repeats, active TEs, high sequence diversity, and whole-genome duplication variation.

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

T15

Coordinated gene upregulation in maize through CRISPR/Cas-mediated enhancer insertion to improve nitrogen use efficiency

(submitted by Hannes Claeys <hclaeys@inari.com>)
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Inari is designing seeds to help address one of the greatest challenges of our times: growing enough nutritious calories for an increasing population while reducing the footprint of agricultural production on the environment. Our platform integrates predictive design and advanced multiplex gene editing tools to develop resilient seeds that will require fewer natural resources and inputs. As part of this platform, we developed a unique technology to precisely and efficiently insert small native elements into maize gene promoters using CRISPR/Cas, creating lines with stable and heritable edits. This capability extends traditional uses of CRISPR/Cas, which typically lead to knock-out or knock-down, but rarely to increased or specific expression of editing targets. We used these methods to insert a small native enhancer element across a wide array of target genes, leading to reliable overexpression. Our technology can easily be multiplexed to upregulate at least four genes in a single line. Little variation has been observed between different lines, thereby providing a number of benefits over traditional transgene-based overexpression. The technology was used to upregulate genes involved in nitrogen use efficiency, leading to observable phenotypes as a proof of concept. Insertion of enhancers or other elements can be combined with knock-out or knock-down of other targets, allowing for sophisticated trait engineering.

T16 @tross_michael

Data driven trait quantification across a maize diversity panel using hyperspectral leaf reflectance

(submitted by Michael Tross <mtross2@huskers.unl.edu>)
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Changes in hyperspectral reflectance patterns is associated with a wide range of plant properties. Estimating plant traits from hyperspectral reflectance data typically involves collecting hyperspectral reflectance data from a large set of samples and, for a subset of the same samples, then collecting expensive and/or labor-intensive trait data using ground truth methods. Here we explore the potential of data driven approaches for analyzing hyperspectral reflectance data which may not require the collection of ground truth phenotypic measurements. A set of 752 maize inbred lines were grown in a randomized complete block design with two replicates and hyperspectral reflectance data was collected from the leaves of plants in each replicate. This produced a high dimensional dataset consisting of the intensities of 2,151 distinct wavelengths measured across 1658 distinct samples. Auto-encoder and principal component analyses were used to reduce the dimensionality of this high dimensional dataset to either 10 principal components or 10 autoencoder latent variables. A substantial proportion of total variance in these variables was explained by genetic factors. Both auto-encoder latent variables and principal components were correlated with molecular traits. The most highly correlated trait with either latent variables or principal components was chlorophyll content. One of the latent variables and principal component 2 were the most correlated with chlorophyll content. However, the relevant latent variable proved to have a much stronger correlation with chlorophyll content (LV8; $R^2 = 0.59$) than the most correlated principal component (PC2; $R^2 = 0.31$). Genome wide association studies were able to identify specific genetic loci in the maize genome associated with variation in latent variables. Future work is currently being undertaken to condition latent variables on numerous traits. This would allow for latent variables that better explain phenotypic variation for traits of interest.

Funding acknowledgement: NIFA-HIPS

T17 @oconnord

A tunable increase in maize kernel row number using DNA base editing

(submitted by Devin O'Connor <doconnor@pairwise.com>)
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The advent of new genome editing tools has enabled the controlled deployment of mutation for crop improvement. However, despite evidence that nucleotide polymorphism is far more common in natural and crop populations than insertions or deletions (indels), the most commonly utilized genome editing tools result in indels from double stranded breaks and error-prone DNA repair. For many crop traits, indels may be too severe or imprecise to achieve the desired trait effect. DNA base editors, which mediate the targeted conversion of nucleotides without causing indels, offer a new opportunity to generate targeted nucleotide polymorphism for crop improvement. We used both cutting and base-editing CRISPR tools to create tunable phenotypic variation in the key maize yield-component trait, Kernel Row Number (KRN). We show that variation at a single conserved amino acid in the predicted FASCIATED EAR2 (FEA2) protein provides a spectrum of kernel row numbers across environments. DNA nuclease cutter-derived deletion alleles in *fea2* resulted in ear fasciation and were too severe to advance to field testing. In contrast, conversion of FEA2 amino acid P477 to F,S,T, or V resulted in a controlled, allele-dependent increase in KRN without fasciation in both controlled environment and multiple field trials. This tunable response is important for trait deployment in commercial agriculture because it could mitigate pleotropic or compensatory phenotypes such as severe fasciation, decreased ear length, plant height, and kernel size. Our results support the utility of base editing for crop improvement and demonstrate the potential for new FEA2 alleles to significantly increase corn yield.

Gene / Gene Models described: *fea2*; Zm00001eb184050

T18

From discovery to smallholder farmers: Ms44 and its 45-year journey to Africa

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While attending graduate school in Dr. Ron Phillips' lab at the University of Minnesota, I helped characterize an EMS mutagenized, M1 maize plant that was not shedding pollen, concluding that it was a dominant mutation that resulted in male sterility. I named the mutant Ms44. Thinking that this was an interesting mutant and that there must be some way it could be useful, I was allowed to take the mutant with me first to Iowa State University and then to Pioneer Hi-Bred. At Pioneer, we eventually cloned the gene and proposed the causal mechanism for sterility. As field trials showed that Ms44 mutants were more productive than their fertile sibs, we initiated studies to determine if commercial scaling was feasible. We had previously developed a hybridization platform called Seed Production Technology (SPT) that enabled the production of nearly 100% male-sterile, non-transgenic progeny from an engineered heterozygous nuclear male-sterile maintainer line that could be used in hybrid seed production. The system is agnostic relative to whether male sterility is recessive or dominant. This meant we could produce homozygous, dominant male-sterile plants, something previously not possible, as well as heterozygous, dominant male-sterile plants. We recognized that these capabilities would be particularly well-suited for producing 3-way hybrids that are commonly sold in Africa. Subsequently, a collaboration among private, public, and government research institutions to deliver this technology to produce high quality, modern maize hybrids for smallholder farmers was developed and funded. I will discuss how engagements with country regulators, government research institutions, and local smallholder farmers have led to the acceptance of this technology for the benefit of smallholder farmers.

Funding acknowledgement: Corteva Agriscience, Bill and Melinda Gates Foundation

SESSION 8 – CELLULAR PROCESSES
Chair: Todd Jones Saturday, April 2. 7:00 PM – 8:20 PM CDT

T19 @plantsdonttweet

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

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The orientation of cell division in plants is critical for proper growth and development. In plants, a microtubule structure called the phragmoplast promotes formation of the cell plate while expanding from the center of the cell towards the division site on the cell cortex. It is unknown how the phragmoplast accurately reaches the division site. Here, we show that microtubules that accumulate at the cell cortex at the telophase transition contribute to positioning of the phragmoplast in maize leaf epidermal cells. Before the phragmoplast reaches the cortex, cortical telophase microtubules transiently pause at the division site near the division site localized protein TANGLED1. Microtubule plus-end pausing of the cortical telophase array at the division site results in orientation of the cortical array perpendicular to the division site. As the phragmoplast expands at the cell cortex, the cortical telophase array is incorporated into the phragmoplast by parallel bundling. Asymmetric accumulation of the cortical array is associated with changes to phragmoplast angle. These data provide new insight into the mechanism by which cortical telophase microtubules and division site proteins fine-tune the positioning of the phragmoplast and the final division plane.

Gene / Gene Models described: *TAN1*; Zm00001d038060
Funding acknowledgement: National Science Foundation (NSF)

T20

A Receptor-Like Proteins PAN2 is required for ABA and dark-mediated grass stomatal closure

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Stomata are small pores on the leaf surface of land plants that facilitate gas exchange. Grass stomata have fast stomatal responses, which may be associated with the dumbbell-shaped guard cells and lateral subsidiary cells. Subsidiary cells are thought to reciprocally exchange water and ions with guard cells. However, the relative contribution of subsidiary cells and the mechanisms of how they contribute is unclear. To untangle the role of subsidiary cells in stomatal closure, we measured stomatal function in *pan1*, *pan2*, and *pan1pan2*, mutants. PAN2 and PAN1 are receptor-like proteins important for correct subsidiary cell formation. In *pan1* or *pan2* single mutants, ~25% of the stomata are abnormal in juvenile leaves, but <5% are abnormal in adult leaves. We measured gas exchange in juvenile and mature leaves of *pan1*, *pan2* and *pan1*;*pan2* mutants to determine if correct subsidiary cell formation was required for stomatal function. Both juvenile and adult leaves from pan2 and *pan2*;*pan1* show slower stomatal closing dynamics in the dark, relative to controls. However, even though *pan1* and *pan2* have similar numbers of defective subsidiary cells, *pan1* gas exchange dynamics were similar to B73. This suggests that PAN2 has a role in stomatal function independent of subsidiary cell function. To determine if stomatal function of individual stomatal complexes is dependent on correct subsidiary cell specification, we measured stomatal aperture in different genotypes plants treated with ABA. In all genotypes, stomata with abnormal subsidiary cells show closure defects in response to ABA. **This is direct evidence that correct subsidiary cells are essential for closing the stomatal pore**. Together, our results indicate that PAN2 is critical for stomatal function and regulation of stomatal aperture, even in normally formed stomata of mature leaves. PAN1, is important for stomatal complex formation, does not seem as crucial for function of normal stomata.

Gene / Gene Models described: *pan1, pan2*; GRMZM5G836190, GRMZM2G034572
Funding acknowledgement: USDA Hatch Grant MAS00570

T21 @Xiaosa_Xu

A comprehensive single-cell atlas of plant shoot meristems facilitates functional analysis

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Single-cell technology has boosted plant development studies in recent years yet still faces many challenges, such as capturing rare stem cells, cross-species comparisons, and integrating multi-modal single-cell data. We constructed a comprehensive single-cell atlas of plant shoot meristems, with over 100,000 cells across diverse species, including *Arabidopsis* and Maize, to tackle these challenges. In specific, we finely dissected the apices of developing inflorescences of *Arabidopsis* and maize and captured stem cell populations that were largely missed in previous plant shoot single-cell studies. We found conserved cell types between the two species, such as stem cells and lateral organ primordia. Besides, we observed a significant overlap of stem cell markers between *Arabidopsis* and maize by constructing cross-species co-expression analysis at single-cell resolution. Plant stem cells are maintained by a conserved CLAVATA-WUSCHEL (CLV-WUS) feedback pathway. We thus also profiled single cells from the inflorescence apices of mutants of crucial regulators in CLV-WUS pathway. We found hundreds of differential expressed genes (DEGs) in the stem cells by comparing these mutants with wild type. We used CRISPR/Cas9 to knock out selected DEGs in a family of sugar kinases, and found a striking meristem termination phenotype, validating the predictive power of our single-cell atlas. In addition, we further finely profiled diverse meristem types for both developing maize ear and tassel. We found that a similar developmental program was re-used in different meristem types across the two reproductive organs. Finally, we conducted a multi-modal and integrative analysis of single-cell RNA-seq and ATAC-seq for developing maize ear and tassel to query chromatin accessibility and gene expression. We uncovered lists of accessible chromatin regions significantly associated with gene expression across cells for both ear and tassel. Together, this comprehensive plant shoot meristem single-cell atlas will be a valuable resource for the maize community to inform functional studies at a fundamentally new level.

Funding acknowledgement: National Science Foundation (NSF)

T22

Revealing Gene Regulation of Cuticular Wax Biosynthesis Using Artificial Transcription Factors

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Cuticular wax is a hydrophobic barrier for protecting the epidermis of land plants. Many genes involved in cuticular wax biosynthesis are referred to as glossy genes as mutants display a glossy leaf phenotype. Expression data from diverse tissues of maize indicated that glossy genes are generally active in young leaves, silks, and tassels and largely inactive in seeds and roots. Gene co-expression network analysis showed that most known glossy genes were tightly clustered, indicative of co-regulation. To understand gene regulation of cuticular wax biosynthesis, we activated *glossy3* (*gl3*), encoding a MYB transcription factor, by designer Transcription Activator-Like effectors (dTALEs) delivered by the bacterium of genus *Xanthomonas*. Through RNA-seq, we found that eight of nine known glossy genes were up-regulated by activation of *gl3*, implying that *gl3* is a master regulator of the cuticular wax pathway. Genes co-expressed with the eight known glossy genes and co-regulated by *gl3* are candidates for new glossy genes. A chemically induced mutant of one new candidate gene, Zm00001d017418, encoding aldehyde dehydrogenase, exhibited a typical glossy leaf phenotype. In summary, we have established a straightforward approach for understanding gene regulation in maize, which, along with co-expression transcriptomic analysis, is effective to identify a promising candidate gene set for cuticular wax biosynthesis.

Gene / Gene Models described: *gl3*; Zm00001d017418
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

T23 @AlonsoFlagella

Ancient Roots: Disruption and rewilding of the Zea rhizosphere microbiome

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Feeding a growing world population amidst global change requires reducing the resource use and environmental impacts of agricultural production. A potential solution to this challenge is harnessing microbiome-associated phenotypes (MAPs) and incorporating them into modern agriculture. Plant genetics play a significant role in shaping the microbiota, yet little work has been done to understand the consequences of microbial selection for ecosystem function. Here we use the expansive genetic variability within the *Zea* genus, which comprises an ecologically diverse collection of plant varieties including domesticated maize and its closest wild relative, teosinte, to understand the relationship between host genetics and the functional rhizosphere microbiome. We started this work by constructing and characterizing the microbiome of a maize chronosequence of elite inbred varieties developed from 1949 to 1986. This study indicated that the chronological development of high-yielding varieties and agronomic management approaches of industrial agriculture inadvertently modified interactions between maize and its *functional* microbiome. Next, we looked further back in time and examined how maize's wild progenitor, teosinte, differed from our modern varieties concerning microbial interaction. In the greenhouse and field, this comparison showed that inbred and wild maize differ dramatically in their interaction with N-cycling microorganisms in the rhizosphere. Specifically, we found that genetic variation in maize altered potential nitrification, incomplete denitrification, and overall denitrification rates. Finally, to assess the viability of this phenotype for crop improvement and to further dissect the genetic basis of these ancestral MAPs, we used teosinte-maize near-isogenic lines (NILs). From this teosinte-maize NIL population, we identified several candidate genetic regions that drove major alterations to the root zone microbiome composition and N-cycling function. The takeaway of this research is that genetic elements originating from maize's wild progenitor teosinte can be used to "rewild" the modern maize microbiome interaction towards agricultural improvement.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Illinois Nutrient Research & Education Council

T24

A tale of two selection methods for resistance to Fusarium ear rot and fumonisin in maize

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Fusarium verticillioides is a common maize fungus that causes Fusarium ear rot (FER) and produces fumonisin (FUM), a mycotoxin associated with various diseases in humans and animals that consume contaminated grain. Phenotypic selection (PS), the primary method of breeding resistance to ear rots and mycotoxins in maize, is hindered by the cost and time required to obtain accurate evaluations of FER and FUM content. Genomic selection (GS) is an alternative method that could reduce that cost and time. To our knowledge, no empirical studies of the relative effectiveness of GS compared to PS for these traits have been reported in maize. This study empirically compared GS and PS for resistance to FER and FUM contamination over multiple selection cycles. Three intermating generations of recurrent GS were conducted in the same time frame and from a common base population as two generations of recurrent PS. Lines sampled from each GS and PS cycle were evaluated for FER and FUM in three North Carolina environments in 2020. We observed slightly greater linear responses to GS than PS for FER and FUM, but also a noticeable decline in response to the last cycle of GS, suggesting the need to retrain our genomic prediction model by this generation. The final cycles of PS and GS did not differ significantly ($\alpha = 0.05$) for FER and FUM content, but both had at least 48% less FER and FUM content than the starting population. We observed a greater decrease in genetic marker variation with GS than PS, indicating the importance of balancing rapid genetic gain and loss of genetic diversity with recurrent GS. The results of this study also highlight some practical challenges involved in executing GS at public-sector breeding programs due to the difficulty in obtaining consistent and reliable marker data in short time frames.

Funding acknowledgement: United States Department of Agriculture (USDA), North Carolina Corn Growers Association

T25

Invisible to Arbuscular Mycorrhizal Fungi: positional cloning and characterisation of the *ina* arbuscular mycorrhizal mutant in *Zea mays*

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Arbuscular Mycorrhizal (AM) fungi engage in mutualistic symbiosis with more than 80% of land plants, including major cereal crops such as rice, wheat, and maize. This mutualistic interaction results in improved nutrient uptake by the host plant, contributing to its nitrogen and phosphate requirements. AM symbiosis is initiated by pre-contact signalling and reciprocal sensing of the symbionts in the rhizosphere. A forward genetic screen identified a *Zea mays* mutant, *independent of arbuscular mycorrhizal symbiosis (ina*), which cannot be colonized when inoculated with the AM fungus *Rhizophagus irregularis*. Extensive phenotyping suggests that this maize mutant is defective in pre-contact signalling, due to its inability to signal to the fungal symbiotic partner. In addition, germinated fungal spores exposed to *ina* exudates display a distinct transcriptional profile compared to WT exudates, further suggesting a qualitative difference in the composition of mutant root exudates. Positional cloning methods have identified a deletion in the *ina* genome containing three candidate genes that may be responsible for this phenotype. These three candidate genes were independently targeted via CRISPR/Cas9 mediated gene KO to determine their individual contributions to the *ina* phenotype.

Funding acknowledgement: Biotechnology and Biological Sciences Research Council, Corteva Agriscience

T26 @Ann_Murithi

Discovery and validation of a recessively inherited major effect QTL conferring resistance to maize lethal necrosis (MLN) disease

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Maize lethal necrosis (MLN) is a viral disease with a devastating effect on maize production. Developing and deploying improved varieties with resistance to the disease is important to effectively control MLN; however, little is known about the causal genes and molecular mechanisms underlying MLN resistance. Screening thousands of global maize lines revealed KS23-5 and KS23-6 as two of the most promising donors of MLN resistance alleles. Selective genotyping of resistant and susceptible individuals within large F2 populations coupled with genome-wide association study (GWAS) analysis identified a major effect QTL on chromosome 6 for MLN disease severity score and AUDPC values in all three F_2 populations involving one of the KS23 lines as a parent. The major effect QTL (*qMLN06_157*) is recessively inherited and explained 55 to 70% of the phenotypic variation with an approximate 6Mbp confidence interval. An additional three $F_{2:3}$ populations involving KS23-5 or KS23-6 confirmed the presence of the major effect QTL on chromosome 6, demonstrating the efficacy of the KS23 allele at *qMLN06.157* in varying populations. Based on the consistent and effective resistance afforded by the KS23 allele at qMLN06.157, the allele has been used extensively in both marker-assisted forward breeding and marker-assisted backcrossing schemes to improve MLN resistance of breeding populations and key lines for Eastern Africa.

Funding acknowledgement: CIMMYT, Bill and Melinda Gates Foundation (BMFG)

T27

Evolution of plant immune receptors

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Plant immune receptors constitute a highly adaptable line of defense that protects plants on a population level from rapidly evolving pathogens. Compared to most other plants, including grasses, cultivated inbred maize lines have a reduced number of disease resistance genes. Interestingly, they still retain a highly variable yet evolutionary heterogeneous cluster of immune receptors on chromosome 10. In our study, we investigate when and how maize experienced a reduction in copy number of immune receptors, including effects from domestication and inbreeding. We specifically focus on immune receptors with a unique capability to rapidly shuffle and acquire new domains, as well as those genes that are undergoing rapid evolution through point mutation. We will present maize immune receptor evolution in the context of other systems, including Arabidopsis, Brachypodium, rice and soybean.

Funding acknowledgement: National Institutes of Health (NIH), Gordon and Betty Moore Foundation, Innovative Genomics Institute

T28

Investigating Dynamic Heat Stress Responses with Diverse Maize Inbreds through Transcriptome Analyses

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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. Our project objectives 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 responses. For transcriptome profiling, 3' mRNA-seq method was applied using 432 leaf samples from 27 inbred lines, collected at 4 time points from developing and mature leaves in a greenhouse heat stress experiment. Samples for lipid profiling and physiological characterization came from the same greenhouse experiment setting. Under natural environments, two existing and three newly developed mapping populations were used for heat tolerance QTL mapping. With the primary focus on generic heat response mechanisms, we classified the 27 inbred lines into two groups (heat-tolerant and heat-sensitive) for downstream investigation. Differential expression analyses and co-expression network analyses consistently uncovered shared and unique heat response patterns for the two groups along the time series. Gene co-expression modules were associated with other traits to bridge the understanding of heat responses from molecular, biochemical, and whole-plant levels. Among the unique modules from the heat-tolerant group, we highlighted one that was identified to be associated with unfolded protein response, supported by gene ontology (GO) enrichment analysis and cis-element over-representation analysis. This module consisted of a high proportion (37.8%) of heat shock transcription factors (HSFs) and heat shock protein genes (HSPs). Through strategic pattern mining from complex datasets, this study provided insights into maize heat stress response mechanisms at multiple levels.

Funding acknowledgement: United States Department of Agriculture (USDA)

T29

Consolidating OMICS and Environmental Database for Maize Yield Predictions in a Changing Climate

(submitted by Parisa Sarzaeim <parisa.sarzaeim@huskers.unl.edu>)

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The performance of an accurate and valid crop growth model depends great extent on the quality of its input data. This study proposes a framework for data quality and consistency controls to release a homogeneous and multidimensional OMICs database with enhanced and expandable spatiotemporal environmental data for short to long-term maize phenotype forecasts and predictions. We use the OMICs records of the Genomes to Fields (G2F) initiative, and weather and climate data for more than 100 maize experimental fields in the US and the Ontario province in Canada. We introduce a pre-processing algorithm to quality-control multidimensional data, including a module for the addition of environmental uncertainties, for maize yield predictions. The G2F provides a diverse set of maize hybrid genotypes' molecular markers (G2F-G), phenotypic measurements (G2F-P), and in situ weather observations (G2F-E) recorded by a weather station located at the field sites during the growing season. Additionally, several missing values in each G2F data dimension have been recorded in the released data files. The pre-processing quality-consistency algorithm corrects the inconsistencies in the data structure and format of datasets with temporal and spatial data gaps. The methodological quality control phase has been designed, developed, and implemented for each data dimension to read the raw files, check the format, correct the data structure, fill the missing values by machine learning techniques in G2F-E, discard the rare variants from G2F-G, remove the unobserved yield in G2F-P, quantify the weather data uncertainty, visualize the input data variability, and finally generate the integrated and improved database. Afterward, the consistency phase modifies the enhanced database suitable for GxE statistical modeling and annual yield simulations. Metadata files (G2F-M) as another dimension have also been generated for each experiment consisting of general information for analytical and geospatial visualization purposes.

Funding acknowledgement: United States Department of Agriculture (USDA)

T30

Unleashing The Potential Of Weather Data For Improving Yield Forecasting In G2F Maize Hybrids

(submitted by Diego Jarquin <jhernandezjarqui@ufl.edu>)
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Genomic Selection (GS) has demonstrated utility for improving the selection of genotypes with desirable characteristics in breeding programs; however, the extend of these improvements in predictive ability is limited by considering only genomic variants in the process. In addition, elaborated prediction models that allow the inclusion of the genotype-by-environment interaction have been proposed in an attempt to leverage availability of weather data. The integration of climate data has increased the expectations for improving predictive ability. However, since all genotypes tested in a location receive the same environmental stimuli (temperature, precipitation, solar-radiation) the simple inclusion of these covariates may result in little or no benefit, providing no genotype-specific information. In this study, we developed a prediction method that uses environmental data to project the amount of environmental stimuli processed by genotypes during the most influential windows of time during the growing season, allowing the inclusion of these as surrogates of secondary traits. Phenotypic (4 years, 40 environments), genomic (inbred), soil, and weather data from the Genomes To Fields initiative were analyzed. Based on the General and Specific Combining Ability terms, two prediction methods were compared: (*i*) M1, the conventional GS model based on main effects and interactions between markers and environments; and (*ii*) M2 the proposed model based on processed environmental stimuli. Four prediction scenarios were simulated for model validation: CV2 (tested genotypes/observed environments); CV1 (untested/observed); CV0 (tested/unobserved); and CV00 (untested/unobserved). Results showed that across traits, environments, and prediction scenarios the M2 method outperformed M1 around 150%. M2, returned average predictive abilities of 0.85 (CV2), 0.77 (CV1), 0.88 (CV0), and 0.62 (CV00) while the corresponding results under M1 were 0.35, 0.31, 0.28, and 0.13. These results show that there is a wide scope for improving predictive ability with the inclusion of predicted values of those projections based on processed environmental stimuli.

Funding acknowledgement: GM02543 - UF RESEARCH FOUNDATION (GATORADE)

Poster Abstracts

P1

Genotype to Phenotype: An authentic maize research experience for high school students

(submitted by Kathryn Parsley <KParsley@danforthcenter.org>)
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Corn is the crop with the highest dollar value worldwide. Since the 1930s, corn has become more productive and prolific due to advances in genetics and breeding. The overall shape of the corn plant is often a limiting factor in how many plants can be grown per unit of land. Corn with leaf angles that are more acute enable higher planting densities and therefore higher yields per acre. While corn canopy architecture has become more upright, there is still room for improvement in, for example, enhancing photosynthetic efficiency. To better understand corn genetics underlying leaf angle phenotypes, high school students participate in what is known as the Genotype to Phenotype authentic research experience. In this project, students grow 3 unique genotypes of corn (with 5 replicates each) to the third leaf stage, and phenotype them. Leaf angles of the second and third leaves of each genotype are measured using a protractor and in parallel are quantified using image-based analyses in Image J. Leaf angles from diverse genotypes measured across a classroom are compared. In a separate molecular component, dCAPs markers are used to genotype lines with contrasting architectures, linking genotype to phenotype. Results of this project will be presented by students who have participated in data collection on behalf of the Eveland Lab at the Donald Danforth Plant Science Center. All data collected from students participating in the program are leveraged in statistical models for predicting corn architecture and physiology in the field. NSF-PGRP award #1733606 is gratefully acknowledged.

Funding acknowledgement: National Science Foundation (NSF)

P2

Community and Cooperation: The MGC Committee on Outreach, Diversity, Inclusion, and Education (CODIE) 2021-22 update

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Formed in 2020, the Committee on Outreach, Diversity, Inclusion and Education (CODIE) is a committee of volunteers from the Maize Genetics Cooperation (MGC) community, charged with fostering a diverse and inclusive community of maize genetics and genomics researchers, breeders, and educators. CODIE is achieving this goal through improving the recruitment, retention, and recognition of scientists from underrepresented groups at different steps of their career; broadening participation in the Maize Genetics Research Community, and 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. In 2021 - 2022, CODIE maintained early initiatives, such as introducing the Diversity and Inclusion speaker at the Maize Genetics Meeting, starting anti-racist training opportunities for the MGC community, and establishing a website with resources promoting diversity, equity, and inclusion (DEI). In addition, CODIE tackled a number of new initiatives. The Committee welcomed the first cohort of graduate student and postdoc members of the committee, expanded the MaGNET alumni network, and joined the NSF-funded "Root and Shoot" research collaboration network connecting seven plant science societies. CODIE highlights "Hot papers" in DEI-relevant literature. CODIE organized the community-oriented Workshop addressing pervasive systemic vulnerabilities exacerbated by the COVID-19 pandemic, including DEI issues and mental health. Initial analysis of information gathered from the ~150 workshop attendees will be presented, pointing toward priorities for 2022.
To learn more about CODIE, including how to get involved, please visit https://maizegdb.org/mgc/outreach/, or meet current members at the conference poster.

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

P3 @thelma_madzima

A course-based undergraduate research experience (CURE) to investigate DNA methylation in maize.

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Course-based undergraduate research experiences (CUREs) provide an opportunity for students to engage in research in the classroom that more accurately reflects the scientific method, as compared to traditional 'cookbook' lab activities. CUREs also serve to expose and recruit students from diverse backgrounds into STEM disciplines. We designed a quarter-long 'maize epigenetics' CURE protocol where students perform bisulfite sequencing and bioinformatic analysis of promoters of maize genes exposed to abiotic stress (e.g., drought) vs. control samples to measure DNA (cytosine) methylation. These genes are often selected from differentially expressed genes (DEGs) from RNA-seq experiments, and students make observations on the effect of the environment on the plant epigenomes and transcriptomes. At the end of the quarter, students present their research in a poster session or oral presentation. This CURE also serves as an opportunity for local collaborations between 'teaching-track' and 'research-track' faculty at research-intensive institutions (R1), primarily undergraduate institutions (PUIs) and Community Colleges. We will describe this maize epigenetics CURE protocol designed for introductory and upper-level undergraduate students, pedagogical and research outcomes, opportunities for collaboration and how this epigenetics CURE designed in maize, can be applied to other biological systems or research questions.

P4

Employing the maize TFome for expanding GRNs while fostering the Integration of Research with Undergraduate Education (F.I.R.E.)

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Gene regulatory networks (GRNs) are central to all cellular processes including those that underly important agronomic traits. Maize is a model system for investigating the architecture of gene 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 Y1H constructs 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 over three semesters is evaluated. This project is funded by NSF grant IOS-1733633.

Funding acknowledgement: National Science Foundation (NSF)

P5 @addie_maize

Seeding public-private partnerships for agricultural genome-to-phenome training

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Training in data analytics in the field of agricultural genomics and phenomics frequently lags behind current, cutting-edge problems, or is structured in a way that is not realistic or relevant to future career success. Correspondingly, private industry encounters difficulty finding experts in this field with experience working and communicating in interdisciplinary teams. The Integrated training Model in Plant And Compu-Tational Sciences (IMPACTS) NSF Research Training program at Michigan State University trains graduate students in the intersection of computational and plant sciences. Graduate students come from a wide range of programs, including basic and applied plant sciences as well as computer science, engineering, and mathematics. To support this program, a certificate program and three new courses have been established. The first course in the series, Foundations in Computational and Plant Sciences, is an introductory course covering the basics of both plants and coding. Drs. Dan Chitwood and Robert VanBuren have created this course and its corresponding materials and have created "Plants and Python" as a public educational resource (Chitwood 2020a-b). The second course in the series, Frontiers in Computational and Plant Sciences, is designed to be entirely hands-on small group projects. The specific projects rotate each year, partnering with individuals or groups with datasets of interest as the end "users" for each module, as well as with experts in data analytics to introduce the students to possible approaches. Partnerships have included both public and private sector scientists and breeders. Developed projects will be adapted and made available to provide public educational modules and training datasets. Survey-based metrics will be used to gauge perceived success, usefulness, and applicability of the new modules from student, instructor, and guest instructor perspectives. Here, we highlight our recent modules, provide links to access the tools created so far, and solicit additional future partnerships.

Funding acknowledgement: United States Department of Agriculture (USDA), Agricultural Genome 2 Phenome Initiative (ISU - USDA)

P6 @hwb333

The Maize-10-Maze Project, an educational public chromosome map garden featuring the mutants of maize.

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The Maize-10-Maze project is a public outreach event, next scheduled for Summer 2023 at FSU in Tallahassee, FL as part of our outreach project (NSF IOS #2025811). The goal is to reproduce our fun and educational self-guided public tour of the maize genome organized along the karyotype of maize. Each of the 10 rows represents a single chromosome and the mutant families are arranged in the same order as their occurrence on each chromosome. This chromosome map garden features mutants that (1) exhibit a visually striking or cool plant or seed phenotype - such as Knotted1 or lazy plant1, (2) are of agronomic importance - such as brittle endosperm1, or (3) have major scientific or historic importance - such as teosinte branched1. Our high school and undergraduate college students developed and updated the weatherproof field placards that describe each mutant, providing information for self-guided public tours of the maize genome. Each placard lists the phenotype, genotype, original reference, mode of inheritance, genetic location, sample photographs, and the status of molecular cloning. We invite members of the maize community to help us review and update what is known about these (placards will be printed and linked online at the poster). The event is co-sponsored with the Florida A&M University's Forestry and Conservation Education (FACE) Summer Program, which celebrates its 25th anniversary in 2023. Photography and web-based resources from the Maize-10-Maze project have been and continue to be developed for use in classroom settings ranging from middle schools to universities. The mutant garden is fascinating and beautiful on its own, while also illustrating the historic importance of maize mutants for advancing plant biology research.

Funding acknowledgement: National Science Foundation (NSF)

P7

Advances in maternal haploid inducer development in maize

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Doubled haploid (DH) technology in maize, obtaining *in-vivo* haploids by inducers, significantly improves the genetic gain for traits of interest in modern maize breeding. With the wider application of DH technology worldwide and in more diverse genetic backgrounds of donor populations, there is a need to constantly improve available haploid inducer lines not only for their haploid induction rate but also for their agronomic performance in different environments. Our Objective is to apply DH technology in haploid inducer improvement. The typical purple color of inducer kernels expressed by the *R1-nj* gene is used as the dominant phenotypic marker for selecting haploids in breeding materials. The *C1-I* gene inhibits *R1-nj* expression epistatically in the embryo and aleurone. We confirmed that the purple embryo of inducer haploids could be obtained by crossing maternal haploid inducers with lines carrying the *C1-I* gene. A *C1-I* maternal haploid inducer line ISURF#5202 with a Haploid Induction Rate (HIR) over 10% has been developed for haploid induction and selection in inducer germplasm fixed for the *R1-nj* allele. Herein, we demonstrate that inducer DH line development is scalable in the field, and feasible for accelerating maternal haploid inducer breeding.

Funding acknowledgement: United States Department of Agriculture (USDA), Plant Sciences Institute, R.F. Baker Center for Plant Breeding, K.J. Frey Chair in Agronomy, and the Doubled Haploid Facility at Iowa State University

P8 @ravi_mural

Assembling and curating multi-environment, multi-trait datasets from community association panels as a prelude to phenotypic yield prediction

LIGHTNING TALK

(submitted by Ravi Mural <rmural2@unl.edu>)
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Many of the relationships between non-yield plant phenotypes and yield outcomes are either unknown or poorly defined. The same value for a given trait can often enhance or decrease yield depending on the environment. Elucidating the relationships between other plant traits and yield across different environments is a necessary precondition for predicting how crop plants will perform in previously untested environments. Here we exploit a key feature of plant quantitative genetics -- the widespread adoption of community association populations -- to assemble a unified dataset by employing a combination of published resequencing data to generate a common set of 18M genetic markers scored across the union of 1014 genotypes present in the SAM and/or WiDiv association panels. We assembled a set of 162 traits which have been scored across different subsets of these 1014 genotypes, including both previously published studies conducted across nine US states and new trait data collected from field trials conducted in Lincoln, Nebraska USA. Using resampling-based GWAS we identified 2,154 confident GWAS hits across these 162 trait datasets including signatures of pleiotropic effects for a number of genetic loci. To test the feasibility of cross-environment phenomic yield predictions, we predicted yields of Lansing, MI 2020 field site using phenotypes from Lincoln, NE 2020 field site with all yield and ear-related traits removed. Permutation analysis suggested the three most important traits for prediction accuracy were: 1) days to anthesis, 2) number of branches per tassel, and 3) plant height. These three traits are all potentially scorable from UAV data, suggesting it may be possible to predict variation in end-season yields using high throughput phenotyping data collected during the growing season. Future work will be needed to assess whether similar plant traits are useful for predicting yield across multiple locations and potentially in future environments.

Funding acknowledgement: Advanced Research Projects Agency–Energy

P9 @MatthewMurph

Assessment of two statistical approaches for variance genome-wide association studies in plants

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Identifying genomic sources responsible for uniformity in poor growing environments and responsiveness in great environments could facilitate the selection of plants that can thrive in increasingly unpredictable environments. The identification of such variance-controlling loci has elusive, primarily due to the inherent statistical and computational complexities arising from the need to identify epistatic and genotype by environment (GxE) interactions that theoretically underlie these genomic sources. While vGWAS has been used for dimension reduction in livestock breeding and humans, searching for variance Quantitative Trait Loci (vQTLs) and higher-order interactions remains underutilized in plants. Two statistical approaches that have already been proven to aid in vQTL identification and potentially higher-order interactions are the Brown-Forsythe Test (BFT) and the double generalized linear model (DGLM). To assure these applications are deployed to vGWAS most advantageously, it is crucial to identify which quantitative genetics factors affect their ability to identify vQTLs and higher-order interactions. We used publically accessible genome-wide marker data in Arabidopsis and maize to simulate traits controlled by epistasis and GxE interactions and on previously published crop vGWAS controlled by variance quantitative trait nucleotides (vQTNs). We then quantified true and false positive detection rates of the BFT and DGLM. We observed that the DGLM consistently yielded similar or higher true positive QTN detection rates than the BFT for the different genetic architectures. While these statistical approaches identified epistatic and GxE interactions, sample size, heritability, and minor allele frequency (MAF) can influence the success of identifying such interactions. We recommend future maize breeding efforts to use larger genotypic datasets than what we used (i.e., n > 2,532) to use vGWAS as a dimension reduction for higher interactions. Such an undertaking should maximize the ability of these two approaches to facilitate future breeding efforts to incorporate epistasis and GxE on a larger scale in maize.

Funding acknowledgement: National Science Foundation (NSF)

P10

Breeding colored and high lysine prime eating stage sweetcorn

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Zea mays subsp. mays L grain proteins are dominated by zein proteins found in the endosperm. Zeins are deficient in essential amino acids, lysine, and tryptophan. The opaque-2 (o2) mutation found in quality protein maize decreases the production of alpha-zein proteins, which increases the production of non-zeins which have a better balance of essential amino acids. Zeins are abundant 20 days after pollination in eating stage sweetcorn which is thus amenable to the beneficial effects of o2. Maize germplasm has a diversity of kernel color in the form of anthocyanins and carotenoids found in the pericarp and aleurone layers. These pigments confer nutritional value and aesthetic appeal, but colored sweetcorn varieties are rare, in part because color can accumulate later than the prime eating stage. This research is focusing on integrating high lysine from several QPM parents and an array of colors from a variety of colored maize varieties into prime eating stage sh2 and su1 sweetcorn. After selecting the best varieties of each, hybrids will be made which confer both the high lysine and color traits. We will present an overview of the breeding scheme and progress up to screening promising color, sweetness, and texture in F3 varieties as well as mapping out future work.

P11
Breeding of diversely colored quality protein popcorn

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Popcorn (Zea mays everta) is a type of flint corn that builds up pressure inside the kernel causing the rapture of endosperm when heated at a temperature of 177 °C. Popcorn is a popular snack food which is healthy but tends to be bland in its natural form and so is rarely consumed without flavor additives. Like conventional maize grain, popcorn lacks essential amino acids such as lysine and tryptophan that the body needs to grow but cannot synthesize. Building on insight gained from previous work which has resulted in high yielding but privately owned Quality Protein Popcorn (QPP), this project aims to generate QPP varieties from publicly available colored popcorn and explore the resulting diversity of intrinsic nutritional value, flavor and texture. To generate QPP, various colored popcorn parent lines were crossed to Quality Protein Maize (QPM) parent lines and are being backcrossed using the recurrent popcorn parent while selecting for glassy kernels that possess the major modifier gene (duplication of the 27 kDa gamma zein gene) and the homozygous opaque-2 allele. We present phenotypic and protein data as far as the BC1 F2 and showcase the ongoing and future hybrid breeding.

P12
Characterization of inbreds with expired plant variety protection – 2022 update

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Formerly proprietary inbreds that enter the public domain after the expiration of their intellectual property protection offer breeders the opportunity to gain access to highly selected material. The main objective of this research was to genetically characterize all available ex-PVP inbreds through September of 2021 and to gather empirical performance information. A total of 498 available ex-PVP inbreds were genotyped using GBS. This produced 519,709 SNP markers that were used to assess relationships. A set of 151 primarily non-Stiff Stalk ex-PVP inbreds were test crossed to LH244 and grown in yield trials at the West Madison Agricultural Research Station (Madison, WI) and the Arlington Research Station (Arlington, WI) in 2021. Performance data allowed ranking of inbred lines for key traits such as yield (9.24-14.24 Mg/ha), harvest moisture (19.10-26.18%), and test weight (49.98-56.11 lbs/bu) and provided comparative information on the potential of new lines in a common experiment. For moisture adjusted yield PHP55, ZS1022, LH287, PHN73, and PHBV8 were the top 5 performing inbreds in combination with LH244. Considering a ratio of Yield/Moisture the top 5 performing inbreds were PHN73, PHP55, 3IIH6, ZS01228 and PHN37. Characterization of ex-PVP inbreds using genetic markers and via comparative yield trials provides breeders with information to determine how a new source of germplasm may potentially be utilized in their internal breeding program, particularly in northern regions of the US Corn Belt.

Funding acknowledgement: United States Department of Agriculture (USDA)

P13

Computer vision for high-throughput quantitative genetics for disease resistance in sweet corn

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Southern Corn Leaf Blight (SCLB) is a foliar disease caused by *Bipolaris maydis* that threatens yield losses every year, especially in warm, humid, sweet corn producing climates of the Southeastern United States. Breeding for multi-gene quantitative disease resistance is necessary as single-gene resistance is not sustainable and breaks down as fungal populations evolve. One of the biggest challenges towards breeding for quantitative disease resistance is the ability to phenotype large populations. Image-based high-throughput phenotyping has the potential to increase phenotyping efficiency by providing quantitative phenotyping to previously qualitative approaches, increasing the speed at which features are measured, and reducing subjectivity, time, and labor. Here, we implement a python-based computer-vision tool that automates phenotyping of diseased leaf area from pictures of detached leaves. This tool was used to phenotype a diverse sweet corn inbred panel of 693 lines over two seasons for resistance to SCLB. We show that a computer vision approach captures a greater proportion of genetic variability in our experiments when compared to traditional qualitative score-based phenotyping. We generate breeding values for the diverse sweetcorn panel, enabling us to characterize the sweetcorn population for disease resistance in a quantitative manner. This information will inform our selections towards inbred development. We leverage robust phenotypic data with whole genome sequences for all individuals in the population to explore the genetic architecture of this trait through a genome-wide association study. We found genetic markers strongly correlated with SCLB resistance. Some markers are located near previously discovered markers associated with SCLB resistance. We also find novel association with SNPs close to known in protein domains involved in plant immune response. This analysis has the potential to further our genetic and biochemical understanding of SCLB toward breeding for resistance in sweet corn.

Funding acknowledgement: National Science Foundation (NSF)

P14 @HongyuJin5

Cross environment yield prediction using genomic and phenotypic data in maize

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Modern plant breeding has depended on data obtained from yield trials in the target environment to identify desirable new crop varieties. More recently, prediction models based on genetic marker data and trained using yield trial data from the target environment have been shown to improve the efficiency and rate of gain in plant breeding programs. However, current approaches to plant breeding are challenged by the increase of volatility of the environments. Selection decisions about future varieties must be made up to 10 years before release and there is limited information of the target environments those varieties will experience in the future. As a result, it has become increasingly urgent to improve cross environment prediction of crop performance to create and sustain resilient agricultural systems for the second half of the twenty first century. In this study, field trials of a set of 752 maize inbred lines were conducted in two different environments: Lincoln, Nebraska and East Lansing, Michigan. With the access to high density genetic marker data and phenotypic data, we were able to benchmark four models which predicted grain yield within and across environments. The coefficient of determination between the observed yield in Nebraska and Michigan was only 0.45. Genomic predicted values trained using only the yield from Nebraska were modestly better predictors of yield in Michigan than the actual observed Nebraska yield data. Two models using either random forest or canonical correlation to integrate measurements of non-yield traits were evaluated. These two models both exceeded the prediction accuracy of genomic prediction, suggesting they may have a promising role to play in future efforts at cross-environment prediction

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

P15

DNA fingerprinting, and evaluation of genetic diversity of indigenous bread and durum wheat using ISSR marker system

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Wheat is one of the most widely cultivated cereal crops, but it endures a limited genetic base. To contribute to effective crop maintenance and sustainability efforts, a better understanding of the genetic diversity of wheat accessions is imperative. In this study, 17 bread wheat (*Triticum aestivum* L.) and 22 durum wheat cultivars (*Triticum durum* Desf.) from different provinces covering most of its natural distribution were investigated using 20 ISSR primers for genetic diversity evaluation. A total of 31 primers on native polyacrylamide gel electrophoresis were successfully identified. Wheat cultivars have diverged into four distinct groups based on Dice dissimilarity matrices and the UPGMA algorithm. High genetic diversity was observed amongst the little-known native bread and durum wheat cultivars. Out of the 20 analyzed primers from the bread wheat cultivars, 16 primers displayed polymorphism. The ISSR analyses identified 123 ISSR loci in the bread wheat cultivars, in which 114 loci (89.9 %) were polymorphic. For the durum wheat cultivars, 15 out of 20 analyzed primers showed polymorphism, detecting 104 loci, of which 92 loci (91.9 %) were polymorphic. This assessment of provenance genetic variation in native wheat using ISSR markers highlights the potential for *ex-situ* conservation and application of genetic resources to wheat breeding.

Funding acknowledgement: National Institutes of Health (NIH)

P16

Dynamics of genetic control of sorghum plant height across developmental stages

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Recent advances in genomics have improved our understanding of the genetic basis of important agronomic traits including plant height. However, the high labor intensity of manual phenotyping has remained a bottleneck. Only the final height is often measured yet crops exhibit different growth rates across developmental stages. We hypothesized that the variation in growth rate across stages has an underlying genetic basis that can be uncovered with time series remote-sensing data captured with an unmanned aerial vehicle (UAV). In this study, we used a UAV to acquire RGB imagery of 377 accessions of the sorghum association panel and 242 recombinant inbred lines of Tx430 x P898012 at three developmental stages. We built a crop surface model from each image collection and estimated plot-based plant height. There were significant correlations of up to 0.89 between UAV and manual measurements indicating the 3D reconstruction of the field successfully captured the genotypic differences in height. Next, we used the logistic function and its first derivative to model the growth trajectories of the genotypes and estimated plant height and growth rate of each genotype at a five-day interval from 20 to 100 days after planting (DAP). Genome scans of the model-estimated plant height and growth rate detected strong genetic signals with dynamic effects. Signals detected on chromosomes 4, 5, and 7 were transient and only expressed at early stage (20-30 DAP) while those co-localizing with *Dw1*, *Dw3*, and *qHT7.1* expressed at later stage (40 -100 DAP) and were persistent. These results demonstrate that we can leverage the high spatial and temporal resolutions of UAV imagery combined with modern genomic technologies to decipher the dynamics of genetic control of plant height.

Funding acknowledgement: United States Department of Agriculture (USDA), National Institute of Food and Agriculture, Plant Science Institute Iowa State University, Iowa Crop Improvement Association

P17

Environmental and genetic basis of phenotypic plasticity in the maize NAM population

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Maize phenotypes are plastic, determined by the complex interplay of genetics and environmental variables including day length, temperature, and precipitation. Identifying an environmental index consisting of an environmental variable measured during a critical window that strongly influences a given phenotype can help to dissect this complexity. When combined with genomic prediction, these environmental indices enable accurate in-season predictions. In this project, the CERIS (Critical Environmental Regressor through Informed Search) method was used to identify environmental indices for 19 traits measured in the Maize NAM population in 11 environments. Identified environmental indices are consistent across training sets and biologically meaningful. The chosen windows show a clear transition from the vegetative to the reproductive stages of maize, with vegetative traits such as leaf length and width affected by windows early in the season while harvest traits such as total kernel weight are influenced by later windows. Identified environmental variables also follow biological expectations; for example, the environmental variable chosen by CERIS for anthesis-silking interval was precipitation, reflecting the important effect of water availability on this trait. Prediction accuracies for flowering time reached 0.92-0.95, even in the most difficult prediction scenario analyzed (untested genotype in untested environment). Finally, GWAS was conducted with more than 20 million SNP and SV markers derived from recent *de novo* sequencing of the 25 NAM founders to identify genes controlling the plastic response to these environmental indices. This systematic approach to uncovering the major environmental factors and the corresponding genetic factors underlying phenotypic plasticity enables improved understanding of complex traits in maize.

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

P18 @CoelhoIgorF

Evaluating the implementation of double haploid technology and genomic selection into a sweet corn breeding pipeline

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Sweet corn is a high value, specialty crop that is produced for processing and fresh vegetable markets. As the breeding process takes a long time, we have established *in silico* simulations to evaluate the implementation of new breeding strategies and guide future breeding decisions. In this study, we simulated the pipeline of the sweet corn public breeding program at University of Florida to investigate the effect of these strategies, in terms of genetic gains, cycle length, and costs. Currently, the conventional breeding program (*CONV*) is an inbred development program that relies on successive self-pollinations and three testcrosses stages. Beyond the *CONV*, five alternative scenarios were evaluated over 30 years of breeding. They were: i) *CONV* implementing genomic selection (GS) (*GS_CONV*); ii) the use of double haploids (DH) instead of the successive selfs (*DH_CONV*); iii) the adoption of DH and GS simultaneously in the breeding program (*DH_GS*); iv) the adoption of DH lines and reduction of one testcross (*FAST_DH*); and, v) use of GS into the *FAST_DH* strategy (*FAST_DH.GS*). Two different genotype-by-environment interactions (GxE) were compared. When the GxE was null, all the scenarios showed similar performances of predicted hybrid gains, with small differences in the total cost required for the implementation of each approach. Under presence of GxE, the *FAST_** scenarios were the most promising strategies in logistic and breeding terms, generating better hybrids, releasing a higher number of lines in a shorter amount of time. However, the total cost of implementing such strategies were also estimated to be higher. Our estimates resulted in a total increase in the cost of up to 60% relative to the budget of the *CONV* strategies. A greater number of inbred lines was released in the FAST_* strategies and the genetic gains increased around 20%.

Funding acknowledgement: NIFA-SCRI 2018-51181-28419

P19

Evaluation of the usefulness of Ex-PVP maize germplasm in hybrid development for organic maize systems using participatory variety testing

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The rapid increase in demand for organic agricultural products has transformed the organic industry from a niche industry into a well-developed alternative to conventional farming systems. However, the current domestic organic food supply does not satisfy the increasing demand. As part of the solution, new crop varieties are needed that perform well in organic systems. The objective of this study was to use a participatory research approach to characterize the performance of maize hybrids developed by conventional and organic breeding programs under real-world organic management practices. Cultivars developed by four breeding programs with varying breeding objectives were evaluated in organic on-farm trials. For this purpose, we created a Variety Testing Network (VTN) collaborating with eight farmers in Illinois and four farmers in Indiana. Eight experimental hybrids and a commercial organic hybrid check were selected and tested by the VTN in 2018, 2019, and 2020. Each farmer planted the hybrids in a four-row 30-meter strip plot using an unreplicated completely randomized design. The hybrids were evaluated for grain yield, grain moisture, test weight, grain chemical composition, and morphological characteristics. Our results show that hybrids differ significantly for all agronomic performance traits across farms, which differ for their crop rotation, nitrogen source, and cultivation practices. The hybrids derived from conventional inbreds had higher grain yields and starch content than hybrids developed from organic inbreds. Their high protein content distinguished organic hybrids. The difference in agronomic management practices across farmers significantly affected the performance of hybrids.

Funding acknowledgement: United States Department of Agriculture (USDA)

P20
Genetic analysis of multiple biomass related traits in sorghum

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Sorghum (*Sorghum bicolor* L.) is a C_4 crop plant used for food and fodder. It can be grown as an energy crop with high biomass yields while lowering agricultural inputs. A variety of genes play important role in complex traits such as biomass that are challenging to map. Genetic analysis of biomass determining traits can lead to the optimal utilization of sorghum as a biofuel feedstock. In this study, we sought to identify genetic loci controlling biomass related traits relevant to breeding in sorghum accessions from the Sorghum Association Panel. We phenotyped 391 different accessions at maturity for leaf dry weight, stem dry weight (SDW), panicle dry weight, bucket weight (BW), total plot fresh weight (TPFW), and total plot dry weight (TPDW). The genotyping-by-sequencing approach was implemented to obtain 234,268 highly accurate SNP markers with minor allele frequencies below 3%. The FarmCPU model together with first two principal components led to the identification of 38 significant markers. The majority of biomass related traits are associated with multiple SNPs, reflecting the polygenic nature of this complex trait. We further identified two markers, 'S08_3545584' and 'S09_56994655', with the allelic variation of T/A and A/G was associated with more than one trait, indicating pleiotropic effects of the loci. Further, SNP-associated genes which were mined within a 50-kb window led the identification of known and novel candidate genes involved in biomass. For instance, SNP marker 'S08_3545584' affects BW and TPDW which co-localized with purple acid phosphatase (Sobic.008G037000.1), while 'S09_56994655' marker affects SDW, BW, and TPFW and co-localized with auxin-responsive protein (Sobic.009G229200.1), xylan biosynthetic gene (Sobic.009G229300.2) and photosystem I (Sobic.009G229700.1) suggesting their role in crop growth and biomass yield. These markers and marker -associated genes identified in this work are expected to be useful for marker-assisted selection for the development of superior sorghum cultivars.

Gene / Gene Models described: ; Sobic.008G037000.1, Sobic.009G229300.2, Sobic.009G229200.1, Sobic.009G229700.1
Funding acknowledgement: Department of Energy (DOE), AG2PI, MSU Plant Resilience Institute fellowship

P21
Genetic analysis of extreme trigonelline levels in maize grain

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Nixtamalization is the process of soaking maize kernels in an alkaline solution to process them into a masa that is easier to grind and more flavorful. Additionally, decreased mycotoxins and increased nutritional quality are observed. Originating in Mesoamerica with the Aztec and Mayan communities, nixtamalization provided a way to convert non-bioavailable niacin to bioavailable nicotinic acid. Thus, providing an important source of essential micronutrients and preventing pellagra, a debilitating disease caused by insufficient dietary intake of niacin. Trigonelline is a N-methyl derivative of nicotinic acid that accounts for 82% of the total, potential vitamin B3 compounds in mature grain in the U.S. maize Ames panel and is inactive and unavailable to humans as a source of vitamin B3. Identifying the genes involved in accumulation of total bioactive vitamin B3 and conversion of nicotinic acid to trigonelline would help in optimizing the bioavailability of vitamin B3 from maize grain for human consumption. Through an analysis of vitamin B3 compounds in grain from ~1,500 inbred lines of the maize Ames panel, three inbred lines were identified to have ~80% lower trigonelline levels and elevated niacin levels. One of these three near-zero trigonelline lines, T232, was crossed with B104 to generate an F2 population of 268 individuals for QTL mapping of vitamin B3 grain levels. A major QTL for trigonelline, which explained 64% of the phenotypic variation, was identified on chromosome 4. Additional experiments and analyses are ongoing to further genetically dissect the QTL interval in an effort to pinpoint the causal variant(s) responsible for extremely low trigonelline levels.

Funding acknowledgement: National Science Foundation (NSF)

P22 @dyl_schoe98

Genetic analysis of natural variation for pericarp color in dent maize

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Maize varieties with dark pericarps are of interest for their association with acetylated anthocyanins and phenolic acid; however, most commercial U.S. maize hybrids lack pericarp pigmentation. We observed variation for pigmentation within the Wisconsin Diversity Panel (WiDiv) and in double haploids (DHs) derived from biparental populations involving ex-Plant Variety Protection (ex-PVP) parents 3IIH6, LH185, LH82, PHP02, PHN46, and PHK76 and University of Wisconsin released line W606S. Pigmentation intensity was rated visually and measured using RGB images to obtain the hue value of the pericarp while applying a random forest model to control for embryo position. Hue heritability ranged from 83% to 90% and was significantly correlated with visual ratings. In the WiDiv Panel, 140 inbreds had detectable pigmentation and 504 inbreds from the biparental DHs segregated for pericarp pigmentation. Genetic mapping of hue and visual ratings revealed many smaller effect QTL with major QTL on chromosomes one, two, and nine. The largest QTL was on chromosome one near the gene *p1*. The QTL peak on chromosome nine was 8Mb from *colored aleurone 1* (*c1*) while the peak on chromosome two was 14Mb from *salmon silk2* (*sm2*). Inbreds with white cobs (colorless glumes) showed significantly darker pigmentation compared to inbreds with red cobs (red glumes). Genetic mapping for pigmentation among 267 inbreds with colorless glume revealed an association with *p1* and *C1,* while genetic mapping among 252 inbreds with red glume were only associated with the QTL on chromosome two. A GWAS of the hue value in the WiDiv panel identified *p1* on chromosome one along with six QTL within 5Mb of QTL peaks in the biparental populations. These results support that the major QTL controlling pericarp color are influenced by the allelic state of *p1*, and many small effect loci contribute to the quantitative variation in pericarp pigmentation intensity.

Gene / Gene Models described: *p1, c1, sm2*; Zm00001eb014430, Zm00001eb373660, Zm00001eb105580
Funding acknowledgement: United States Department of Agriculture (USDA)

P23 @WilkerJen

Genetic control of maize aerial node root number and diameter

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Exploring natural diversity for biological nitrogen fixation in maize and its progenitors is a promising approach to reducing our dependence on synthetic fertilizer and enhancing the sustainability of our cropping systems. Specific maize landraces from Sierra Mixe, in Oaxaca, Mexico, can acquire 29%–82% of their nitrogen from the air by hosting nitrogen-fixing bacteria in a mucilage produced by aerial nodal roots after the rain [1,2]. Aerial root number and diameter correlate well with mucilage abundance and nitrogenase activity in the Sierra Mixe landraces. The number of nodes with aerial roots seems well-correlated with the duration of high nitrogen fixation activity. These landraces have limited utility in a wide range of cropping systems in North America due to their long growing season and adaptation to tropical cropping systems. Our research aims to elucidate the genetic basis of this trait and to identify quantitative trait loci associated with N fixation. To find quantitative trait loci associated with nitrogen fixation, $F_{2:3}$ mapping populations are being developed between ex-PVP (plant variety protection) genotypes and Oaxacan landraces differing in the number of nodes with aerial roots and aerial root diameter. We will evaluate the mapping populations at two field locations in Wisconsin in 2022. Genome resequencing a Oaxacan accession will enable the identification of genes controlling the trait. Phylogenetic analyses using modern maize varieties, teosinte, and Sierra Mixe landraces are also in progress. The development of nitrogen-fixing maize varieties adapted to North American production environments is a long-term goal of this project.

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Funding acknowledgement: United States Department of Agriculture (USDA)

P24

Genetic variation for photosynthetic efficiency and leaf hyperspectral imaging in Sorghum

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Sorghum bicolor (L.) is a major cereal crop that ranks fifth in terms of importance for food and feed around the world. Global population is expected to reach 9 billion by 2050, making increasing cereal yield potential a critical goal for ensuring food security. It appears that crop yield improvement through enhanced photosynthetic efficiency is a promising strategy for meeting future food demand under climate change conditions. To achieve this goal, we quantified the natural and genetic variation in photosynthetic performance in the flag leaf of 45 sorghum accessions from the Sorghum Association Panel. The following six vegetation indices were calculated through non-invasive hyperspectral imaging (453-937 nm): Normalized Difference Vegetation Index (NDVI), Normalized difference NIR/Red edge Index (NDRI), Leaf chlorophyll index (LCI), Hyperspectral vegetation index (HVI), Anthocyanin reflectance index (ARI), and Pigment specific simple ratio (PSSR). While infrared gas analyzers were used to quantify four gas exchange parameter, net photosynthesis rate (A), internal CO2 concentration (Ci), stomatal conductance (gsw), total conductance to water vapor (gtw), transpiration rate (E), and electron transport rate (ETR). Pearson correlation analysis showed significant positive correlation among NDVI, NDRI, LCI, HVI, and PSSR, while ARI had a negative association with all the traits. Further from the gas exchange parameter, A showed positive association with Ci (r = 0.68) and gsw (r = 0.87), Ci with gsw (r = 0.91), and gtw with E (r= 0.94), indicating that these traits can be exploited to enhance photosynthesis. Our next step is to investigate marker –trait associations, and genomic prediction using multi-trait model to improve photosynthetic performance in sorghum which can be integrated into sorghum breeding programs.

Funding acknowledgement: Department of Energy (DOE)

P25

Genome Wide Association Study of selected commercial maize germ plasms under environmental stress

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Maize is an economically important cereal crop consumed across the globe. However, with changing climatic and environmental conditions which threatens food security, it is crucial to uncover the genetic architecture underlying complex traits of agronomic value for crop improvements to mitigate the effects of these environmental stressors. Phenolic compounds in maize have been identified in response to biotic and environmental stresses. The aim of this study was to identify the key genetic markers that are associated with responses to environmental stresses such as drought. First, we measured the phenolics concentration in several maize lines in response to drought after which, we conducted a GWAS to identify the regions in the selected maize genome linked to drought tolerance. This study will provide additional information on the underlying genetic basis that accounts for phenotypic variations observed across different maize germ plasms and how they can be exploited in future plant breeding and crop improvement programs.

Funding acknowledgement: United States Department of Agriculture (USDA)

P26

Genome wide association studies of auxin responses in maize seedlings using the Wisconsin Diversity panel

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Root system architecture is a key component of nutrient usage and can impact yield in maize. Auxin is a key plant phytohormone involved in root morphogenesis but it is not well understood how auxin contributes to maize root formation. In order to discover key genetic drivers of auxin-dependent seedling morphogenesis we have performed genome wide association studies (GWAS). For this study we used the well-characterized Wisconsin Diversity Panel (627 lines) to conduct phenotypic screens across several seedling traits, including auxin responses, and then performed GWA to uncover genes of interest for shoot and root auxin response. In order to assess auxin response across lines, the entire panel was grown out in using a random complete block design and our rolled towel growth assay protocol, with 6-10 replicates per genotype and treatment. After planting, kernels were scored for germination and synchronized to initiate auxin or mock control treatments on 3-day-old seedlings, which were then continued for 4 days. Seven-day-old seedlings were imaged, generating over 12,500 images, and traits were manually curated in ImageJ. Six traits were quantified from these data (1) shoot length, (2) auxin treated shoot length, (3) root length, (4) auxin treated root length, (5) shoot-to-root ratio and (6) auxin treated shoot-to-root ratio. We then performed GWAS with >20 million single nucleotide polymorphisms (SNPs), applied a threshold cutoff of p-value

Funding acknowledgement: United States Department of Agriculture (USDA)

P27

Genome-wide association study of maize tassel architecture phenotypes under nitrogen deficit stress

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Nitrogen is an essential nutrient for optimal growth and yield in many crops including maize and also an expensive input for farmers. Optimizing nitrogen use efficiency along with minimizing nitrogen application benefits farmers while reducing negative environmental impacts. Responses to nitrogen deficiency stress are plastic in maize, differentially impacting many agronomically important traits, including tassel architecture traits. Previously, tassel branch number has been found to have an inverse relationship with grain yield, where a decrease in branch number correlates to an increase in yield. A field experiment was conducted over two years with approximately 230 diverse lines from the Buckler-Goodman maize association panel under normal and deficient nitrogen conditions. Four tassel architecture traits were measured, including tassel length, branch zone length, spike length, and tassel branch number. In addition to these measurements, flowering time was also collected. A Genome-Wide Association Study (GWAS) was conducted to identify potential significant single nucleotide polymorphisms (SNPs) associated with tassel morphology under differing nitrogen conditions. We are currently working to identify possible candidate genes associated with significant peaks detected in the GWAS. These findings have the potential to aid the advancement of maize germplasm by optimizing nitrogen use efficiency and nitrogen stress response, thereby increasing yield under suboptimal conditions.

Funding acknowledgement: National Science Foundation (NSF)

P28 @zhikai16

Genome-wide mediation analysis: an empirical study to connect phenotype with genotype via intermediate transcriptomic data in maize

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Mapping genotype to phenotype is an essential topic in genetics and genomics research.
As the Omics data become increasingly available, two-variable methods have been widely applied to associate genotype with the phenotype (genome-wide association study, or GWAS), gene expression with the phenotype (transcriptome-wide association study, or TWAS), and genotype with gene expression (eQTL).
However, signals detected by these two-variable association methods suffer from low mapping resolution or inexplicit causality between genotype and phenotype, making it challenging to interpret and validate the molecular mechanisms of the underlying genomic variations and the candidate genes.
Under the context of genetics research, we hypothesized a causal chain from genotype to phenotype partially mediated by intermediate molecular processes, i.e., 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 the exposure to the mediator to the outcome, and implemented it with the maize association panel (N=280 lines).
Using 40 publicly available agronomy traits, 66 newly generated metabolite traits, and published RNA-seq data from seven different tissues, our empirical study detected 736 unique mediating genes.
Noticeably, 83/736 (11%) genes were identified in mediating more than one trait, suggesting the prevalence of pleiotropic mediating effects.
We demonstrated that several identified mediating genes are consistent with their known functions.
Additionally, our results provided explicit hypotheses for functional validation and suggested mediation analysis is a powerful tool to integrate Omics data to connect genotype to phenotype.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), UNL Start-up fund, Nebraska Research Initiative

P29 @BioBDub

Hyperspectral based prediction of nutrient content in maize leaves

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Hyperspectral imaging is a promising method to predict crop traits in a high-throughput manner and unlock quantitative genetic studies by lowering the cost to collect phenotypes. A single hyperspectral image can be used to predict several unrelated traits at once. Researchers have successfully modeled different physiological traits in maize such as vegetative Nitrogen content but the effect of different development stages, genotypes, and treatments on modeling power remains unclear. This study explored the ability to model leaf macro- and micro- nutrient content in addition to leaf water content from hyperspectral transmittance data from 350nm - 950nm collected with a LeafSpec imaging device developed by Jian Jin at Purdue University. Three different machine learning algorithms were compared: Partial Least Squares Regression, Random Forest and a Neural Net. Traits were collected from twenty-two hybrids throughout the 2020 field season under normal and low Nitrogen conditions. Fourteen genotypes excluded from model training were used to externally test model performance, representing a train-test split of 76% to 24%. Sulfur, Nitrogen, Manganese, Copper, and Iron leaf concentrations were the most amenable nutrients to prediction with coefficient of determination scores up to 0.78. The most accurate method was dependent on the trait being modeled. These findings demonstrate the ability to predict nutrient content in field grown maize over a variety of developmental stages, genotypes, and treatments from a handheld hyperspectral imaging device.

Funding acknowledgement: MSU - Plant Science Fellowship, AgSpectrum

P30

Identification of a genomic region influencing early development and cold tolerance in an adapted maize landrace

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Genetic improvement of early development and cold tolerance are important goals for corn breeding in temperate climate zones. Locally adapted landraces constitute a promising source for the identification of novel alleles influencing these traits. To make landrace diversity accessible, we generated doubled-haploid (DH) lines from three preselected European landraces and used them to identify genes influencing cold tolerance related traits. Here we present the genetic dissection of a genomic region that is significantly associated with early plant height, early vigor and the maximum potential quantum efficiency of Photosystem II in the Austrian landrace 'Kemater Landmais Gelb'. We generated a bi-parental fine mapping population by crossing two Kemater DH lines with high overall genomic similarity but contrasting haplotype alleles in the target region. We phenotyped the resulting recombinants for target traits in field experiments and under controlled conditions. Phenotype-haplotype associations could be validated in the recombinants and fine-mapped to a genomic segment containing less than 20 genes (Zm-B73-REFERENCE-NAM-5.0). We show that contrasting recombinants have a difference of almost one week in the start of their exponential growth phase. In addition, clear differentiation was visible between recombinants after severe cold treatment. After verifying our findings by mutant analysis, we will evaluate allelic effects of the selected candidate genes and develop makers to use them in breeding. Our work is a successful example on how to extract genes associated with quantitative traits of interest from locally adapted landraces.

Funding acknowledgement: Federal Ministry of Education and Research (BMBF)

P31

Identification of epistasis by environment interaction in maize

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Genotype-by-environment interactions can substantially influence QTL effects. Epistatic interactions can also influence QTL effects. Because both of these forces are challenging to investigate on their own, studies investigating epistasis by environment interactions are rare. The Genomes to Field (G2F) initiative has provided a wealth of data that we used to search for epistasis by environment interactions in maize. The data include trials conducted in over 29 locations and measurements of several phenotypes, including yield. The population comprises a "mini-Nested Association Mapping" panel, with three biparental families sharing one common parent. Three hundred and twelve individuals were included in our study. We clustered the 30 locations into five mega-environments using k-means clustering implemented through the learnMET/R package. Genotyping was done with skim sequencing and imputed with Practical Haplotype Graph (PHG). After filtering, QTL mapping was conducted based on Simple Interval Mapping (SIM). Epistatic interactions were identified with the scan-two function in R/QTL. For further statistical analysis, significant main-effect and epistatic markers within each ME were identified. We used mixed linear models to calculate marker by ME interactions (MxE) and epistasis by ME interactions (MxMxE). In this study, we aim to illustrate how environmental factors influence QTLs and epistatic QTLs on phenotypic characteristics of maize. We found from four to 11 significant main-effect QTLs and epistatic QTLs for pollen and silk emergence date. For both traits, we also found at least one and up to five significant MxE interaction terms and MxMxE interaction terms. The same analyses are ongoing for plant height (PH), ear height (EH), stand count, and yield.

P32 @spalalidelen

Identification of the genetic locus to decouple the genetic linkage between harmful heavy metals and essential minerals in maize

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The production of high-quality maize grain is critical to meeting food demand for the world's growing population. However, heavy metal accumulation in cereal crops caused by soil pollution has created a widespread health problem and has a negative impact on grain quality. Cadmium (Cd), as one of the harmful heavy metals accumulating in grain, has a negative impact on human and animal health. In contrast to Cd accumulation, deficiency of essential metal elements, such as zinc (Zn) and iron (Fe), chemically similar to Cd, is usually lower in concentration than the necessary level, causing malnutrition for people in many developing countries. Current literature suggested that Cd may have a shared genetic and molecular mechanism with essential minerals, making it hard to breed crops with low Cd and high Zn and Fe concentrations. Here, using the genome-wide association study (GWAS), we identified a genetic locus strongly associated with Cd concentration, but the signal is insignificant for both Zn and Fe. This locus associated with Cd concentration but not with Zn and Fe provides a great opportunity to study the specificity of heavy metal assimilation and to genetically decouple the heavy metal and essential minerals. Further functional study of the locus will help understand the mineral accumulation and transportation process and improve the grain quality by decreasing the Cd accumulation and increasing the essential mineral contents in the maize kernels.

Funding acknowledgement: United States Department of Agriculture (USDA)

P33

Identifying the genetic regulators of leaf $\delta^{13}C$ and stomatal traits in *Zea mays*

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Leaf stable carbon isotope composition ($\delta^{13}C$) is an integrated measurement of CO_2 uptake efficiency and plant water-use strategies. In C_3 species, leaf $\delta^{13}C$ has become well established as a proxy trait for breeding improved water-use efficiency. However, the carbon concentrating mechanism present in C_4 species makes the relationship between $\delta^{13}C$ and water-use more complex. Elucidation of the genetic regulators controlling leaf $\delta^{13}C$ will enable us to identify important C_4 biological processes contributing to variation in isotopic fractionation. Here we present the use of a biparental mapping population to identify QTL controlling leaf $\delta^{13}C$ and stomatal traits in *Zea mays*. An Expired Plant Variety Protection (ExPVP) inbred that displays an extreme negative leaf $\delta^{13}C$ signature was identified in a multi-year field trial. An F_2 mapping population was produced by crossing to another ExPVP line that has an average leaf $\delta^{13}C$ value. A genotyping by sequencing library, containing the 314 F_2 individuals, was generated and produced a marker set containing ~900 filtered SNPs. Segregation of leaf $\delta^{13}C$ in the F_2 population indicates that the extreme $\delta^{13}C$ value is likely controlled by a single recessive allele. Using the software package *Rqtl*, a single significant QTL was identified on chromosome 3 for $\delta^{13}C$. Optical topometry coupled with machine learning algorithms were used to phenotype stomatal traits as a possible mechanism underlying the $\delta^{13}C$ phenotype. Although three significant QTL were identified for stomatal density (chromosome 1, 4, and 5), no significant QTL for stomatal traits colocalized with the QTL for $\delta^{13}C$. A fine mapping approach to reduce the QTL region for $\delta^{13}C$ is currently underway. Dissecting the genetic regulators will improve our understanding of the relationship between carbon fractionation and C_4 photosynthetic mechanisms.

Funding acknowledgement: United States Department of Agriculture (USDA)

P34
Impact of exotic introgressions on maize hybrid performance in multi-environment trials

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Maize landraces are highly diverse and may contain alleles that could improve a variety of traits such as disease resistance, stress tolerance, and grain quality. However, introgressions from landraces that are adapted to other parts of the world could reduce overall fitness and productivity of the resulting genotypes when grown in US environments. This study sought to characterize the effects of introgressions from Latin American landraces on hybrid performance in trials located across the US. The Germplasm Enhancement of Maize (GEM) project crossed landraces with PHZ51. The resulting hybrids were backcrossed to PHZ51 and doubled haploid (DH) induction was performed on the backcross families by GEM. A collection of 56 of those DHs, which represent 27 different landraces from 10 countries of origin, was testcrossed with tester LH195. Field trials were conducted as part of the maize Genomes to Fields initiative at 15 locations in the US and Canada in 2016 and at 10 US locations in 2017. The recurrent parent (PHZ51) was also testcrossed and included in the trials. Considerable phenotypic variation was observed for all traits measured, including ear height, flowering time, and grain moisture, but none of the experimental hybrids had significantly greater yield than the PHZ51 X LH195 hybrid. In aggregate, the experimental hybrids had better performance in southern US environments than northern ones relative to a group of reference hybrids. We hypothesized that exotic alleles might decrease phenotypic stability. However, for most traits, the stability of the experimental hybrids did not differ significantly from that of well-adapted reference hybrids. Genetic data revealed that the introgressions found in the DHs do contain haplotypes that are rare or absent in US ex-PVP lines. DH libraries derived from exotic by adapted crosses may be a useful tool for identifying beneficial genetic variation from exotic sources.

Funding acknowledgement: United States Department of Agriculture (USDA), Germplasm Enhancement of Maize Project, National Corn Growers Association, Iowa Corn Growers Association

P35
Investigating rapid progeny screening approaches using hyperspectral imaging

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Maize is the most prolifically produced agricultural crop in the United States; in 2021, 15.1 billion bushels were produced domestically. To support and improve production, significant resources are dedicated to maize breeding in both the public and private sectors to increase desirable traits. Traditional breeding often involves introducing beneficial alleles or loci from a donor parent into elite germplasm. A disadvantage of this approach is linkage drag – when the donor parent brings background deleterious alleles along with the region of interest into the recurrent parent. High-throughput tools to help identify differences in the offspring could assist in determining both the successful introduction of beneficial genetic regions and the presence of undesirable ones.

Previous work has demonstrated that hyperspectral imaging can be used to distinguish contrasting varieties of maize by examining the transmittance and/or reflectance of different wavelengths of light, caused by different biochemical properties of the leaves. In this study we tested whether spectral data could be used directly to estimate the percent parentage within the intermated B73 Mo17 recombinant inbred lines (IBM RIL 302), demonstrating usefulness of the approach in selection of offspring by providing an idea of the amount of genetic information inherited from a donor versus a recurrent parent. However, results so far indicate that partial least squares regression using the transmittance values from inbred lines do not yield a model capable of estimating percent parentage. Current analysis is focused on identifying spectral transmittance wavelengths that can be associated directly with specific loci, with the goal of finding spectral traits that can identify the presence of advantageous or deleterious genes in breeding populations.

P36

Is local adaptation of highland maize from Mexico and South America associated with different genomic regions?

(submitted by Miriam Nancy Salazar-Vidal <msalazarvidal@ucdavis.edu>)

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After the domestication of maize about 9000 years ago in the Balsas region of southwest Mexico, it began to adapt to different environments, allowing its dispersion and diversification. The spread of maize to the Mexican highlands (~2400 m.a.s.l.) has been described as the movement of maize from the Mexican lowlands to the highlands aided by hybridization with the highland adapted teosinte *Zea mays ssp. mexicana*. This hybridization and subsequent introgression enabled adaptation to low temperatures, low humidity, and high UVB light, among other environmental factors. The maize of Mexican highlands exhibits regions of *mexicana* genomic introgression, sometimes conserved as inversions, which have been associated to flowering time, macrohairs, pigmentation and UVB protection traits. Lowland maize also spread to South America, and then adapted to highlands of South America (~3000 m.a.s.l) acquiring characteristics similar to those of Mexican highland maize, but without the help of teosinte *mexicana,* suggesting convergent evolution. In this project, two F2:F3 populations derived from crosses of lowland and highland maize lines from Mexico and South America were evaluated for 20 agronomic and fitness traits in reciprocal transplant experiments at three different elevations and the same latitude. The QTL mapping is ongoing to uncover QTLs associated with adaptation to the highlands in each population and each elevation, and to compare genomic regions associated with their adaptation between populations from Mexico and South America. Results from this study will reinforce the route of spread of maize to South America and elucidate the independent mechanisms of highland adaptation. Ultimately highland maize serves as a model for adaptation that can be extended to maize adaptation to other environmental conditions, where adapted germplasm and adaptation mechanisms are much less defined.

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

P37
K-mers based GWAS in sweet corn

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Sweet corn (*Zea mays L.*) is an important vegetable worldwide and is present in many food cultures. The University of Florida sweet corn breeding and genomics lab is interested in understanding the genetic mechanism underlying variation in commercially relevant sweet corn traits, and in its application in molecular breeding initiatives. One approach to achieve this goal is the use of single nucleotide polymorphisms (SNPs) in genome-wide association studies (GWAS). However, we hypothesize that such an approach may miss important genomic regions associated with structural variation (SV) types, or with genomic regions not presented in the reference genome used for DNA alignment. Here we derived k-mers, short substrings sequences, whose presence and absence pattern can identify a broad range of genetic variants in GWAS. Whole-genome short reads from a sweet corn panel with 693 genotypes with ~9X coverage depth were used to build the k-mers and modeled against a variety of phenotypic data for traits measured during two years. From the raw sequences, the occurrence count (KOC) for k-mers of 31 bp in length was calculated for each individual. A joint table was created containing the presence/absence and the counts of each k-mers in the population. The kinship matrix of relatedness between accession was calculated with EMMA using the k-mers covariables. GWAS was conducted using a linear mixed model (LMM) in GEMMA, with k-mers filtered for minor allele frequency (MAF) 0.01. To identify known genomic associations, the k-mers were aligned to the Ia453-sh2 genome using Bowtie2. For the seed germination trait, an important sweet corn trait, our results showed promising k-mers associated with known genes in chromosomes 3 and 5. Future analysis will include the comparison of SNP-based GWAS with k-mer-based GWAS.

Funding acknowledgement: United States Department of Agriculture (USDA), National Institute of Food and Agriculture (NIFA)

P38
Kernel color variation in heirloom varieties and their properties in food.

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Corn kernels come in many colors despite being associated with yellow in the public's imagination; this is especially true for heirloom varieties. This is due to different pigments at varying levels of intensity in different tissues of the kernels. These beautiful colors can have commercial benefits due to the perceived or actual value of the kernels. For example, blue and red kernels are sought after by craft whisky makers because the color is associated with making a superior flavor in the distilled product. Orange colored kernels can make a vibrantly orange colored corn meal; this can be caused by the relationship between carotenoid pigments and the vitreous endosperm type. This project aims to investigate how different colors are produced in corn kernels and how this affects the texture, flavor and aroma of food produced from them. Many heirloom varieties lack the desirable agronomic characteristics of their commercial counterparts, so we are implementing a breeding program to improve these agronomic characteristics in heirloom varieties while maintaining the colors that make them so special.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Maximizing Access to Research Careers (MARC), Initiative for Maximizing Student Development (IMSD)

P39

Leveraging variant functional annotations to explore the role of allelic heterogeneity in genomic prediction

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A central tenet of quantitative genetics is that genetic similarity among individuals generates covariance among relatives; thus phenotypic similarities should partially reflect similarities due to allele sharing at relevant QTL. Quantitative geneticists estimate genetic similarity between individuals in a population based on allele sharing at genome-wide SNP markers, and leverage these similarities to estimate important genetic parameters (e.g. heritability) and make predictions for untested, related individuals (i.e. genomic prediction). While genetic evaluations from such estimates are used effectively in plant breeding, in cases when independent variants can have the same functional effects on a gene -- a phenomenon referred to as allelic heterogeneity -- allele sharing at SNP markers may inadequately capture the underlying genetic basis of the trait. To account for these issues, we devised a strategy using variant-level annotations to aggregate functionally-equivalent alleles to gene-level alleles. We used three large resequencing datasets in maize, Arabidopsis and soybean to identify loss-of-function (LoF) variants and collapsed multiple LoF variants in each gene into gene-level LoF alleles. Genomic relationships between individuals were estimated based on the degree of allele sharing at each LoF gene. These data were used for genomic prediction of 86 traits in maize, and six and three traits in Arabidopsis and soybean respectively. We show that predictions for 21 of the traits in maize were significantly improved after accounting for allelic heterogeneity, while no improvements were observed in the other species. These results suggest that there may be some value in accounting for allelic heterogeneity in genomic prediction, but the benefit is likely dependent on the genetic architecture of the trait, as well as the prevalence of LoF variants and genes in each population.

P40

Maize genetic diversity and microbial interactions

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Manipulating crop microbiomes has exciting potential for agriculture. There is, however, a lack of research in how the plant host affects the interaction with both individual beneficial microbes and the entire microbiome. We quantified how different maize genotypes respond to and affect the microbiome in a series of experiments. First, we compared the effects three growth-promoting endophytes on diverse maize varieties in the greenhouse. Growth assessment of 3- week-old seedlings found significant growth promotion in a subset of maize genotypes, with the specific phenotypes affected depending on the maize genotype. We also found that the abundance of one endophyte, *Serendipita bescii*, correlated with increased growth recorded in the greenhouse. Despite these individual differences, the overall effect of host genotype on the interaction was low. In a second experiment, we compared the rhizosphere and endophyte communities of inbred, hybrid, and open-pollinated maize grown in the greenhouse and field. We found significant differences in community diversity, makeup, and predicted function based on the experimental group. Finally, inoculating greenhouse-grown maize with microbiomes from two inbred maize lines (B73 and Mo17) and their F1 hybrid had the unexpected result of decreasing growth when one parent (B73) and the hybrid were used as inoculate sources. Overall these results demonstrate that maize genotype has a small but significant effect on interaction with individual endophytes, and that these differences have the potential to impact later generations of crops.

Funding acknowledgement: FFAR, University of Georgia Graduate School

P41

Mapping QTLs contributing to germination and seed vigor in *Sorghum Bicolor*

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Germination is a process beginning with a seed's ability to imbibe water and ending with its extension of its radicle. A seed's ability to germinate and emerge, even under adverse environmental conditions, is referred to as seed vigor, which can be quantitatively and phenotypically measured by stand count. The objectives of this study were 1) to identify quantitative trait loci (QTLs) affecting seed germination and seed vigor, and 2) to compare the shared or distinct genetic regions between these two traits. A sorghum population containing 242 recombinant inbred lines derived from Tx430 and P898012 were used in this study. Seed vigor was measured by counting the plot stand in the field 22 days after planting (DAP) and repeated for three years. Seed germination experiment was conducted in the lab with Petri dish using filter paper and deionized water under dark condition. Germination rate was determined by the number of seeds with a visible radicle after two weeks. QTLs were identified for both traits with composite interval mapping in Windows QTL Cartographer. We identified six QTLs that likely control seed vigor; three of which showed up in all three years, and two were identified on chromosome (Chr) 7 as qHT7.1 and Dw3. These two QTLs have previously been identified as contributing to plant height heterosis. Three QTLs were identified for germination; two of which were co-localized with the seed vigor QTLs on Chr 4 and 6. The correlation between germination rate and stand count showed a significant correlation of 0.68 (p

P42

Mapping freezing tolerance in *Tripsacum* by bulk segregate analysis

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One consequence of climate change is increased variability in weather patterns. Some agronomic solutions include shifting planting zones north or planting earlier to avoid excessive heat during flowering. These solutions, along with more common weather extremes, are making crops more likely to encounter freezing events. For these reasons it is imperative that research is conducted to improve freezing/chilling tolerance in maize to maintain yields in the years to come. *Tripsacum dactyloides*, a close relative of maize, having diverged less than 1MYA, can be found across the Americas. As a perennial, this species must withstand elongated freezing temperatures in northern latitudes to survive. The close relationship of Tripsacum to maize may indicate a transferability of this freezing tolerance trait. To identify causal freezing tolerance genes we are using a bulk segregant analysis to find QTL within a diverse Tripsacum population. An initial founder population of 304 clones was collected from across the US and used to generate an F1 population from crosses between northern and southern clones. F2 seed was then generated for use in freezing screens where seedlings were brought to -7°C overnight. The diversity and structure of this population allows for both linkage and association mapping studies to be conducted. Illumina short read sequencing was performed on the founders, F1s and the F2 bulks. To identify founder contributions to each bulk, Pac-Bio Hifi genomes have been generated for two founders to be used as reference genomes in a custom pipeline for variant calling and phasing. Preliminary linkage mapping results within some crosses have shown founder contributions segregate between bulks. Expanding the analysis to the remaining crosses should improve the resolution of linkage mapping and allow for identification of potential causal freezing tolerance QTL.

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

P43

Missouri heritage corn: A flavorful source for distillation & food products

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Many US whiskeys and bourbons are distilled from standard commodities such as No. 2 yellow dent corn. However, the craft distilling industry in Missouri and elsewhere is exploring the variation present in "landrace" (our preferred term is "heirloom") populations to create novel whiskies with distinct taste and/or aroma profiles. Other maize-related industries may also benefit by developing niche products using heirloom populations. In 2021, we initiated The Missouri Heritage Corn Project focused on identifying suitable candidates for use in distillation and food products and exploring the diversity present in each heirloom population. The primary goal is to investigate variation among 50 different Missouri heirloom populations and evaluate agronomically important traits such as flowering time, plant/ear height, and overall performance in Missouri. We are testing grain characteristics relevant to food and alcohol production using NIR spectroscopy and will create test batches of each population to examine flavor and aroma. Another goal is to determine if heirlooms with similar common names such as "St. Charles" and "Big St. Charles White" have the same or independent origin, based on phenotypic contrasts between suspected populations. These populations were historically grown in Missouri climates; therefore, we may reveal traits associated with adaptation specific to various regions of Missouri. Heirloom maize populations provide an untapped resource for food and alcohol industries to create flavorful and/or potentially more nutritious maize derived products.

P44

Nested function-value traits as a framework to explore quantitative traits

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Function-value traits (FVTs), parameter-phenotypes that describe models fitted to time series data, are proving to be an increasingly valuable tool for exploring genetics and physio-development. However, the impact ontogeny has on FVTs resulting from serially produced organs such as leaves has been a neglected area of study. Here we present a second-order function-value trait (2FVT) model capable of describing growth of organs across ontogeny which we developed from pseudo-landmark phenomics data. Using the framework provided by this model, we explored the genetics underlying culm height in a Setaria RIL population. We found that the 2FVT parameters describing variation in stature were heritable, and that their breeding values could be reconstituted to describe 88% of the heritability underlying height. In addition, 26% to 51% of the phenotypic variance for the 2FVT parameters could be explained by 13 shared QTL that variably colocalized between these parameters. When the additive effects of the QTL were tested using simulations that assumed phytomers appear progressively over time, we were able to discern that some QTL play major roles across the development of the plant causing them to disproportionately influence life history strategy. Furthermore, morphospace-based simulations provided further evidence of major loci that are prone to selective sweeps when maximizing attributes related to height. Finally, structural equation models were able to show that these QTL represent two orthogonal gene networks from which height results as a composite trait. Our results demonstrated the capacity of the 2FVT model to provide novel insights in physio-developmental genetics by explicitly modeling the ontogenetic relationships. We also discuss potential applications of the 2FVT model in phenomics and in understanding genome-to-phenome interactions across ontogeny and varying environments.

Funding acknowledgement: National Science Foundation (NSF)

P45

Optimization of sweet corn speed breeding for advancement of fresh kernel nutritional quality traits

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In today's world, 2 billion people suffer from hidden hunger, the lack of adequate micronutrients in a diet with adequate caloric intake. Even in the US, up to 88.5% and 43.0% of the population suffer from deficiencies in micronutrients vitamin E (tocochromanols) and vitamin A (carotenoids), respectively. However, biofortification of crops offers an avenue to increase micronutrients via breeding. Sweet corn is an excellent candidate for biofortification of micronutrient traits in the US because it is one of the most commonly consumed vegetables in the US, and because significant variation in vitamin E and provitamin A has been found in temperate sweet corn germplasm. As in all plant breeding efforts, genetic gain for sweet corn biofortification is limited by generation time. We address this constraint with speed breeding, a method in which abiotic environmental factors such as light quality and quantity, nutrient availability, and temperature are manipulated to reduce time from planting to flowering, thus reducing generation time in a breeding program. In this work, we manipulated light quality and photoperiod in combination with high daily temperatures and rigorous foliar supplement application to decrease the amount of time to flowering on a set of sweet corn inbred lines that have been selected from a diversity panel for their content and composition of tocochromanols and carotenoids in fresh kernels. Moving forward, this speed breeding protocol will be used to advance breeding populations generated from the same set of inbred lines, and will be combined with genomic selection to rapidly improve fresh kernel nutritional quality.

Funding acknowledgement: National Science Foundation IOS-1546657, The National Institute of Food and Agriculture, USDA Hatch under accession number 1023660

P46

Phenotyping a new maize shoot mutant in inbred and F1 hybrid genetic backgrounds

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Maize mutants are best characterized by first converging mutant alleles into a uniform, typically inbred genetic background and then phenotyping, to understand specific effects of a mutation independently of other factors that may segregate in a population. Similarly, researchers working with mutants are anecdotally familiar with mutant phenotypes differing between inbred and hybrid genetic backgrounds. On the other hand, in industry hybrid maize is used for crops and an F1 hybrid displays phenotypes distinct from its inbred parents. Therefore, we are quantifying phenotypic differences for a shoot architecture mutant allele between inbreds and hybrids by conducting field phenotyping and comparing observed traits. We have addressed this question by studying a novel, recessive mutant initially discovered in a B73/W22 hybrid background given the temporary name leafy* (lfy*) due to some resemblance to mutants of the maize zfl genes, homologs of the Arabidopsis LEAFY gene. lfy* mutants have a range of phenotypes including altered leaf number, shortened internodes and feminized tassel branches, making them appropriate for studying this question. The original mutant allele was introgressed into four standard corn belt inbreds (A619, B73, Mo17 and W22) which were then used to also produce segregating wildtype and mutant lfy* progeny within uniform, F1 hybrid backgrounds. Inbred and hybrid populations were phenotyped in the field for developing and mature plant phenotypes such as phase change, plant height and other potentially mutant-affected characteristics. From these results we will present an overview of the lfy* mutant phenotype and compare it in hybrids versus inbreds. We are also working to clone the causal gene for this mutant phenotype. From map-based approaches we previously narrowed to a genomic interval of about twelve candidate genes. By using a nearby Ds transposon in a targeted tagging approach we have now identified an additional allele which should aid in gene identification.

P47 @lindachidao

Phenotyping for cold tolerance in sweet corn seedling emergence

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Sweet corn is a warm-season crop; however, cold tolerant varieties are desired for early spring plantings. Historically, commercial sweet corn germplasm contained sugary1 (su1) or a double mutant of su1 with sugary enhancer1 (se1). Contemporary sweet corn predominantly employs shrunken2 (sh2) germplasm, which has poor emergence in cold soil conditions. We evaluated seedling emergence in 584 lines from a diversity panel of sweet corn germplasm. Kernels were treated with a Pacific Northwest blend of insecticides and fungicides. The kernels were sown in potting mix and grown in a 14°C/14 hour 10°C/10 hour daily cycle for 35 days. Seedling emergence was recorded daily. Additionally, emergence at 24°C for eight days was scored by VIGOR, a high-throughput soil-based machine vision platform. Emergence time and emergence frequency were measured in both conditions. Consistent with prior studies, sh2 lines showed poor emergence in cold conditions, while su1 lines fared better. Cold stress growth results are positively correlated (r = 0.65) to a cold stress field trial in central Washington using the same lines (N = 550) and seed treatment. The top ten performing sweet corn lines in terms of emergence frequency were su1 mutants, su1 se1 double mutants, and control field corn lines . Genome-wide association (GWAS) of emergence time found significant associations at three loci (after accounting for su1, sh2, and se backgrounds). Our results identify additional genetic variation that can be investigated for use in improving sh2 seedling vigor.

Funding acknowledgement: United States Department of Agriculture (USDA)

P48

Plasticity of sorghum panicle architecture in response to nitrogen deficit stress

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Nitrogen is an essential nutrient required for growth and development in plants, and insufficient nitrogen availability can reduce both vegetative growth and grain yield in grain crops such as maize and sorghum. However, nitrogen is a costly input for farmers, is energy intensive to manufacture, and run off of excess nitrogen fertilizer impacts water quality. Compared to its close relative, maize, sorghum [*Sorghum bicolor* L. Moench] exhibits much greater resilience to nitrogen, drought, and heat stress, allowing it to be grown with fewer inputs and on marginal land. Research has shown that variation in grain yield between sorghum accessions, as well as between nitrogen conditions, can be partially explained by differences in inflorescence architecture traits. Previous genome wide association studies (GWAS) of inflorescence traits have reported genetic markers associated with known genes controlling growth and development, but not under varying nitrogen conditions. To investigate the effects of nitrogen stress on inflorescence development, eight inflorescence traits, including six panicle architecture traits, were phenotyped on 347 diverse inbred lines of the Sorghum Association Panel (SAP) grown under both standard nitrogen application and no nitrogen application treatments. Rachis length, upper and lower rachis diameter, primary branch number, primary branch first internode length, and magnitude of branching were all significantly decreased under nitrogen deficient conditions ($p \leq 0.05$), suggesting these traits are negatively impacted by nitrogen stress conditions. All eight traits show high broad-sense heritability for both nitrogen treatments ranging from 0.43 to 0.86, indicating a notable portion of the variation in these traits can be attributed to genetic factors. Currently, genome wide association studies are being conducted to identify genetic markers associated with these panicle architecture traits in order to better understand the genetic factors involved in nitrogen stress response for potential use in breeding improved sorghum varieties.

Funding acknowledgement: Department of Energy (DOE)

P49 @burnsmj7

Predicting moisture content during maize nixtamalization using machine learning with NIR spectroscopy

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Lack of high throughput phenotyping systems for determining moisture content during the maize nixtamalization cooking process has led to difficulty in breeding for this trait. This study provides a high throughput, quantitative measure of kernel moisture content during nixtamalization based on NIR scanning of uncooked maize kernels. Machine learning was utilized to develop models based on the combination of NIR spectra and moisture content determined from a scaled-down benchtop cook method. A linear support vector machine (SVM) model with a Spearman's rank correlation coefficient of 0.852 between wet lab and predicted values was developed from 100 diverse temperate genotypes grown in replicate across two environments. This model was applied to NIR spectra data from 501 diverse temperate genotypes grown in replicate in five environments. Analysis of variance revealed environment explained the highest percent of the variation (51.5%), followed by genotype (15.6%) and genotype-by-environment interaction (11.2%). A genome-wide association study identified 26 significant loci across five environments that explained between 5.04% and 16.01% (average = 10.41%). However, genome-wide markers explained 10.54% to 45.99% (average = 31.68%) of the variation, indicating the genetic architecture of this trait is likely complex and controlled by many loci of small effect. This study provides a high-throughput method to evaluate moisture content during nixtamalization that is feasible at the scale of a breeding program and provides important information about the factors contributing to variation of this trait for breeders and food companies to make future strategies to improve this important processing trait.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Iowa Agriculture and Home Economics Research Station

P50

PyBrOpS – Python Breeding Optimizer and Simulator

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Choosing a suitable breeding strategy is essential to the success of a plant breeding program. Simulations are an important tool that allow plant breeders to propose and assess the merits of alternative breeding strategies. The Python package PyBrOpS provides a highly flexible and modular framework to make optimized breeding selection decisions and perform stochastic simulations of plant breeding programs. PyBrOpS utilizes a customizable scripting-based approach to constructing breeding simulations and optimizations. Through the use of software interfaces that allow for extensibility, the user may implement custom PyBrOpS modules that provide additional functionality. PyBrOpS offers pre-built subroutines for selection strategies such as conventional genomic selection, weighted genomic selection, optimal contribution selection, optimal population value selection, and optimal haploid value selection. Additionally, PyBrOpS is capable of both single- and multi-trait selection. For multi-trait selection scenarios, PyBrOpS offers the novel capability of mapping trade-off frontiers through the use of multi-objective evolutionary algorithms. Here, we describe the main features of PyBrOpS and provide example use cases for breeding program simulation and optimization.

Funding acknowledgement: National Science Foundation (NSF), NSF Research Traineeship Program (DGE-1828149), MSU Plant Science Fellowship, MSU Plant Resilience Institute

P51

QTL mapping of seedling tolerance to exposure to low temperature in the maize IBM RIL population

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Maize is a cold-sensitive crop that exhibits severe retardation of growth and development when exposed to cold spells during and right after germination, including the slowdown in the development of new leaves and in formation of the photosynthetic apparatus. Improving cold tolerance in maize would allow early sowing to improve crop yield by prolonging a growing season and by decreasing the negative effects of summer drought, diseases, and pests. Two maize inbreds widely incorporated into American maize germplasm, B73 and Mo17, exhibit different levels of tolerance to low-temperature exposure at the seedling stage. In addition, thirty-seven diverse inbred maize lines showed large variation for seedling response to low-temperature exposure with lines with extremely low tolerance to seedling exposure to low temperatures falling into stiff stalk, non-stiff stalk, and tropical clades. We employed the maize intermated B73×Mo17 (IBM) recombinant inbred line population (IBM Syn4 RIL) to investigate the genetic architecture of cold stress tolerance at a young seedling stage and to identify quantitative trait loci (QTLs) controlling this variation. A panel of 97 recombinant inbred lines of IBM Syn4 were used to measure, and score based on several traits related to chlorophyll concentration, leaf color, and tissue damage. Our analysis resulted in the detection of two QTLs with high additive impact, one on chromosome 1 (bin 1.02) and the second on chromosome 5 (bin 5.05). Further investigation of the QTL regions using gene expression data provided a list of the candidate genes likely contributing to the variation in cold stress response. Among the genes located within QTL regions identified in this study and differentially expressed in response to low-temperature exposure are the genes with putative functions related to auxin and gibberellin response, as well as general abiotic stress response, and genes coding for proteins with broad regulatory functions.

Funding acknowledgement: National Science Foundation (NSF)

P52 @S_Oliver17

Reducing free asparagine content in maize to minimize acrylamide formation potential

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When starchy crops with high free asparagine content are cooked in low moisture, high temperature environments such as baking, frying, and roasting, the Maillard reaction occurs. It is beloved in the culinary community for producing the flavors, aromas, and browned colors associated with delicious foods including chips and bread. However, it also produces the carcinogenic and neurotoxic compound acrylamide. Since this discovery in 2001, there has been a push in the plant breeding community to reduce the amount of acrylamide precursors in food crops. In particular, free asparagine content. Asparagine is vital for nitrogen transport throughout plant during growth, maintenance, and senescence. Though it can be greatly influenced by factors such as nutrient stress, average free asparagine content appears to be heritable in most species. Most of the work done thus far has been on potatoes and wheat. Despite maize being one of the most important staple crops worldwide, there is a lack of research conducted on it regarding this topic. We performed genome wide association study (GWAS) on free amino acids in the 282-association panel. We found 74 significant SNPs for free asparagine, corresponding to 477 candidate genes, based on a 200kb confidence window around the SNP. We also performed weighted protein correlation network analysis to associate protein expression with free asparagine content during kernel development. Here, we identified 2578 proteins/genes with a significant correlation with free asparagine. Comparison of the two analyses yielded an overlap of 39 genes, many of which are in amino acid synthesis pathways. Several of which are involved with the translational machinery such as ribosomal proteins. With this data, we hope to narrow down the top genes that regulate the free asparagine in maize seeds and potentially engineered it in a way that does not compromise flavor and quality of its products.

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

P53
Relating leaf area index to sorghum canopy traits

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In a world with growing population and scarcity of natural resources, efficiency is vital. Sorghum, a C4 grass native from East Africa, has potential to meet this modern world requirement. With over 7 million acres planted in the US and about 1 million acres planted in Eastern and Southern Africa, sorghum is a plant well adapted to high temperatures and dry conditions. It is largely used as feed for cattle, both as silage and grain, as a source of nutrition for humans and more recently, as a source of biodiesel.Leaf Area Index is defined as the total leaf area per unit of ground area; for sorghum, a typical range was found to be between 3 and 5 in fully developed canopies. LAI is an important indicator of canopy architecture development, and relates to light interception as higher LAI indicates greater capacity for light capture. This increased energy is then transformed into biomass and grain. The focus of this project was to relate LAI to detailed phenotypic data collected in 391 lines of the Sorghum Association Panel (SAP) during Summer 2020. This study seeks to understand how canopy phenotypic traits such as plant height, leaf number, leaf area, leaf size, stalk diameter, panicle number, and tiller number, etc. relate to LAI and biomass production in the diverse sorghum genotypes. Overall, we found that individual traits are not highly correlated with LAI directly, but may be combined to model and predict LAI. This work will increase our understanding of the impact of component traits that contribute to overall canopy architecture and therefore light capture. Future work will leverage high-throughput phenotyping tools and drone-based imagery to model canopy architecture and biomass production.

Funding acknowledgement: Department of Energy (DOE), Michigan State University College of Agriculture and Natural Resources URP

P54
Resistance of maize genotypes against *Fusarium verticillioides* isolates with different pathogenicity in artificial inoculation experiments

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The benefit of artificial inoculation experiments using highly aggressive isolates is the predictable severity of ear rot disease, which is the basis of effective resistance breeding. In 2019-2020, we examined the pathogenicity of 8 *Fusarium verticillioides* isolates on eight maize hybrids with different resistance properties based on our previous studies. There was a twofold difference in regards of infection severity between different *F. verticillioides* isolates, and a fourfold difference regarding ear rot coverage of the hybrids tested. There were large (3.85-21.00 mg/kg) differences between the fumonisin concentrations produced by the isolates and in the mycotoxin contamination of the tested hybrids (4.71-17.26 mg/kg). There was no significant correlation between the ear rot coverage data and the toxin determination results of the samples (R^2= 0.1339), which confirmed the weak relationship between these two factors. To determine which experimental model might be suitable for resistance breeding, the extent of infection caused by the eight isolates was compared with systems, which consist of two, three, and four most pathogenic *F. verticillioides* isolates, respectively. Based on the correlation analysis of the mean infection values the four isolates model showed the highest correlation (r = 0.9901) with the eight isolates model, while the three and two isolates models had slightly lower coefficients (r = 0.9888 and r = 0.9790). The highest correlation was observed between the three and four isolates models, respectively (r = 0.9987). There was a significant difference between the pathogenicity of the isolates in each case, but the level of significance increased in direct proportion to the number of isolates used. There were also significant differences in regards of resistance against *F. verticillioides* isolates of the experimental hybrids in each case. Therefore, the proposed methodological recommendation (considering economic factors) is the use of three isolates per toxic fungal species in artificial inoculation studies.

Funding acknowledgement: Ministry for Innovation and Technology (ITM); National Research, Development and Innovation Office (NRDI Fund); Science Patronage Programme (MEC_R 140708)

P55 @martincostamon

Selection signatures underlying genotype by environment interaction during modern hybrid maize breeding

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Since the advent of hybrid maize, breeders have made remarkable progress in the productivity and stability of cultivars. However, understanding sources of "good" alleles for performance and plasticity could benefit breeders in selecting varieties for diverse environments. In this study, we analyzed the 2018-2019 data set released by the Genomes to Fields Initiative. This study used a group of three populations with PHW65 as a reference parent and PHN11, Mo44, and MoG as alternate parents. The common parent, PHW65, is an ex-PVP Lancaster-type line, PHN11 is an ex-PVP line from the Iodent group; Mo44 is a tropical-derived inbred from the cross of Mo22 and Pioneer Mexican Synthetic 17; and MoG is an unimproved line derived from the variety Mastadon. Hybrids were produced by crossing segregating progenies to Stiff Stalk testers PHT69 and LH195, and trials were grown across the United States. Firstly, a two-stage analysis was conducted to compare the hybrid performance and genotype by environment interaction (GxE) variance of the populations. We hypothesized that the population involving ex-PVP would have the least GxE variance, and this was confirmed across all eight traits analyzed. In addition, a Finlay Wilkinson Regression Analysis of grain yield was performed, and the inter-quantile range of the mean-squared error values was 13% more dispersed for the PHW65-MoG family. Ongoing analysis will determine if less-selected parents contain some useful alleles to contribute to stability despite their overall lower breeding value.

Funding acknowledgement: United States Department of Agriculture (USDA), Iowa Corn Growers Association, United States Department of Agriculture - Economic Research Service, Foundation for Food and Agricultural Research

P56

Speech-based phenotyping methods for field studies

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Field-based phenotyping of maize is time-consuming, cumbersome, and generally requires the evaluation of predefined traits of interest. We aim to expand researchers' field phenotyping "toolbox" by developing methods for collecting computationally tractable speech-based phenotyping useful for applications including, but not limited to, association genetics research. As a proof of concept, we designed an experiment that compares speech-derived trait concepts with traditional quantitative trait data collected by hand using the Wisconsin Diversity panel (grown in Ames, Iowa summer 2021). Details on methods, expectations, and current status for the project will be described.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), ISU PSI, P3 NSF NRT, AIIRA NSF USDA, IOW04714 Hatch funding to ISU

P57

Spontaneous haploid genome doubling (SHGD) mechanism to accelerate crop breeding

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Growing population and shrinking agricultural resources are one of the most important challenges facing food production. To meet the projected increase of global demand for food, feed, and fiber (100% by 2050), the linear progress will need to be increased by accelerating genetic improvement. Doubled Haploid (DH) technology can produce homozygous and homogeneous lines in two rather than five or more generations - a breakthrough to reduce cycle time for pure line and hybrid crop breeding. Large-scale production of DHs in maize has become economically viable within the last ca. 20 years. In a traditional DH program, F_1 is made between two inbred parents, which is then pollinated with a maternal haploid inducer to produce haploid kernels. In maize haploids, there is a decent amount of haploid female fertility, while haploid male fertility (HMF) is the bottleneck. This requires the haploid seedlings to be treated with colchicine (or other chemical treatments) for genome doubling and then transplant them, typically using a vegetable transplanter – in contrast to direct sowing of maize kernels. The current process is very time consuming and labor-intensive, making it a bottleneck for making DH technology in maize more effective. The alternative is spontaneous haploid genome doubling (SHGD) mechanism(s), which can restore HMF. Our group has identified one line with SHGD, and a major QTL has been mapped to chromosome 5. The underlying genes have not been identified yet. Our current efforts are focused in identifying gene(s) contributing to SHGD, which can then be used in breeding programs.

Funding acknowledgement: United States Department of Agriculture (USDA), FFAR, Plant Sciences Institute (ISU)

P58

Study of *Aspergillus flavus* resistance in maize Italian breeding varieties

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Aspergillus flavus is a saprophytic and opportunistic fungus that infect multiple crops such as maize. The fungus grows in the maize kernels producing high concentration of aflatoxins, potent carcinogenic toxins. Aflatoxin contamination not only reduces the value of grain as an animal feed and as an export commodity but also has been linked to increased mortality in farm animals and increased incidence of liver cancer in humans. In this contest, we consider crucial to establish a methodology to measure fungal resistance in new maize varieties under procedure to be registered in the Italian catalogue. Here we show some preliminary results on the characterization of resistance levels of maize varieties after inoculation of the kernels with a fungal strain belonging to *Aspergillus* section *Flavi*. The selected genotypes for the analysis are part of our reference collection composed by breeding varieties registered in the Italian register since 2000. Disease phenotypes were scored on artificially inoculated kernels using rolled towel assay in which the severity of the symptoms was determined using a five-point scale. We measured - after 7 days of incubation at 25°C in the dark - germination percentage, seedling length and weight. Our first observations showed that the tested inbred lines responded differently according to the fungal infection. A limited fungal colonization was found in genotypes where the seedling growth were reduced compared to the control and the germinability was slightly affected. Otherwise, in other inbred lines, a pronounced fungal infection was recorded, impacting on morphophysiological development of the plant. No resistant genotypes were identified so far. As maize resistance to *A. flavus* is a quantitative and complex trait controlled by high number of genes with small effect, we are interested to consider the data collected with the laboratory screening to perform a genome wide association study.

P59

Temporal analysis of maize canopy cover using aerial high-throughput phenotyping

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Canopy closure is an important agronomic trait influencing photosynthesis, weed suppression, biomass accumulation, and yield. Plants capable of maximizing early season light interception have a competitive advantage during vegetative and reproductive developmental stages. Canopy closure is difficult to accurately quantify as conventional in-situ methods rely on time-consuming, subjective, manual ratings. Little is known about the stability of this trait across growing seasons, the genetic architecture of canopy closure, or how causal factors change throughout the growing season. We used unoccupied aerial vehicles to quantify plot-level canopy closure for a temporal analysis of the genomic landscape of this trait. Aerial RGB images of the maize Wisconsin Diversity Panel were acquired between 300 and 1650 growing degree days (GDD) in 2018-2021 at the University of Minnesota, St. Paul campus experimental station. A loess curve was modeled for each plot, estimating closure at 50 GDD intervals to allow for equivalent comparison of canopy cover at parallel growth stages across years. Several traits were extracted and calculated from the images including percent closure at each GDD, rate of closure, and area under the growth curve at terminal GDD. An additive main-effects and multiplicative interaction (AMMI) model and Bayesian regression model were used to calculate multiple AMMI stability metrics and Finley-Wilkinson coefficients for each genotype to study phenotypic plasticity of canopy cover between years. In addition, a temporal GWAS was performed to associate markers across time and between trait estimators. This study explores the phenotypic plasticity of canopy closure in maize across years, provides a temporal dissection of the changing genomics influencing canopy cover throughout maize development, and highlights the extraction and extrapolation of multiple trait metrics from aerial images.

Funding acknowledgement: United States Department of Agriculture (USDA), Bayer Crop Science

P60

Terminal ear1 and *PhytochromeB1; PhytochromeB2* act independently to regulate leaf initiation in *Zea mays*

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Leaf initiation is an essential part of development in higher plants, yet very few genes are known to regulate this process directly even in model species. There are two maize mutants, however, *terminal ear1* (*te1*), and the p*hytochrome B1*, p*hytochrome B2* (phyB1; *phyB2*) double mutant that exhibit dramatic differences in total leaf number and leaf initiation rate. *Te1* negatively regulates the initiation of new leaves, as the *te1* mutant exhibits a significant increase in total leaf number. *PhyB1*, and *PhyB2* act redundantly to promote leaf initiation, as *phyB1; phyB2* double mutants have drastically fewer leaves than wild-type. We crossed these distinct mutants to test whether they interact to influence leaf initiation rate and total leaf number. We created an F2 population segregating for these three genes and quantified differences in leaf number, leaf initiation rate, plant height, leaf length, leaf width, number of juvenile leaves, stalk diameter, and dry shoot biomass. The total number of leaves in the triple mutant, as well as the rate of leaf initiation, fell between the extremes of the two parents, suggesting an additive genetic interaction between *Te1* and *PhyB1; PhyB2*. Additionally, we found that the *phyB1; phyB2* double mutant exhibited a dramatic environmental response between greenhouse and field trials. The triple mutant showed a similar environmental response as well, suggesting the regulation of leaf initiation by *PhyB1* and *PhyB2* is particularly sensitive to external cues.

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

P61

The exploration of UAS-based multispectral imaging in field phenotyping

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Maize is one of the most widely distributed of the world's food crops. To maximize breeding efficiency, accurate estimates of phenotypes are required at a large scale. Maize has high phenotypic plasticity, with phenotypic variance reflecting genetic, environment, and genetic x environment interactions. The goal of this study was to investigate high-throughput phenotyping methods to determine their relationship to agronomic and developmental traits, as well as their genetic control. This study was conducted in the 2018-2019 Michigan location of the Genomes 2 Fields project. We collected traditional ground-based phenotypic traits and utilized an unoccupied aerial system (UAS) equipped with natural and multispectral cameras to capture images throughout the whole growing season. Based on these images, we extracted canopy cover throughout the season and calculated vegetative indices such as NDVI, GDNVI, NDRE, SAVI, EVI, BGI and TVI. We then established the relationship between hand measured traits and vegetative indices and attempted to predict the hand measured traits. Vegetative indices were also treated as novel traits, with considerably high heritability. Genome-wide mapping studies across both trait datasets revealed that regions associated with spectral reflectance itself were different from those associated with hand measured traits. Future work will compare accuracies of genomic prediction, phenomic prediction, and a combination of approaches.

Funding acknowledgement: Corn Marketing and Promotion of Michigan, Iowa Corn Board

P62

The impact of structural variation on heterosis and combining ability in maize

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The growing demand for grain and increased strains on agricultural production systems necessitate the development of superior maize hybrids. The role of genomic structural variation (SV), specifically genic copy number and presence/absence variation, in the expression of heterosis in maize is poorly understood. The objective of this study was to determine the proportion of heterosis and specific combining ability in agronomically important maize traits caused by structural variation. Our hypothesis is that structural variants play a significant role in heterosis. 400 RIL-derived maize hybrids were produced and phenotyped in 11 environments over 2 years. Several phenotypic traits were measured, including yield, plant height, ear height, and kernel test weight and moisture. The amount of variation in GCA and SCA explained by structural variants was quantified using a 2-stage mixed linear model approach. In the first stage, BLUEs were calculated on a single environment basis for each trait. In the second stage, parent relationship matrices and hybrid dominance matrix were included in a multi-environment model. The genotypic markers used to calculate the dominance matrices were split into five categories: SNPs+SVs, only SNPs, only SVs, SNPs in LD with SVs, and SNPs not in LD with SVs. The second stage resulted in GCA and SCA variances for each trait and marker type. Except for moisture for the "Parent A" group and yield for the "Parent B" group, GCA variances were greatest in SV models for all traits. GCA variances were generally smaller than SCA variances.

Funding acknowledgement: United States Department of Agriculture (USDA), National Institute of Food and Agriculture (NIFA), Illinois Corn Marketing Board

P63

The sugary enhancer1 (se1) allele is associated with significant decreases in the amount of lutein, zeaxanthin, and tocotrienols in yellow (Y1) sugary1 (su1) kernels

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A distinctive phenotype of homozygous *sugary enhancer1* (*se1*) sweet corn inbreds and hybrids is a light yellow color in mature kernels. Carotenoid pigments contribute to yellow endosperm color in maize and are of nutritional importance in the human diet, along with the related tocochromanols, or the E vitamins. Tocochromanols, including both tocopherols and tocotrienols, are antioxidants important in human nutrition and cardiovascular health. Production of tocotrienols, like carotenoids, occurs in the endosperm. Identifying an interaction between *se1* and carotenoid or tocochromanol synthesis will both generate hypotheses for future studies and inform breeders on the role of *se1* in improving the nutritional quality of sweet corn. Using the Wisconsin Sweet Corn Diversity Panel, we evaluated the effect of the *sugary enhancer1* allele on carotenoid and tocochromanol levels in homozygous *su1*, *Y1* endosperm. While population structure was present in the panel, the majority of variation in carotenoid levels was explained by genotype at the *se1* locus. *se1* was associated with significant decreases in the amount of lutein (54%) and zeaxanthin (36%) and decreases of tocotrienols by 12-65%. There were no significant differences in β-carotene and tocopherol levels between the two groups. Given that the biosynthesis pathways for carotenoids and tocochromanols are well-defined, these differences in carotenoids and tocotrienols between *su1Se1* and *su1se1* inbreds further suggest a broader role of *Se1* alleles in metabolic pathways beyond endosperm starch production.

Funding acknowledgement: United States Department of Agriculture (USDA)

P64

Towards the understanding of the genetic control of lateral root development in adult maize plants

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In maize, the genetic control of root system architecture in the adult stage of the plant is largely unknown, despite its importance to sustain plant growth and contribute to yield potential. We used a large population of doubled-haploid (DH) lines derived from three European maize landraces to map quantitative trait loci (QTL) for seedling root traits evaluated in a high-throughput phenotyping platform. In a subset of the DH lines, we collected additional data on root system architectural traits in stage R3 and observed significant genetic variation for lateral root density and length. We selected two DH lines differing in haplotypes associated with root traits throughout plant development but showing high molecular similarity in the rest of the genome. We generated a bi-parental mapping population with the objective to investigate the genetic control of lateral root development in adult maize plants and found four significant QTL. Currently, QTL regions are fine-mapped and a greenhouse assay that can represent field conditions is established for faster screening of recombinants.
In the future, we will investigate the relevance of lateral root development in maize for agronomic traits such as nutrient and water use efficiency as well as plant anchorage and aim to transfer new favorable alleles from landraces into maize breeding germplasm.

Funding acknowledgement: Federal Ministry of Education and Research (BMBF)

P65 @mandmeier

Uncovering links between maize genetics and root-colonizing microbes under nitrogen stress

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Root-colonizing microbes have been shown to promote the growth and development of the host plant. However, it remains largely unknown to what extent the host genome affects root microbial communities. A set of 230 diverse maize inbred lines were grown under standard agronomic practices and nitrogen (N) deficient conditions in a two-year field experiment and the composition of rhizosphere microbial communities was assessed through 16S amplicon sequencing of 3,300 rhizosphere samples. We constructed a core microbial community that consists of 150 highly abundant and consistently reproducible microbial groups at the family, genus, and species level. Distinct compositions of microbial communities were observed between genotypes, and between the same genotypes grown under different N treatments. Our analysis suggests that 79 microbial groups (i.e. "rhizobiome traits") are heritable under either or both N treatments and that 34 traits are under adaptive selection. Genome-wide association studies (GWAS) using a genetic marker set of approximately 20 million SNPs identified a set of 467 genetic loci that are strongly associated with the abundance of 115 microbial groups. Integration of RNA sequencing and aerial phenotyping data further revealed that genes near the microbe-associated loci are preferentially expressed in roots, and that the abundance of 62 genome-associated microbes correlates with plant performance in the field. A better understanding of these plant gene-microbe interactions may open avenues to sustainably improve crop performance in the agricultural industry.

Funding acknowledgement: National Science Foundation (NSF)

P66

Understanding the genetic basis of shattering in pearl millet

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Pearl millet (*Cenchrus americanus*) is the world's sixth most important cereal crop and was domesticated in sub-Saharan Africa, in the region of eastern Mali and Western Nigeria. Studies for understanding its genetic basis of shattering has not been done. The wild relative, *Cenchrus americanus* ssp. *violaceum* (*monodii*), easily shatters by breaking at the base of primary branch where the pedicel of spikelet joins the rachis while domesticated accessions do not break at this point, making it non-shattering. A histological and SEM analysis of the shattering zone shows a unique indentation of the epidermis that is present from early development of the primary rachis branches in both accessions, although the diameter of the branches differs. To understand the genetic base of shattering in pearl millet, we crossed domesticated *Cenchrus americanus* (*Tift 23DB*) and wild; *monodii* (*Tift 5120*) and created 387 F_2 lines. Qualitative phenotyping of F_2 and $F_{2:3}$ population through a simple hand-grasping method suggested that it followed a 15:1 segregation ratio implying that two loci might be responsible for the shattering trait in pearl millet. A QTL analysis of the qualitative data using a high-density SNP map identified two QTL regions, on chromosomes 3 and 5. The F_2 population was phenotyped using force gauge measurements 27 days after heading, and the same two QTLs were identified. The chromosome 3 and chromosome 5 QTLs from the quantitative analysis explained ~22% and ~6% phenotypic variance respectively. Comparative genome analysis of major QTL region with the closely related C4 model grass, *Setaria*, revealed no conserved QTL shattering regions. Comparison with *Oryza sativa* revealed that the major QTL in pearl millet has a conserved QTL region on rice 4, containing the candidate gene, Sh4, a myb domain transcription factor, previously characterized as major shattering gene in rice. Genotypic analyses of descendant populations will help narrow the QTL regions in pearl millet, helping us understand better this unique shattering mechanism.

Funding acknowledgement: National Science Foundation (NSF)

P67

Understanding the relationship between genetic architecture and genetic marker selection for improved genomic prediction accuracy

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Due to the current low sequencing costs, breeders can predict the performance of untested lines more efficiently by using genetic rather than phenotypic information. SNPs are the most commonly used genetic information in genomic prediction, but other types of markers (e.g., transcriptomic and metabolomic data) can provide additional information to the model. Structural variants (SVs) - large deletions, insertions, inversions, duplications, and translocations - can also be used in genomic prediction models, but little is known about how they impact prediction accuracy. Given that not all SVs are in linkage disequilibrium to SNPs and that they are associated with environmental adaptation, SVs have the potential to bring additional information to multi-environment models that are not captured by SNPs alone. We evaluated different marker types (SNPs, SVs, or SNPs with varying degrees of linkage disequilibrium to SVs) on prediction accuracy across a wide range of genetic architectures (variable numbers and types of quantitative trait loci, heritability, and effect sizes) using simulations across multiple environments. After performing a two-stage analysis with GBLUP prediction model for each marker type and genetic architecture, we obtained prediction accuracies using two types of cross-validation (CV1 and CV2). Our results show that SVs can improve prediction accuracy, but it is highly dependent on the genetic architecture of the trait and the type of cross-validation. For example, when predicting performance of untested genotypes (CV1) from a trait that has high heritability and a high number of SVs as causative variants, SVs increase prediction accuracy up to 20% compared to SNPs. However, no difference was observed when the trait had low heritability and low number of SVs as causative variants. Our findings demonstrate the importance of knowing the genetic architecture of a trait in deciding what marker types to use in large scale genetic prediction modeling in a breeding program.

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

P68

Using Generalized Linear Model (GLM) and Power analysis to investigate mutations associated with aberrant sexual reproduction in maize

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The experimental model in this project is maize pollen, representing the haploid male gametophyte, a crucial element of sexual reproduction and seed production. The impact of a mutation on pollen function can be measured indirectly by analyzing ratios of progeny seeds resulting from an outcross from a heterozygous pollen parent. If a mutation alters pollen function, the outcome will deviate from the expected 1:1 ratio (mutant:wild type). The goal of the overall project is to predict the genetic functions of a set of maize mutations (>400) via analysis of a large dataset of kernel counts. A Generalized Linear Model (GLM) method was used to analyze an initial dataset of 72 different mutations with multiple data points for each mutation (a total of >750 data points, each representing the counts of two kernel phenotypes on a single ear, corresponding to mutant and wild-type alleles). The GLM approach is advantageous compared to a traditional chi-square analysis, as the model incorporates overdispersion to account for potential ear-to-ear variation in ratios. The analysis has identified nine alleles with p-values below $\alpha = .05$, with those alleles having an estimated transmission rate ranging between 30% and 46%. Confidence intervals for transmission rate of statistically significant alleles were about 2-3% difference from the estimate. To help ascertain the sensitivity of this method, a post hoc power analysis has also been completed. The power analysis was developed with the intention of guiding the assessment of new mutations in the future, determining statistical power with respect to sample size, effect size, alpha level, and overdispersion. The results indicate that five independent outcrosses per mutation provide ~80% power, if the transmission rate is 9% (effect size) or more away from the expected.

Funding acknowledgement: National Science Foundation (NSF)

P69

Validating genetic resistance to maize tar spot in a stiff-stalk MAGIC population

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Tar spot of maize is caused by the fungal pathogen *Phyllachora maydis*. The signs of *P. maydis* manifest as small, raised, black stromata. Symptoms include the development of lesions on the foliar tissue of maize plants, in small regions around the stromata. Under significant tar spot pressure leaf blighting can occur. In-season management of tar spot is challenging, as once it is established it can spread rapidly in conducive environments and cause significant damage, reducing stalk integrity, grain yield, and forage quality. The most cost-effective management option in the long term will be to plant resistant hybrids; for this, alleles from resistant genotypes must be identified and incorporated into breeding programs. Previously, genome-wide association mapping was conducted in a subset of the Wisconsin Diversity Panel, and candidate loci were identified. In this study, a stiff-stalk multiparent advanced-generation intercross (MAGIC) population of 500 maize lines was assessed in three different trial locations - Indiana, Michigan, and Wisconsin - to validate previously observed marker-trait associations and enable identification of new quantitative trait loci (QTL) in the parental genotypes. The stiff-stalk MAGIC population of maize lines contains a balanced mix of the six founder genotypes: B73, B84, LH145, NKH8431, PHB47, and PHJ40. Performing stromatal severity ratings on the ear leaves of this population in the different locations coupled with the marker data for the population and sequence data for the founders allowed for QTL analysis to validate and discover new resistant loci conferring tar spot disease resistance. Identifying markers linked to validated QTLs for tar spot resistance will enable plant breeders to leverage these discoveries, breed for resistant varieties, and minimize the devastating impact of this fungal disease.

Funding acknowledgement: United States Department of Agriculture (USDA)

P70

Cytosine methylation patterns associated with *pl1* paramutation are reestablished and *trans*-chromosomally adopted during embryogeny

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Paramutation describes specific meiotically heritable changes in gene regulation dependent on *trans*-homolog interactions. A highly expressed state (termed *Pl-Rh*) of the *purple plant 1 (pl1) Pl1-Rhoades* allele can be heritably suppressed in *trans* by a transcriptionally and post-transcriptionally repressed state (termed *Pl'*). This leads to heritable reduction in 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 repression (rmr)* and *mediator of paramutation (mop)* loci affecting the paramutation process. Because most of the currently known RMR and MOP proteins are likely orthologs of Arabidopsis RNA-directed DNA methylation (RdDM) pathway components, it's hypothesized that a similar pathway contributes to paramutation behaviors through 24-nucleotide (24nt) RNA-based recruitment of chromatin modifications like 5-methylcytosines (5mC). In day 8 seedlings, we found *Pl'* states have significant enrichment of 24nt RNAs at a small region of a penta-repeat ~12kb 3' of the *pl1* coding region – coincident with 5mC in primarily CG and CHG contexts. Identical profiles were also seen in *Pl-Rh / Pl'* and *Pl' / Pl-Rh* seedlings indicating that *Pl'*-specific 5mC patterns are adopted by *Pl-Rh* during early development. Similar comparisons with 15 and 21 days after pollination (DAP) *Pl-Rh / Pl'* and *Pl' / Pl-Rh* developing embryos show a progressive reestablishment of *Pl'* like 5mC patterns with parent-of-origin differences that appear independent of 24nt sRNA levels. At 21 DAP in particular, 5mC in both CG and CHG contexts remained sparse in *Pl-Rh / Pl'* relative to *Pl' / Pl-Rh*. While the nature of these parent-of-origin differences remains speculative, our results point to the mid stages of embryogeny where 5mC patterns specified by meiotically-heritable information are elaborated.

Funding acknowledgement: National Science Foundation (NSF)

P71
DNA demethylation in endosperm
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Demethylation of transposons can activate expression of nearby genes and cause imprinted gene expression in endosperm. It is also hypothesized to function in genome defense by activating expression of mobile transposon siRNAs that reinforce silencing in the embryo. In maize, wild-type function of maternal derepression of r1 (mdr1) is required for maternal expression of the imprinted gene R1. We found that mdr1 encodes one of two endosperm DNA glycosylases in maize with homology to Arabidopsis DEMETER. Similar to DEMETER, mdr1 is partially responsible for demethylation of thousands of small genomic regions in endosperm. Surprisingly, however, these demethylated regions are depleted from the majority of repetitive elements in the genome and produce few siRNAs. Consistent with the essential role of maternally imprinted gene expression in other plants, a functional maternal allele of at least one of the two maize endosperm glycosylases is required for fertility. Our results, however, suggest an unidentified role for DNA demethylation in endosperm, as the majority of genes that overlap the demethylated regions are not imprinted.

Gene / Gene Models described: *mdr1, dng101, dng102, r1*; GRMZM2G123587, GRMZM2G422464, Zm00004b040676
Funding acknowledgement: National Science Foundation (NSF)

P72 @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). It is often noted that 85% of the maize genome consists of TEs, and TE alleles have been linked to major phenotypic changes in plant architecture, flowering time, and day length adaptation. Across maize individuals, the proportion of the genome derived from TEs is broadly consistent, yet there is extensive variation in the abundance of TE families and the position of individual TE copies – only about 20% of all TE copies are found at the same position. Despite this great variation, it is unclear the degree to which these differences in TE content affect plant phenotypes and fitness. We project TE copy number inferred from genome assemblies of NAM parents 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 yield. This association is stronger for TEs closer to genes, consistent with mechanisms of local epigenetic and genetic disruption. Altogether, while the maize genome can tolerate a large TE load, TEs continue to contribute to quantitative variation in agriculturally relevant phenotypes.

Funding acknowledgement: National Science Foundation (NSF)

P73

Exploring endosperm regulation through the DNA glycosylase maternal de-repression of R1(mdr1)

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Endosperm is a complex tissue with distinct methylome and transcriptome dynamics. While DNA methylation is generally stable across tissues and across generations, endosperm is a major exception to this pattern. DNA demethylation by specific glycosylases occurs in the central cell prior to fertilization, establishing differentially methylated regions (DMRs) and imprinted (parent-of-origin biased) expression of genes and transposable elements(TEs). Though specific glycosylases involved in imprinted expression have been identified as key regulators, we are still working to understand how they are targeted and what genes they regulate. One of the glycosylases found to be involved in genomic imprinting in arabidopsis is demeter. Recently, the maize gene mdr1 , a homolog of demeter, was cloned and confirmed to play a role in demethylation of genes in the endosperm. Thousands of DMRs were identified between wild type and mdr1 mutant endosperm, 91% of which were hypermethyated in the mutant. To understand the impact of these DMRs on expression, RNA-seq was performed to identify differential expression (DE) between the mutant and wild-type. We then compared this information to imprinting calls in wild-type to better understand the role of mdr1 in endosperm development and imprinting control. 98% of DE genes were down-regulated in the mutant, consistent with the role of DNA methylation in gene silencing. We found that 45% of DE genetic elements overlap DMRs, 51% genes and 49% transposable elements. An additional 8% of DE genes are within 2 kb of a DMR, suggesting that proximal changes to DNA methylation is a major contributor to differential expression. Finally, we discovered that around 40% of the DE genetic elements were also imprinted, including 39 maternally-expressed genes and 33 maternally-expressed transposable elements. This new information can help us determine how mdr1, and DNA methylation, regulates expression in endosperm and contributes to genomic imprinting.

Funding acknowledgement: National Science Foundation (NSF)

P74

Genic DNA methylation in maize

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DNA methylation contributes to inaccessible chromatin structure of repetitive DNA and represses transcription initiation in genes and transposable elements (TEs). It is depleted from promoters and other cis regulatory elements of expressed genes. However, exons of many expressed genes are methylated in the CG context specifically. This form of methylation, called gene body methylation (gbM), does not have a clear function but is widespread and is conserved in homologous genes across species. Exons of many genes are also methylated in both CG and non-CG contexts in a form called TE-like methylation (teM). We are using the resource provided by recent developments of high-quality maize NAM founder genome assemblies, gene and TE annotations, and transcriptomes and methylomes to study gene methylation. Here we present our findings on the expression and structural features of methylated genes, both gbM and teM, as well as the stability of gene methylation across the NAM founders.

Funding acknowledgement: National Science Foundation (NSF)

P75

Genome-wide analysis of maize hybrids and their progeny to understand the initiation and maintenance of epigenetic silencing in maize

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In plants, the initiation of DNA methylation at CG, CHG (H = A, C, or T), and CHH can be triggered by small interfering RNAs (siRNAs), known as RNA-directed DNA methylation (RdDM). Recently, it was reported that in hybrids, siRNAs produced by RdDM can interact with homologous sequences from either parent and result in significant changes in the DNA methylome of F1 derived from the two progenitors, including *trans*-chromosomal methylation (TCM) and *trans*-chromosomal demethylation (TCDM). While a great deal is known about the role of RdDM in the initiation and maintenance of the overall DNA methylation at all cytosines, relatively little is known at specific sequence context, especially at CHH sequence context, which is generally methylated at a relatively lower level. To understand the initiation of DNA methylation, we performed high-throughput sequencing of DNA methylome, small RNA, and mRNA of the F1 hybrid plants of two mutants including *mop1* (*mediator of paramutation1*) and *lbl1* (*leafbladeless1*) as well as of F1 wild type siblings. We found that at both CG and CHG TCM sites, the changes in the DNA methylome of F1 hybrids are mainly caused by the increased methylation of the lower alleles. In contrast, the methylation levels of both higher and lower alleles are increased at CHH TCM sites. Furthermore, the methylation of these CHH TCM sites is largely removed by the *mop1* mutation that reduces 24 nt siRNAs in these regions. Our next step is to examine the methylation levels of these CHH TCM sites in the F2 progeny that are derived from the F1 hybrid mutants and the wild-type plants to understand the maintenance of DNA methylation in maize.

Funding acknowledgement: National Institutes of Health (NIH)

P76

H2A.Z and transcription influence neocentromere positioning in CEN5 of Zea mays.

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Centromeres are the structures required for the faithful segregation of chromosomes during cell division. They are defined epigenetically by the histone variant cenH3 which forms the foundation of the kinetochore. Centromeres typically contain long stretches of tandem repeat (centC in *Zea mays*). During domestication many maize centromeres lost centC resulting in cenH3 either expanding into formerly pericentromeric regions or jumping to a new location entirely. In CEN5 of *Zea mays* we observe transcribed genes flanking the ancestral CEN5M which prevented cenH3 expansion and resulted in the centromere jumping to either the pericentromeric CEN5L or CEN5R locus. Both CEN5L and CEN5R are suboptimal neocentromere loci containing both transcribed sequence and H2A.Z which are shown to be disruptive to cenH3. However, comparisons of H2A.Z peaks among the diverse NAM founder lines suggest that H2A.Z is reduced in active centromere regions, and that H2A.Z can be suppressed by cenH3, stabilizing the neocentromere. Furthermore, two deletions were uncovered in CEN5L that resulted in the loss of H2A.Z loci which may have also had a stabilizing effect on the neocentromere. These mechanisms coupled with the insertion of centromere targeting CR2 retrotransposons, further stabilize the neocentromeres by pushing H2A.Z/genes out of the neocentromere and increasing repeat content. CEN5L and CEN5R are not ideal centromere loci but through suppression/loss of H2A.Z/transcription, and the accumulation of repeats, the loci are stabilized.

Funding acknowledgement: National Institutes of Health (NIH), United States Department of Agriculture (USDA)

P77

Helitron sequence dynamics across the maize genome

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The maize genome is replete with repetitive, non-coding DNA. These sequences, mostly transposable elements (TEs), play important roles in gene regulation, genome structure and mutation. Understanding how these sequences themselves change is a continuing area of investigation. Using the whole genome sequence data for twenty-six parental lines from a maize nested association mapping (NAM) population, we identified the nature, number and timing of recent sequence change in one class of relatively understudied TEs, the *Helitrons*. Phylogenetic trees of orthologous *Helitrons* and of their flanking regions (including genes) were generated and used to describe the evolutionary relatedness of orthologous chromosomal segments, particularly vis-à-vis ongoing recombinational and other rearrangement events. This analysis provides insights into the dynamics of non-coding regions relative to coding regions (differentiating exons, introns, etc.) at several locations across the maize genome.

Funding acknowledgement: Department of Energy (DOE)

P78

High-resolution crossover maps to understand the role of DNA methylation in meiotic recombination

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Meiotic recombination is a process through which homologous chromosomes exchange their parts and reshuffles the alleles. Such phenomenon plays a significant role in evolution and artificial breeding. Meiotic recombination is regulated by both genetic and epigenetic factors. While a great deal is known about genetic factors, relatively little is known about epigenetic factors, such as DNA methylation. Our recent publication indicates that loss of function in the *Mop1 (Mediator of paramutation1)* gene, a putative RNA-dependent RNA polymerase, resulted in a significant increase of the frequency of meiotic recombination in the chromosomal arms and decreased recombination frequency in the pericentromeric regions. Whole genome Bisulfite sequencing of *mop1* mutants strongly suggests that such redistribution of crossovers (COs) was driven by the loss of CHH (where H = A, T, or C) near the transcriptionally active genes of the chromosomal arms in the mutants. Our analysis of recombination was informative, but due to the large regions assayed and limited numbers of backcross (BC1) individuals, the resolution of the data is too low to determine the specific genomic and epigenomic features influencing meiotic COs in *mop1* mutants. To obtain COs at high resolution, we re-sequenced the genomes of168 and 132 BC1 individuals derived from parents of *mop1* mutants and their wild type siblings. All the single nucleotide polymorphisms (SNPs) were identified in these 300 individuals and meiotic recombination events will be determined throughout the genome using these SNP markers. We will compare the high resolution COs between *mop1* mutants and their wild type siblings. We wish to identify smaller regions in which recombination frequency is changed because of loss of DNA methylation in *mop1* mutants. Such finding will help us to understand how DNA methylation affects meiotic recombination at both global and local scales in maize and perhaps in many other crop species.

Gene / Gene Models described: *mop1*; GRMZM2G042443
Funding acknowledgement: National Institutes of Health (NIH)

P79 @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. LTR retrotransposons make up the majority of the maize genome, but only a handful of new insertions have ever been identified in controlled experiments. Long read sequencing has allowed for better capture of long insertions, however existing tools used to annotate LTR retrotransposons could not be applied to sequence insertions outside of the context of an assembled genome. The goal of this project was to write a script in python that can determine whether inserted sequences are likely to be LTR retrotransposons based on having expected structural characteristics. Using Biopython, insertions are analyzed for the presence of LTRs and primer binding sites, filtered to eliminate short sequences and those representing simple repeats, and high-quality candidates are output in table and graphical formats. The output includes a calculated similarity score and an estimated LTR length. To explore the accuracy of this process, we used LTR Predictor on all structurally annotated TEs in the B73v4 maize genome to estimate false positive and false negative rates. The tool was successful in selecting LTR retrotransposons as candidates, with very few false positives. We also applied the tool to an experimental dataset of insertions found in irradiated maize and identified with long-read sequencing. This experimental dataset yielded several LTR candidates that can be further validated experimentally. In the future this tool can be used to identify active or novel LTR transposons so we can have a better understanding of their impact on the maize genome.

Funding acknowledgement: Iowa State University College of Liberal Arts and Sciences

P80

Mutator transposable element behavior across maize genotypes

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Transposable elements (TEs) are small pieces of DNA that are capable of moving to new positions in genomes. When activated, TEs generate substantial genetic variation in individuals and populations. Despite their abundance in maize and instrumental role in producing genetic variation, little is known about what influences the distribution of TEs throughout genomes or the impact of TE accumulation on plant fitness. Here we use Mutator TEs, the most active class II transposon family, as a model system to investigate the impacts of TE behavior on plant fitness, whole plant phenotypes, and gene expression. We genotyped the Goodman association panel and identified 8-266 Mutator copies per inbred. These copies were found at low frequencies, often in a single inbred. PCA indicated that Mutator population structure differs from SNP population structure, which suggests that selection has influenced the distribution of Mutator throughout these inbreds. Mapping identified Mutator insertions to the B73 reference genome confirmed that Mutator TEs preferentially insert in and around the 5' UTR and transcription start site of genes in maize. To determine the degree to which these insertions are affecting gene function, we further characterized the impact of Mutator insertions through analysis of gene expression and associations with phenotypes in the Goodman panel. The effects of more recent insertions were investigated by comparing our findings in the Goodman panel with Mutator TE behavior in BonnMu, a maize population with activated Mutator in a B73 background. The same analyses used on Goodman panel data were applied to companion genotype and phenotype data of ~2,000 BonnMu maize seedlings. Significant correlations between more Mutator copies and lower germination were identified, suggesting a cost to TE accumulation. Collectively, these analyses provide insight into whether particular maize genotypes resist mutagenic effects of TE activity, laying a foundation for future plant breeding applications.

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

P81

Mutator transposon insertions within genes often provide a novel outward reading promoter

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The creation and maintenance of the sequence-indexed UniformMu transposon population is a vital resource for maize functional genomics. Prior studies have found evidence for *Mutator* (*Mu*)-suppressible alleles that provide conditional phenotypic effects based on the chromatin state of *Mu* elements, which may lead to activity of an outward reading promoter within the *Mu* elements that can generate functional transcripts. The frequency of outward reading promoters in *Mu* elements has not been well characterized. We performed RNAseq on a set of 35 UniformMu alleles that represent insertions of *Mu* elements into the transcribed regions of genes (5' UTRs, exons or introns). Transcriptome assembly data revealed 21/35 mutant alleles have transcripts initiating from within the *Mu* sequence. The presence of outward reading transcripts was observed for several different types of *Mu* elements and does not seem to be entirely dependent upon *Mu* orientation relative to the gene. In many cases there is a short transcript that begins at the gene promoter and terminates within the *Mu* element and a second transcript that initiates within the *Mu* element and reads through to the gene transcription termination site. These independent transcripts were confirmed using a combination of RT-PCR and Nanopore long-read sequencing experiments. The tissue-specific pattern of transcripts that originate from within the *Mu* elements were assessed for several genes that exhibit differential gene expression patterns. The abundance of the *Mu*-initiated transcript was often lower than the endogenous transcript but maintained very similar tissue specific patterns. This suggests that the outward reading *Mu* regulatory region acts as a minimal promoter interacting with native cis-regulatory elements. Our findings suggest that silenced *Mu* elements can minimize the consequences to genes by providing a minimal promoter that can often result in production of a similar gene expression pattern.

Funding acknowledgement: National Science Foundation (NSF)

P82

Predominantly inverse modulation of transposon element expression in haploid and diploid aneuploidies in maize

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Genomic imbalance refers to the more severe phenotypic consequences of changing part of a chromosome compared to changing the whole genome set. Previous genome imbalance studies in maize have identified prevalent inverse modulation of the genes on the unvaried chromosomes (trans) no matter the addition or subtraction of chromosome arm. Nevertheless, the modulation of transposon elements (TEs) in aneuploidy has not been systematically studied in any organism. Here, we analyze the TEs expression using the RNA-seq data of aneuploidy and ploidy series and found that most aneuploidies showed an inverse modulation of TEs, but reductions in monosomy and increases in disomy and trisomy are also common. The TEs in disomies in haploids were modulated to a greater extent than in monosomy and trisomy in diploids. On the other hand, the ratio distributions of the ploidy series showed little TE modulation. Next, we examined the modulation difference between TEs and genes in the same experimental group and found that the TEs showed a greater modulation than that of genes, especially in disomy. Furthermore, by separating the TEs into different classes, we find that Class I and II were differentially modulated in most aneuploidies and some superfamilies under each TE class also showed differential modulation. Finally, the significantly up-regulated TEs in three disomies (TB-7Lb, TB-9Lc, and TB-10L19) did not increase the proportion of adjacent gene expression when compared with that non-differentially expressed TEs. Taken together, these results suggest that the prevalent inverse TE modulation in aneuploidy is a result of stoichiometric upset of the regulatory machinery used by TEs similarly to the response of core genes to genomic imbalance.

Funding acknowledgement: National Science Foundation (NSF)

P83

Quantifying the insertion frequency of the mutator transposon across tissue types and over developmental timing

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The Mutator transposon (Mu) is an autonomous DNA element that has been heavily studied in maize since the discovery of MuDR in the late 1970s. Previous research has suggested that Mu insertion activity is temporally regulated, and that most new insertions occur late in development. To examine Mu insertion timing, we utilized next generation sequencing to quantitatively assess the frequency of new Mu insertions within matched leaf and pollen samples. Our preliminary data are consistent with a simple statistical model in which Mu has a constant rate of new insertions per cell division throughout development. Counterintuitively, the high frequency of late insertions in both somatic tissue and pollen grains may simply be driven by the exponential nature of cell divisions and does not require an increase in Mu activity over time. Overall, this research demonstrates how modern sequencing technology can be utilized to revisit previously established research and further develop our understanding of a heavily studied transposon. Going forward, we plan to develop Mu as a tool to study the impact of new mutations in haploid pollen grains.(Note: the data is only consistent with Mu activity being constant throughout development for new insertions; Mu excisions are more complicated)

P84

RNAi mediates drive in teosinte-maize hybrids

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Meiotic drivers subvert Mendelian heredity through targeted exploitation of the haploid stage, biasing their own transmission at the expense of competing gametes. The majority of drive complexes are hypothesized to exist in cryptic states, making it difficult to assess the full extent to which these selfish genetic elements impact natural populations. However, hybridization can lead to the production of naïve individuals where drive is unleashed. As such, cryptic drivers likely play important roles in reinforcing reproductive isolation and influencing patterns of gene flow between populations. Here, we report the discovery and characterization of Teosinte Pollen Drive (TPD), a selfish genetic system that occurs in hybrids between maize (*Zea mays ssp. mays*) and teosinte (*Zea mays ssp. mexicana*). Using direct sequencing of individual pollen grains as well as long-read *de novo* genome assembly, we define a *mexicana* inversion haplotype that causes severe transmission ratio distortion. Furthermore, we demonstrate that promiscuous RNA interference (RNAi) plays a critical role in mediating this phenotype through ectopic silencing of a lipase gene that is essential for male fertility. The rapidly evolving nature of germline non-coding RNAs, as well as the blurring of 'self' versus 'non-self' in outcrossing populations, suggests that RNAi plays a broad and underappreciated role in potentiating genetic conflict.

Funding acknowledgement: National Science Foundation (NSF), Howard Hughes Medical Institute (HHMI)

P85 @ClaireCodes

TIPs and tricks for identifying transposable element insertion polymorphisms in large genomes at the population-level

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Transposable element insertion polymorphisms (TIPs) are TE sequences not found in the same location between individuals. Across many plant species, TIPs have been shown to contribute to differences in phenotype which has compelling implications for crop improvement. Historically, a major challenge with studying TIPs on a genome-wide level is access to high quality genome assemblies that accurately assemble the TE portions of the genome and precise annotation of elements within those assemblies. Now, with access to 30+ maize assemblies, the challenge lies in studying TIPs at the population-level (500+ individuals). There are several methods that use short-read resequencing data and known TE annotations from genome assemblies to accurately identify novel insertions in other individuals. These include programs such as TIP_finder, TEfinder, SPLITREADER and TEPID that were designed for use in small, less complex genomes. Here, we benchmark seven different existing bioinformatic tools to identify TIPs in resequencing data from *Arabidopsis* and maize based on accuracy, precision, runtime and memory efficiency, and discuss which are best suited for large genomes.

Funding acknowledgement: National Science Foundation (NSF)

P86 @spklein2

Transposable element-derived genotypic variation is likely associated with diverse root responses to nitrogen stress

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Transposable elements (TEs) comprise a large portion of the maize genome and are a major contributor to genotypic variation. Novel insertions of transposable elements have been associated to significant phenotypic alterations and there is growing evidence supporting their role as potential regulators of transcriptional networks, particularly in response to abiotic stress. Recent work has identified several TEs and TE families containing promoters or enhancers rendering neighboring genes stress-responsive, making TEs a largely untapped potential for crop improvement via transcriptional rewiring. While responses to some abiotic stresses (cold, heat, drought) have already been observed, no research has yet explored TE responses to a mineral-based stress, like limited nitrogen (N) availability. Using the NAM founders, we are investigating potential associations between TEs and root growth angle, a root architectural trait that has been directly linked to improved N acquisition, under variable N availability. We planted the NAM founders in the field under high and low N and observed substantial variation in root system architecture and plant performance measures under N stress. We identified B73 as a steep-angled and non-responsive genotype whereas Oh7B was shallow-angled and N-responsive. In parallel, we are adapting a bioinformatic approach enabling us to quantify TE presence/absence variation in the NAM founders. Ultimately, we will integrate these phenomic and genomic efforts to associate TE insertional polymorphisms with root growth angle phenotypes, which will further our understanding of the role TEs play in mediating transcriptional and phenotypic responses to environmental stress.

P87 @YinjieQiu

Whole-genome variation of transposable element insertions in a maize diversity panel

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Intact transposable elements (TEs) account for 65% of the maize genome and can impact gene function and regulation. Although TEs comprise the majority of the maize genome and affect important phenotypes, genome-wide patterns of TE polymorphisms in maize have only been studied in a handful of maize genotypes, due to the challenging nature of assessing highly repetitive sequences. We implemented a method to use short-read sequencing data from 509 diverse inbred lines to classify the presence/absence of 445,418 nonredundant TEs that were previously annotated in four genome assemblies including B73, Mo17, PH207, and W22. Different orders of TEs (i.e., LTRs, Helitrons, and TIRs) had different frequency distributions within the population. LTRs with lower LTR similarity were generally more frequent in the population than LTRs with higher LTR similarity, though high-frequency insertions with very high LTR similarity were observed. LTR similarity and frequency estimates of nested elements and the outer elements in which they insert revealed that most nesting events occurred very near the timing of the outer element insertion. TEs within genes were at higher frequency than those that were outside of genes and this is particularly true for those not inserted into introns. Many TE insertional polymorphisms observed in this population were tagged by SNP markers. However, there were also 19.9% of the TE polymorphisms that were not well tagged by SNPs that potentially represent information that has not been well captured in previous SNP-based marker-trait association studies. This study provides a population scale genome-wide assessment of TE variation in maize and provides valuable insight on variation in TEs in maize and factors that contribute to this variation.

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

P88

pl1 paramutation requires an SNF2-type ATPase encoded by the *rmr13* locus

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Paramutations are defined by *trans*-homolog interactions that result in meiotically heritable changes in gene regulation. While the mechanism(s) responsible remain unclear, an RNA-directed DNA methylation (RdDM) pathway provides a working model. In maize, one example of paramutation occurs at the *purple plant1* (*pl1*) *Pl1-Rhoades* (*Pl1-Rh*) allele which encodes a regulator of anthocyanin production. Relative to the highly expressed reference state (*Pl-Rh*), a paramutant derivative (*Pl'*) confers weak anther color and is exclusively transmitted from *Pl'* / *Pl-Rh* heterozygotes in apparent violation of Mendelian segregation. In a screen for genetic factors *required to maintain repression* (*rmr*) of *Pl'* states, ethyl methanesulfonate (EMS)-induced recessive mutations have defined at least sixteen distinct *rmr* loci. All known RMR proteins, except RMR12, are involved in RNA polymerase IV-derived 24 nucleotide sRNA biogenesis. Curiously, no RdDM-like sRNA effector proteins have been identified. Here we characterize mutations defining a novel locus (*rmr13*), which genetic tests show is necessary for both the establishment and maintenance of *Pl'* repression. Analysis of whole genome sequence from independently-derived *rmr13* mutants identified two transition-type nonsense mutations in the same *4L* gene model. Phylogenetic analysis of this putative *rmr13*-encoded protein places it in a family of SNF2 ATPases containing RING finger domains associated with E3 ubiquitin ligase activity. Intriguingly, the closest rice ortholog, BIT-responsive Histone-interacting SNF2 ATPase 1 (BRHIS1), suppresses rice innate immunity through binding to monoubiquitinated H2A and H2B variants. The closest Arabidopsis ortholog, SNF2-ring-helicase-like2 (FRG2), also suppresses the immune response and functions downstream of sRNA biogenesis in RdDM. These findings support a role for *rmr13* in establishment and maintenance of *Pl'* repression through chromatin remodeling and variant histone ubiquitination. RMR13 thus represents the first possible sRNA effector central to the *pl1* paramutation mechanism.

Gene / Gene Models described: *rmr13*; Zm00001eb171780
Funding acknowledgement: National Science Foundation (NSF)

P89 @NadiaNazihM

A new sorbitol dehydrogenase mutant in maize

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Sorbitol dehydrogenase (SDH, EC 1.1.1.14) can catalyze both the biosynthesis and catabolism of sorbitol in mammals, plants, and bacteria. Unlike Rosaceous species that translocate sorbitol in their phloem, kernels rapidly synthesize, transport and metabolize their own sorbitol. The physiological roles for this process remain elusive. Because maize SDH catalyzes the reversible interconversion of fructose + NADH ← → sorbitol + NAD, the reaction would likely favor sorbitol synthesis in the high-sugar, low-oxygen interior of the endosperm and possibly the reverse in other locales. Maize SDH is encoded by a single copy *SDH1* gene, which is strongly expressed early in the endosperm-filling stage. Preliminary analysis of a new, *Ac/Ds*-induced *sdh1* mutation indicates that it confers a small-kernel phenotype. Since availability of NAD is typically limited under the low-O_2 conditions in maize endosperm, the capacity for SDH to generate NAD provides a mechanism for protecting redox balance and sustaining kernel growth. To address this hypothesis, we are characterizing the *sdh1* mutant and generating *SDH1* over-expression lines for biochemical and molecular genetic analysis. Meanwhile, to study the physiological role of SDH in high-sugar environments, double mutants that combine *sdh1* with the high-sugar endosperms of *sh2* are being grown for morphological and biochemical analysis. Outcomes will determine the suitability of *SDH1* and its regulators as possible targets for genetic manipulation or metabolic engineering to alter quality and/or quantity of maize kernels.Research support by NSF-IOS-PGRP-1748105

Funding acknowledgement: National Science Foundation (NSF)

P90

Adapting corn to heat stress via carbohydrate metabolism modification

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Heat stress in maize reduces grain fill. During kernel development a large fraction of glucose that is incorporated into starch passes through the oxidative pentose phosphate pathway prior to be incorporated into starch. The last step of the oxidative phase of the pentose phosphate pathway is catalyzed by 6-phosphogluconate dehydrogenase (6PGDH). Maize has three isoenzymes of 6PGDH. Thermostable PGD1 and PGD2 are located in the cytosol while thermal-labile PGD3 is located in the plastid. Loss of PGD1 or PGD2 have no effect on seed phenotype; however, loss of PGD3 results in severe defective kernel phenotypes. Synthetic 6PGDH genes targeting PGD1 or PGD2 to the endosperm amyloplast with the maize waxy1 chloroplast targeting sequence provide both heat stable enzyme activity and complement *pgd3* mutants. These WPGD1 and WPGD2 transgenes were tested in a B73 x W22 hybrid field corn trial to compare yield of transgenic and non-transgenic plants under heat stress. Preliminary results from this trial show WPGD increases 6PGDH activity compared to non-transgenic hybrid controls. This increase in activity is correlated with increased ear weight in transgenic plots under heat stress. These results suggest thermostable 6PGDH activity can mitigate heat stress yield losses in hybrid field corn.

Funding acknowledgement: United States Department of Agriculture (USDA)

P91

Altering the binding site of a plant hormone in corn proteins affects how fast they break down

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Plants, like animals, are able to sense and respond to the presence of small molecule hormones, such as auxin, that trigger signaling pathways that initiate growth and developmental processes. Within the nuclear auxin response pathway lies an elegant transcriptional control system: under the absence of auxin, a repressor halts developmental gene transcription, and in the presence of auxin the repressor becomes degraded and transcriptional repression is relieved. Mutations in the repressor's auxin-binding domain (degron) or rate motif regions can be used to tune the degradation rate and alter the pace of downstream developmental events in Arabidopsis. To begin testing whether this was also true in maize, we made two sets of degron mutations in ZmBIF4 designed to slow or completely abolish auxin-induced degradation. We then measured their degradation rate in the presence of two different maize auxin receptors using a yeast synthetic auxin signaling system. The BIF4 degron G->R mutation completely abolished auxin-induced degradation, while the PVV->AAA mutation slowed but did not prevent degradation. With this in vitro confirmation of variable auxin-induced degradation rates, we soon will assess the in vivo effects to determine the impact of slowed vs. abolished BIF4 degradation on maize organ developmental progression.

Funding acknowledgement: National Science Foundation (NSF), Arnold & Mabel Beckman Foundation

P92

An expanded gene regulatory network governing 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. Here we focus on the regulation of the phenylpropanoid genes that are most highly expressed throughout maize development. We report on Y1H screens that uncover upstream TFs that regulate 13 TF genes previously shown to bind multiple phenylpropanoid promoters in maize. We have found candidate "Master TFs" that include novel members of the MYB, MYBR, DOF, bZIP, MADS, GBP, TRAF, Homeobox, and NAC TF and Co-regulator families. This project is funded by NSF grant IOS-1733633.

Funding acknowledgement: National Science Foundation (NSF)

P93

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 land plants. It provides the first line of defense from biotic and abiotic stresses that are detrimental to plant health. The cuticle is composed of a network of lipids that are both intercalated within and laid atop an insoluble cutin polyester matrix. The solvent extractable cuticular wax mixture, depending on organ and stage of development, is comprised of combinations of different lipid classes, such as 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 focuses 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 unclear, homozygous *gl2* mutant seedlings exhibit cuticular waxes of shorter chain lengths, presumably due to the inability to fully elongate VLCFAs. Recently, GLOSSY2-LIKE (encoded by *Gl2-like*), which shares 63% amino acid similarity to GL2, was also shown to play a role in VLCFA elongation when heterologously expressed in Arabidopsis. To assess the *in planta* physiological function of *Gl2-like,* five unique maize 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 are being investigated using protein preparations from *E. coli* that is recombinantly expressing both homologs. This combination of genetic and biochemical strategies is unraveling the roles that *Gl2* and *Gl2-like* serve in maize cuticle biosynthesis.

Gene / Gene Models described: *gll1, gl2*; GRMZM2G315767, GRMZM2G098239
Funding acknowledgement: National Science Foundation (NSF)

P94

Analysis of functional and compositional diversity of the adult maize leaf cuticle reveals a key role for wax esters in water barrier function

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The cuticle, a hydrophobic layer of cutin and waxes synthesized by epidermal cells, has many functions in protection of shoot tissues including restricting water loss. Transpiration across the cuticle (cuticular conductance, or g_c) is the major source of water loss at night and in water-limiting conditions when stomata are closed. g_c is therefore a plausible target trait for breeding efforts to improve maize drought tolerance. It is well-established that cuticular waxes are important for minimizing g_c, but not what features of wax composition are important. Multiple lines of evidence in our studies support the conclusion that wax esters (one of several classes of cuticular wax) have a key function in restricting transpiration across adult maize leaf cuticles. (1) Analysis of cuticle maturation along the developmental gradient of partially expanded adult maize leaves showed that maturation as a water barrier coincides with replacement of alkanes with wax esters as the dominant wax class. (2) Natural variation in mature adult leaf g_c values across the Wisconsin Diversity Panel is strongly associated with the abundance of several wax esters; collectively, this group of waxes explains about 30% of the variance in g_c. (3) A genome-wide association study identified a Rab-GAP gene as a candidate determinant of adult leaf g_c. The same gene was identified in a transcriptome-wide association study (TWAS) searching for genes whose transcript abundance in the cuticle maturation zone is associated with the abundance of ~50 different cuticular wax molecules in mature leaves. Specifically, this Rab-GAP gene was associated by TWAS with six wax esters. In addition to strengthening the evidence of a role for wax esters in minimizing g_c, this finding implicates a predicted regulator of vesicle targeting in cuticle construction as a functional water barrier.

Gene / Gene Models described: ; Zm00001d38404
Funding acknowledgement: National Science Foundation (NSF)

P95 @scichi007

Autoactive and pathogen-dependent enhanced disease resistance mutants of 'Kronos' durum wheat

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More than 50 lesion mimic mutants of maize have been described, informing our knowledge of autoimmune loci in grass crops. Previously, a TILLING population of tetraploid 'Kronos' wheat was screened for enhanced disease resistance (EDR) to the yellow rust pathogen, *Puccinia striiformis* f. sp. tritici. More than 30 EDR mutants were identified, about a third of which showed lesions in the absence of the pathogen. Here, we show that cold treatment in a growth chamber promoted lesion formation in many of these lines. Suppressed immunity at low temperatures has been described and is sometimes due to repression of SA. Genes induced by *P. striiformis* infection were mined from transcriptional data. We evaluated expression of a subset of these genes over time in our autoactive lines grown in cold conditions. All mutant lines were also evaluated for TKW over two years and in most, lesion formation was not correlated with decreased kernel weight under pathogen challenge conditions.

Funding acknowledgement: National Science Foundation (NSF)

P96 @natekorth

Breeding food for health: Identification of substrates from quality protein popcorn that promote growth of specific beneficial bacteria in the human gut microbiome

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There is tremendous interest in understanding how major food components affect the form and function of the human gut microbiome. Though the effects of fiber and complex carbohydrates are becoming clearer, much less is known about the interactions of dietary protein. Here we studied the effects of major seed protein composition on the microbiome using quality protein popcorn (QPP), centered around the *opaque-2* mutation. This mutation blocks synthesis of the major maize seed proteins (α-zeins) and causes compensatory synthesis of new seed proteins that are much higher in content of essential amino acids (lysine and tryptophan). We show that gut microbiome fermentation patterns of popped corn from QPP derivatives compared to parental, non-QPP lines, have significantly higher amounts of the major bacterial metabolite butyrate and corresponding increases in abundances of members of the butyrate-producing family Lachnospiriaceae, including the genera Coprococcus and Roseburia and this signature was detected in microbiomes from three diverse subjects. We further show that lysine-enriched seed protein can stimulate growth of microbes through multiple pathways, including transport and degradation of lysine in organisms carrying these pathways (Coprococcus), whereas in organisms lacking these pathways (Roseburia) utilization depends on fructoselysine produced during thermal processing (popping) by the Mailliard reaction. Thus, the combination of seed composition in QPP and interaction of protein adducts with carbohydrate during thermal processing can stimulate growth of health-promoting, butyrate producing organisms in the human gut microbiome through different pathways.

Funding acknowledgement: FFAR, Nebraska Food for Health Center, Raikes Foundation

P97

Conserved function of RNA Binding Motif Protein48 (RBM48) Armadillo Repeat Containing 7 (ARMC7) in maize and human U12 splicing

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Splicing of pre-mRNA is fundamental for genes containing introns. Emerging data points to a deeply conserved role of this process in eukaryotic cell differentiation and proliferation. The vast majority of introns termed U2-type introns are spliced by a major spliceosome; however, there also exist rare and more conserved U12-type introns that are spliced by a minor spliceosome. Mutations that disrupt U12 splicing inhibit cell differentiation in both maize endosperm and human blood cells. However, the mechanism underlying this process is not well understood. The maize RNA Binding Motif Protein 48 (RBM48) plays an essential role as a U12 splicing factor and is required for proper maize kernel development. Using human cell lines, CRISPR-Cas9 knockdown of RBM48 demonstrated a conserved function in human U12 intron splicing. RBM48 and Armadillo Repeat Containing 7 (ARMC7) protein interact in both maize and human as part of the activated minor spliceosome. Here we show RBM48 and ARMC7 co-localization in the nucleus of human cell lines. Vertebrate ARMC7 is normally localized in the cytosol, whereas RBM48 is found in the nucleus. Our data suggests that the interaction plays a role in regulating ARMC7 localization or the efficiency of U12 intron splicing. We also performed comprehensive transcriptome profiling and identified a common subset of conserved Minor Intron Containing Genes (MIGs) impacted in both human and maize RBM48 knockout mutants. Of importance, the vast majority of these MIGs are associated with developmental defects in both plants and animals. This suggests that aberrant splicing of these targets has a high likelihood of mediating abnormal cell phenotypes. These data support evolutionarily conserved U12 splicing mechanisms between maize and humans with both RBM48 and ARMC7 having roles in the activated spliceosome.

Gene / Gene Models described: *RBM48, ARMC7*; GRMZM2G163247, Zm00001d054077, Zm00001eb209860
Funding acknowledgement: National Institutes of Health (NIH), National Science Foundation (NSF)

P98
Cutting up a maize auxin repressor determines the regions most important for degradation

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The hormone auxin is a major regulator of developmental and signaling pathways in plants. In maize, auxin facilitates the development of proper ear and tassel growth. Following identification of the putative the maize nuclear auxin signaling proteins, we previously used a synthetic yeast system to show that auxin repressors show a range of degradation rates and the maize auxin receptor is highly sensitive to auxin. To narrow in on the regions of the repressor that control degradation rate, we made truncations and mutations in two regions known to act as rate motifs in Arabidopsis: (1) the highly-conserved KR motif and (2) a more variable region further downstream. When the maize auxin repressor ZmIAA25 KR motif was removed or mutated (KR->AA), the degradation of ZmIAA25 slowed slightly. Only when the more variable rate motif region was removed did degradation completely stop. The ZmIAA16 repressor has KA in place of the KR motif. When removed or mutated (KA->AA or KA->KR), no change in degradation rate was observed. However, removal of the more variable rate motif region downstream dramatically slowed degradation. These results affirm that auxin repressor rate motifs identified in Arabidopsis function similarly in maize and may also be amenable to manipulations that could allow for genetic engineers to fine-tune ear and tassel development.

Funding acknowledgement: National Science Foundation (NSF)

P99
Deciphering the morphological, geometric, and metabolic components of stalk lodging resistance in maize (*Zea mays* L.)

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Stalk lodging results from the breakage/buckling of stalks and severely affects yield and quality in maize (*Zea mays* L.). Stalk lodging resistance (SLR), ability of stalks to avoid mechanical failure, is a complex trait governed by an interplay of genetic and environmental factors. Phenotyping SLR under field conditions has remained challenging due to lack of phenotyping platforms that reliably reproduce natural stalk lodging patterns. We have recently shown that, under field conditions, stalk bending strength (SBS) measured with DARLING (Device for Assessing Resistance to Lodging IN Grains) reliably predicts the natural lodging incidence (Sekhon et al., 2020, Field Crops Research, DOI: https://doi.org/10.1016/j.fcr.2020.107737). We have been evaluating multiple morphological, geometric, and metabolic traits, termed intermediate phenotypes (IPs) that can be used to assess SLR in field and laboratory conditions. Herein, we studied variation for different IPs in sixteen maize hybrids in two environments and examined the relative ability of these phenotypes to predict SLR. Evaluation of multiple plant-level and internode-level traits showed substantial intra- and inter-plot variation indicating pervasive underlying genetic and environmental interactions (GxE). Moreover, substantial intra-plot variation within certain hybrids indicated variation in the GxE effect among the hybrids. Among different IPs examined in the study, flexural stiffness, plant height, major diameter, rind thickness, internode length, section modulus, and linear density were found to be the key predictors of SBS. Evaluation of two distinct internodes, the internode below ear-bearing node (EIN) and the lowest elongated internode (LIN), showed abundant variation in the IPs both between internodes and among hybrids, and that EIN was comparatively more predictive of SBS. Analysis of metabolic phenotypes is currently underway. These data will be combined using advanced statistical/machine learning techniques to identify the most informative phenotypes underlying SBS. Overall, our study will contribute to generating a comprehensive picture of the genetic architecture of SLR.

Funding acknowledgement: National Science Foundation (NSF)

P100

Disruption of *Zea mays isochorismate synthase1* suppresses PHENYLALANINE AMMONIA LYASE activity and hypersensitive response induced metabolism

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In plants, ISOCHORISMATE SYNTHASE (ICS) catalyzes the isomerization of chorismate to isochorismate, an essential percussor in the biosynthesis of the photosystem I electron carrier phylloquinone. Plants deficient in phylloquinone are pale, have stunted growth, and exhibit seedling lethality. Isochorismate is also a percussor in one of two pathways for the biosynthesis of the defense response hormone salicylic acid (SA) and mutants of ics are often utilized to study the consequences of inhibiting SA biosynthesis in plants. In this work, we characterized two mutant alleles of *Zea mays ics1* (B73v4: Zm00001d020220). We generated double mutants between *ics1-1* and *Rp1-D21#4*, a hypersensitive response (HR) autoactive allele of the nucleotide-binding leucine-rich repeat (NLR) gene *Resistance to Puccinia sorgi1* (*Rp1*) to test the role of SA and *ics1* during HR. We found that despite not accumulating SA, *Rp1-D21#4 ics1-1* double mutants displayed similar HR-induced lesion formation as *Rp1-D21#4* single mutants. Through untargeted metabolomic analyses of *Rp1-D21#4* mutants, *ics1-1* mutants and *Rp1-D21#4; ics1-1* double mutants we identified that most *Rp1-D21#4* responsive metabolism requires *ics1* by a mechanism independent of SA. Lastly, we found that disruption of maize *ics1* suppresses PHENYLALANINE AMMONIA LYASE activity, coincident with increased accumulation of phenylalanine and decreased production of phenylalanine derived metabolites in *ics1-1* mutants. These findings demonstrate that high SA accumulation is not required for HR in maize and indicate that additional factors are required for HR initiation and progression. Our work also expands on the current knowledge of ICS in the direct biosynthesis of phylloquinone and SA and indicates that ICS is required for many plant enzymatic reactions, including phenylpropanoid metabolism, by a yet unknown mechanism.

Gene / Gene Models described: *ics*, *Rp1*; Zm00001d020220, Zm00001d023317
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P101

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

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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 sugars and signaling pathways in senescence. To understand the genetic architecture and the underlying molecular mechanisms, we have performed a systems genetic analysis of SSRS in maize. Through characterization of the natural diversity for SSRS in a diversity panel, and evaluation of a recombinant inbred line 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, abscisic acid, and hexokinase activity. Natural diversity analysis identified 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 maize and by overexpression of maize *ccp4* in Arabidopsis. Promoter analysis of *ccp4* in maize identified allelic variation in ABA responsive elements associated with *ccp4* activity during SSRS. These findings were confirmed by differences in the effect of ABA treatment on distinct ccp4 alleles. Characterization of the novel genes and pathways presented in this study will enhance the mechanistic understanding of senescence in maize and other cereals.

Funding acknowledgement: National Science Foundation (NSF)

P102

Distinct roles of plastid and cytosolic pathways for aromatic amino acid biosynthesis in kernel and plant development

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Understanding mechanisms that determine organ size is a fundamental theme in plant development. In addition, ratio of embryo and endosperm size is an important determinant of grain composition. Overall seed size is known to be predominantly regulated by maternally expressed genes, and many genes regulating seed size have been identified. In contrast, zygotic mechanisms that determine relative size of embryo and endosperm, but not overall seed size, are little understood. So far, only four genes, including two identified by our group, have been identified in regulation of relative size of embryo and endosperm in cereals. Here we report *big embryo 6* (*bige6*) mutant isolated in a screen for maize mutants that alter relative size of embryo and endosperm without affecting overall kernel size. Mature *bige6* embryos have greater fresh weight and dry weight than wild type embryos, whereas size and weight of the whole kernel is unaffected indicating that embryo size increases at the expense of the endosperm. The increase in embryo size is primarily due to enlargement of the scutellum, whereas growth of the embryo axis is less affected. Homozygous *bige6* kernels germinate slowly with a reduced frequency producing plants that are much smaller than wild type at flowering. *BigE6* encodes a key enzyme in a plastid localized pathway for biosynthesis of aromatic amino acids. We attribute the non-lethal phenotype to a close paralog, *BigE6like*, that encodes a cytosolic form of the enzyme. Counter-intuitively loss of *BigE6*, the predominant form expressed in the embryo, increases embryo growth at the expense of the endosperm. We hypothesize that bige6 disrupts a critical metabolic balance maintained by plastid and cytosolic pathways in the developing embryo and endosperm. Distinctive roles are further revealed in developing leaves.

Funding acknowledgement: National Science Foundation (NSF)

P103

Dolabralexin-deficient maize mutant provides insight to maize defense and root architecture

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Two major groups of maize (*Zea mays*) specialized diterpenoid metabolites, the kauralexins and dolabralexins, serve either known or predicted functions as chemical defenses against pathogens, herbivores, and other environmental stressors. To critically examine the physiological roles of dolabralexins, we more closely examined the diversity, tissue-specificity, and stress-elicited production of dolabralexins. Evidence supports a larger diversity and number of dolabralexin end-products. We identified dolabradienol as a major, yet previously undetected, pathway metabolite and characterized its enzymatic production. Transcript and metabolite profiling showed that dolabralexin biosynthesis and accumulation predominantly occurs in primary roots and shows quantitative variation across genetically diverse inbred lines. Generation and analysis of a loss-of-function CRISPR-Cas9 derived Kaurene Synthase-Like 4 (*Zmksl4*) mutant had dolabralexin production deficiencies, demonstrating that ZmKSL4 is the sole maize diterpene synthase that converts the common geranylgeranyl pyrophosphate precursor into dolabradiene and downstream pathway products. *Zmksl4* mutants further display altered root-to-shoot ratios and root architecture in response to water deficit, consistent with an interactive role of dolabralexins in maintaining plant vigor in response to abiotic stress.

Gene / Gene Models described: *ZmKsl4*; Zm00001d032858
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P104

Engineered 6-phosphogluconate dehydrogenase in amyloplasts 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 heat stress and potentially is a limiting pathway for grain yield under heat stress. In addition to enzymes 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. Maize encodes two cytosolic isozymes, PGD1 and PGD2. These isozymes are heat stable, while 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 plastid targeting sequence to the Pgd1 and Pgd2 open reading frames. Previous work showed that WPGD1 and WPGD2 transgenes complement the pgd3 defective kernel phenotype suggesting the fusion proteins are targeted to the amyloplast. Initial field trial suggests WPGD1 and WPGD2 mitigate part of the yield losses due to heat stress. We generated B73 x W22 *Wpgd* 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: NIFA

P105

Enhancing transposon resources: Probing Robertson's originals and other unexplored Mu-active lines

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In addition to the UniformMu National Public Resource, other mutant phenotypes from Mu-active populations are curated by the Maize Genetics Cooperation Stock Center. Among these diverse phenotypes are those of unexplored mutant lines from Donald Robertson's original selections. Here we report a preliminary exploration and feasibility appraisal for identifying causal genes in these resources. A thorough screen of databases and historic data yielded over 200 mutant phenotypes with unidentified causal genes. Because these were generated in Mu-active populations, we surmised that a substantial fraction would be Mu-tagged and thus accessible to the Mu-seq, UniformMu pipeline for linking phenotype to genotype. Over 50 different mutant phenotypes were selected, ranging from leaf pigment to seedling morphology, and from kernel composition to grain development. From these we selected 6 lines for a pilot study. All selections showed clear heritability and allowed establishment of a stable, Mu-off state, typically in less than 3 generations. We anticipate that Mu-seq analysis of these and other unexplored lines will identify mutants not yet observed in the UniformMu population given differences we have observed between Mu-active populations. This approach will also allow delineation of allelic relationships within mutant groups, most of which include subtle to pronounced variations. In addition to enhancing the array of currently-available, Mu-tagged mutants, the value of other extant resources would be enhanced by this phenotype-to-genotype effort.

Funding acknowledgement: National Science Foundation (NSF), (NSF-IOS-PGRP-1748105)

P106

Establishment of *Ustilago maydis* infection in maize anthers

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Ustilago maydis is a biotrophic fungus that causes tumors in all aerial maize organs. In anthers, visible tumors appear approximately seven days post infection; however, there is limited information on how the infection progresses in the period between host plasma membrane invagination and visible tumor formation. Transcriptomics analysis of single timepoints during infection has identified a subset of genes that are differentially expressed in infected anthers; however, without more complete time courses of expression data, it is unclear how the infection and plant disease response develop over time. We have collected more detailed time course data from the first half of the infection in W23 infected with wild type U. maydis to better characterize the course of a normal infection. Additionally, we have collected transcriptomics data from infections with mutant U. maydis strains or male sterile maize mutants where the infection fails to progress to tumor formation to better identify key genes for infection success.

Funding acknowledgement: National Science Foundation (NSF)

P107

Exploring the metabolome diversity for staygreen in maize

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Staygreen is an important trait that improves plant productivity by prolonging the period of photosynthetic assimilation. The physiological and metabolic components that accurately discern phenotypic variation for staygreen in maize remain poorly defined, thus making elucidation of the genetic architecture of this trait difficult. To systematically define the physiological and metabolic underpinnings of stay-green, we performed an in-depth analysis of phenotypic variation captured in a set of 19 genetically diverse inbred lines. Time-course phenotypic analysis of physiological phenotypes that capture different components of the C4 photosynthetic process revealed novel phenotypic variation. Changepoint analysis revealed that differences in staygreen phenotype are manifested both as the time of onset and the rate of progression of senescence. This approach identified inbred lines that show staygreen due to one or both parameters. These observations indicate the presence of multiple mechanisms that regulate staygreen and highlight the limitation of relying on a snapshot of a single physiological phenotype in genetic studies. Based on these analyses, we identified a set of five staygreen and five non-staygreen inbred lines and performed a time-course analysis of changes in the leaf metabolome during senescence. Remarkably, the substantial variation observed in the leaf metabolome was strongly associated with the observed phenotypic differences for staygreen, thus indicating a crucial role of the metabolic process in senescence. The identity of metabolites and metabolic pathways underlying staygreen will be presented and discussed. Our findings will identify novel components of biological organization that regulate staygreen in maize.

Funding acknowledgement: National Science Foundation (NSF)

P108

Fertility restoration of maize CMS-C altered by a single amino acid substitution within the *Rf4* bHLH transcription factor

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Type C Cytoplasmic male sterility (CMS-C) is the most commonly used form of CMS in maize hybrid seed production. *Restorer of fertility 4* (*Rf4*), the major fertility restorer gene of CMS-C, is located on chromosome 8S. To positionally clone *Rf4*, a large F3 population derived from a cross between a non-restorer and restorer (n = 5,104) was screened for recombinants and then phenotyped for tassel fertility, resulting in a final map-based cloning interval of 12-kb. Within this 12-kb interval, the only likely candidate for *Rf4* was GRMZM2G021276, a basic-helix-loop-helix (bHLH) transcription factor with tassel specific expression. The *Rf4* gene product contains a nuclear localization signal and likely does not interact directly with the mitochondria. Sequence analysis of *Rf4* revealed four encoded amino acid substitutions between restoring and non-restoring inbreds, however, only one substitution, F187Y, was within the highly conserved bHLH domain. The hypothesis that *Rf4* restoration is altered by a single amino acid was tested by using CRISPR-Cas9 Homology Directed Repair (HDR) to create isogenic lines varying for the F187Y substitution. In a population of these CRISPR-Cas9 edited plants (n = 780) that was phenotyped for tassel fertility, plants containing F187 were completely fertile indicating fertility restoration, and plants containing Y187 were sterile, indicating lack of fertility restoration. Structural modeling shows this amino acid residue 187 is located within the four helix-bundle core, a critical region for stabilizing dimer conformation and affecting interaction partner selection.

Gene / Gene Models described: *Rf4, Ms23*; GRMZM2G021276

P109 @bhattarays

Identification and comparative analysis of resistance against *Ustilago maydis* in maize, teosinte, and near-isogenic lines

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Ustilago maydis (*U. maydis*), the causal agent of corn smut, is an important fungal pathogen responsible for significant corn yield losses infecting all aerial plant parts and locally inducing tumors. Yield losses and the lack of *U. maydis*-resistant maize cultivars necessitate identifying new sources of resistance to this pathogen. The phenotypic evaluation identified two near-isogenic lines (NILs) from a maize (the inbred line B73)-teosinte (*Zea mays* ssp. *parviglumis*) introgression population that are resistant to *U. maydis*. Genotypic analysis of the two resistant NILs identified a 3.9-Mbp teosinte introgressed region on the short arm of chromosome 9 that was present only in the two resistant NILs, suggesting the teosinte introgressed region is responsible for the resistant phenotype. Comparative analysis of *Z. mays* ssp. *parviglumis*, B73, and a nested association mapping population identified genes present only in the *Z. mays* ssp. *parviglumis* parent, which may contribute to the resistant phenotype in both NILs. Candidate genes were identified in the 3.9-Mbp teosinte introgressed region. To make gene predictions and assess the expression of candidate genes, RNA sequencing was performed. RNA-seq data analysis is ongoing for the two NILs, B73, and *Z. mays* ssp. *parviglumis* inoculated with *U. maydis*. These data will identify candidate genes expressed in two NILs derived from the teosinte parent that potentially confer resistance to *U. maydis*.

Funding acknowledgement: Georgia Association of Corn Commission

P110

Identification of genomic regions associated with phenolic metabolism

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Maize is one of the most cultivated and important crops worldwide for food, feed and biofuels production. In the USA alone, maize grain production in 2021 was 12.5 billion bushels valued at more than $49 billion dollars (https://www.nass.usda.gov/). Its productivity has increased because of improved genetics and agronomic practices to satisfy a rapidly growing population. However, the process of selective breeding applied to increase productivity has resulted in the unintended selection of crop varieties lacking important compounds for plant protection against abiotic and biotic stresses, which can result in significant losses. One good example is provided by the loss of phlobaphenes in most cultivated maize varieties. There is a growing interest in endogenously produced compounds that can act as buffers to defend plants and mitigate productivity losses under stressed conditions. A goal of this work was to identify genomic regions involved in the formation and regulation of phenolics in maize by conducting a Genome-Wide Association Study for a set of 33 phenolic compounds profiled across 597 genetically diverse inbred lines. The set of inbreeds was grown under controlled environment room under high-intensity LED lights condition, with 3 biological replications, and the stem of the plant was sampled. The phenolic evaluation was conducted by liquid chromatography coupled with mass spectrometry (LC-MS) using a 10 min targeted method. Initial results have identified a number of candidate genes belonging to UDP-glycosyltransferase and cytochrome P450 families that are predicted to be involved in the formation of phenolic compounds reported previously to protect the plant against pathogens attacks and UV light exposure. In addition, new members of the MYB and BHLH transcription factors families previously reported to control flavonoids production were identified. A subset of promising candidate genes are currently being experimentally validated by metabolic engineering in yeast and *Nicotiana benthamiana* systems. Our emerging results will provide a resource for improved plant breeding for stress tolerance and maize improvement.

Funding acknowledgement: National Science Foundation (NSF)

P111

Investigating the contribution of the endogenous *Zea mays* aquaporin *ZmPIP1;5* in regulating water-use efficiency

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The tradeoff between water loss and carbon acquisition represents a fundamental balance that plants must optimize for survival. Maximizing the amount of carbon gained per water lost is integral for sustaining crop productivity in the face of a changing climate. Aquaporins are water-permeable membrane proteins implicated in regulating both transpiration and photosynthetic carbon assimilation. In plants with a C_3 photosynthetic pathway, members of the plasma membrane-intrinsic protein (PIP) subclass of aquaporins have previously been implicated in CO_2 transport affecting CO_2-responsive stomatal signaling and mesophyll conductance. However, the definitive functional relevance of this CO_2 transport property has yet to be demonstrated in C_4 species, including *Zea mays*. Only two PIPs in *Z. mays* have been characterized as CO_2-permeable; of these, *ZmPIP1;5* was selected as a target for mutagenesis due to its proximity to an *Activator* transposable element. A reverse genetic screen of 533 individuals recovered two exonic insertion alleles. RT-PCR demonstrated that only one allele confers a stably inherited complete knockout. Gas exchange analyses evaluating this mutant allele showed no significant difference in steady state net assimilation values, yet nearly a 10% increase in stomatal conductance, transpiration, and intercellular CO_2 concentrations relative to wild type was observed. Stomatal closure in response to low light is nearly 40% slower than wild type, while opening in response to restored light is only 10% slower. Stomatal closure in response to high CO_2 is 25% slower in the mutant, while stomatal opening in response to low CO_2 is not significantly different. Overall, wild type has a 7% higher intrinsic water-use efficiency relative to the mutant. Future evaluation of *ZmPIP1;5* will explore the interaction of individual CO_2 and water transport components in conferring the observed phenotype. Collectively, optimizing the expression profile of *ZmPIP1;5* and other aquaporins provides an avenue for improving crop water-use efficiency.

Gene / Gene Models described: *ZmPIP1;5*; Zm00001eb190950
Funding acknowledgement: United States Department of Agriculture (USDA)

P112

Ketols produced by a 9-Lipoxygenase play a role in defense against insect herbivores.

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The major defense hormone governing resistance to insect herbivory is jasmonic acid (JA), one of the oxylipins produced in the 13-lipoxygenase (13-LOX) pathway. However, the roles of a large group of 9-oxylipins produced by 9-LOXs in insect resistance is unclear. Here, we report a novel anti-herbivory role of a 9-LOX, ZmLOX5, and its oxylipin product, an alfa-ketol, 9-hydroxy-10-oxo-12(Z),15(Z)-octadecadienoic acid (9,10-KODA). We show that *lox5* knock-out mutants are susceptible to beet and fall armyworms. Although JA levels are reduced in the *lox5* mutants, exogenous JA did not rescue resistance. In contrast, the 9-oxylipin, 9,10-KODA, completely rescued resistance in the *lox5* mutant. Furthermore, transfusion with xylem sap supplemented with 1 uM 9,10-KODA primed maize for a strong resistance response against herbivory primarily due to increased biosynthesis of 12-OPDA, the precursor to JA. Wounding of 9,10-KODA-treated plants induced a rapid or stronger defense gene expression. In addition to priming antiherbivore defense, 9,10-KODA supplemented to artificial diet arrested insect growth. The biosynthesis JA is required for 9,10-KODA-priming effect on plant defense as the JA-deficient opr7opr8 mutant susceptibility could not be rescued by this ketol. Our observation shed a light on a previously unknown antiherbivore defense mechanisms mediated by α-ketols.

Gene / Gene Models described: *LOX5*; Zm00001d013493
Funding acknowledgement: United States Department of Agriculture (USDA)

P113
Low-cost photosynthesis phenotypic solutions for GWAS

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Plant photosynthetic productivity is a key constraint on plant growth, development, and yield. Photosynthetic activity is highly sensitive to changes in environment including osmotic status, temperature and light intensity. The rapid changes in photosynthesis combined with the high cost and long measurement times required to measure photosynthetic parameters have limited the study of natural variation in photosynthetic parameters under field conditions. Here we evaluate the feasibility of a low-cost instrument for quantifying photosynthetic phenotypes I a large maize diversity panel. Measurements of Fv/Fm made with the low-cost instrument and gold standard sensors across a small panel of diverse genotypes under multiple treatments in controlled environments were highly correlated. In the field, a large proportion of total variability of observed Fv/Fm was explained by variation in light density and temperature throughout the three-day collection period required to phenotype 1,680 plots, as well as instrument to instrument variation. In a second experiment, conducted over three nights a large proportion of variation in many photosynthetic parameters was explained by genetic factors. Genome wide association studies conducted with the resulting photosynthetic traits identified a number of significant loci distributed across the maize genome.

Funding acknowledgement: United States Department of Agriculture (USDA), 2018 Wheat Innovation Program, Hatch Multistate Research Capacity Funding Program of the USDA

P114
Maize accessions exhibit considerable variation in phenylalanine-derived metabolites

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Untargeted metabolomics has the power to describe the metabolic space of a plant, and when combined with genome wide association (GWA), can provide information about the genetic factors involved in the synthesis and regulation of metabolites. Phenylalanine (Phe) is a precursor for a diverse array of soluble specialized metabolites. Here, we identified Phe-derived metabolites that are produced in the leaves of 23 NAM founders by feeding $^{13}C_6$-ring labeled Phe to each line, and tracking the incorporation of the label into metabolites that were detected through untargeted LC-MS. This approach identified over 800 Phe-derived metabolite features, many of which exhibited extreme variability in at least one NAM founder. This included variation among multiple types of hydroxycinnamoyl esters, feruloylsucroses, flavones and flavonols. Post hoc tandem MS analysis of the ^{13}C-labeled mass features led to structural predictions for over 100 metabolites. This library was then used to annotate Phe-derived metabolites within the leaf metabolomes of members of an F1 hybrid population of 220 diverse inbred lines crossed with H95. Following GWA analysis, these annotations led to the identification of multiple Phe-derived metabolites that had strong associations to SNPs in, or linked to, annotated phenylpropanoid pathway genes. Overall, this dual labeling and GWA approach contributes a comprehensive accounting of the nucleotide variation that influences metabolite variation within a single metabolic pathway in maize

Funding acknowledgement: United States Department of Agriculture (USDA), Department of Energy (DOE)

P115

Maize genetic engineering and gene editing research and services at the Wisconsin Crop Innovation Center (WCIC)

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The goal of the Wisconsin Crop Innovation Center (WCIC) at the University of Wisconsin-Madison is to develop and offer crop genetic engineering (transformation) and gene-editing services in elite, commercially relevant germplasm and cultivars in crops to facilitate functional genomics research, crop improvement applications, and technology transfer. Research aimed at development, optimization, and deployment of enhanced transformation and editing systems for maize has focused on three main approaches: 1) Optimization of "standard" maize transformation/editing protocols to achieve efficient transformation of maize inbred line LH244, 2) Establishment and optimization of Bbm/Wus2 morphogene-based protocols licensed from Corteva Agrisciences to achieve efficient transformation of multiple diverse maize inbred lines, and 3) Development and optimization of novel, efficient, genotype-flexible maize transformation/editing systems. Maize inbred line LH244 is our current primary recommended line and is a tissue culture-responsive, elite B73-type Stiff Stalk inbred line made available to the public by Bayer Crop Science. Its use is supported by Bayer's release of a complete genome sequence. Through optimization of "standard" (Type 1 callus-based) transformation protocols, we were able to establish routine, efficient transformation of LH244 and now offer LH244 transformation/editing services to the public at WCIC. Research aimed at establishment and optimization of the Bbm/Wus2 morphogene-based systems resulted in successful transformation of several dent and sweet diverse maize inbreds, (including very high efficiency transformation of LH244) and WCIC will be offering morphogene-based transformation/editing services for specific maize inbreds, and via collaboration for non-standard materials. In addition to expanded maize transformation/editing services, we have generated maize/monocot-optimized, GoldenGate-compatible vectors and components for use in our vector construction services and for distribution to the public, and evidence of efficacy for various uses will be presented. Copy number assay services have been established and are available to the maize community. Further details on WCIC maize service offerings, and updates on technology advances can be found at cropinnovation.cals.wisc.edu.

Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Department of Energy (DOE), Wisconsin Alumni Research Foundation (WARF), CALS-University of Wisconsin

P116

Metabolomic analysis of maize pollen during storage

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Pollen is a defining feature of flowering plants and contributes to the reproductive success of many key agricultural crops. Decades of genetic and molecular research have contributed to our understanding of pollen morphology and development. While pollen samples are prevalent in the fossil record, most pollen types are known to exhibit short timeframes of viability. PowerPollen has recently developed novel proprietary technology to facilitate removing grass pollens from the natural pollination system and keep pollen viable under storage until pollination is needed. An outstanding question in the field is what molecular changes occur within pollen after maturation and before death. To determine if altered metabolic profiles are associated with maize pollen viability we performed quantitative metabolomics of fresh maize pollen at maturity and under storage conditions known to prolong viability. To assess phenotypic differences in metabolite accumulation patterns associated with maize inbreds we also profiled two unrelated inbred genotypes with three technical replicates. Non-targeted GC-MS based total metabolite profiling identified 969 metabolites in total, including amino acids, fatty acids, sugars, and sterols. To assess whether key metabolite(s) may be associated with mature and/or viable pollen post-storage I have implemented the R-based package MetaboAnalyst 5.0. Key metabolites of interest which are altered in response to pollens storage and/or genotype were identified from univariate and multivariate methods. This study enhances our understanding of pollen biology and will advance our understanding of molecular factors that contribute to reproductive success.

P117 @LaurenJenx_Sci

Opaque-2 mutant development provides insight into protein rebalancing mechanism

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Attempts to increase essential amino acids through protein over-expression or knockout had limited success mainly due to a phenomenon known as rebalancing. This study aims to shed light on aspects of this protein bound amino acid (PBAA) maintenance phenomenon in maize by looking across development at the amino acid and proteomic responses in the classic re-balanced mutant, Opaque-2 (o2). Opaque-2 mutants have a major decrease in the 22 kDa alpha-zein but relatively comparable PBAA composition to WT with a slightly higher lysine content. Toward this goal, we analyzed lyophilized kernels ranging from seed filling to mature seed stages (14-46 days after pollination, DAP) of B73 and o2/B73 maize for amino acid and proteome composition.Consistent with previous findings, despite minor differences, amino acid levels and composition remained largely similar to WT levels. Those differences were more pronounced towards the end of the seed maturation. The developmental proteome, on the other hand, showed 2512 differentially expressed proteins (DEPs) at some point across development with each timepoints ranging approximately 200 to 400 DEPs. GO analysis of developmental DEPs indicates key processes in energy metabolism, nitrogen compound processes, and carbon metabolism differ across the whole development period. Proteins involved in stress response are differentially expressed earlier in development, while proteins involved in sulfur/thioester compounds are differentially expressed later in development. These results indicate different mechanisms and biological processes were involved in the proteome rebalancing of o2 mutants.

P118 @amaizeingcorn

Paternal imprinting of *dosage-effect defective1* contributes to seed weight xenia in maize

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Historically, xenia effects were hypothesized to be unique genetic contributions of pollen to seed phenotype, but most examples represent standard complementation of Mendelian traits. We identified the imprinted *dosage-effect defective1* (*ded1*) locus in maize (*Zea mays*) as a paternal regulator of seed size and development. Hypomorphic alleles show a quantitative seed weight reduction when *ded1* is transmitted through the male. *Ded1* encodes an R2R3-MYB transcription factor expressed specifically during early endosperm development with paternal allele bias. DED1 directly activates early endosperm genes and endosperm adjacent to scutellum cell layer genes, while directly repressing late grain-fill genes. These results demonstrate xenia as originally defined: Imprinting of *Ded1* causes the paternal allele to set the pace of endosperm development thereby influencing grain set and size.

Gene / Gene Models described: *ded1*; Zm00001d033265
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P119
RAMOSA3 determines inflorescence branching and undergoes liquid-liquid phase transition

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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 of correlation between TPP enzymatic activity and branching phenotypes. Interestingly, RA3 localizes to speckles in cytoplasmic and nuclear compartments. RA3 contains intrinsically disordered regions, which are predicted to induce the formation of biomolecular condensates in a process called phase separation. Notably, we found that RA3 undergoes liquid-liquid phase transition in vitro. These results suggest that the in vivo RA3 nuclear puncta may be phase-separated condensates. Mass spectrometry-based targeted proteomics found that RA3 can associate with two closely related RNA binding proteins localized in nuclear speckles. Consistently, bimolecular fluorescence 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 the nuclear condensates in modulating meristem determinacy. These findings provide a potential mechanistic link between metabolic signals and gene regulation in inflorescence architecture.

Gene / Gene Models described: *ra3*; Zm00001d022193
Funding acknowledgement: National Science Foundation (NSF)

P120
REL2 mediated plant-pathogen response

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Protein acetylation is a major post-translational modification that modulates many different cellular processes, including plant immunity and stress responses. *Cochlibolus carbonum,* Northern Corn Leaf Spot, produces the effector HC-Toxin, a histone deacetylase inhibitor required for pathogen virulence. RAMOSA1 ENHANCER LOCUS2 (REL2) is a transcriptional corepressor homologous to TOPLESS (TPL) in *Arabidopsis.* These corepressors act in different pathways including auxin (TPL-IAA-ARF) and jasmonate (TPL-JAZ-MYC2) signaling. We identified a lysine acetylation site on REL2 using global acetylome profiling of corn treated with HC-Toxin or *C. carbonum.* Furthermore, we found that *rel2* loss of function mutant plants are susceptible to infection, demonstrating that REL2 is directly related to plant immunity. 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; Zm00001d024523; Zm00001eb415530
Funding acknowledgement: National Science Foundation (NSF)

P121

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

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

Funding acknowledgement: National Science Foundation (NSF)

P122

Single-cell RNA sequencing reveals tissue localization of the trehalose-6-phosphate pathway in maize leaves

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Trehalose-6-phosphate (Tre6P) has been proposed to act as a sucrose availability signaling metabolite by informing the metabolic status of the plant. It has an essential role in developmental processes by regulating sink-source tissue relationships. Also, it has been implicated in stress tolerance in crops. The Tre6p synthesis pathway is composed of two enzymes, trehalose-6-phosphate synthase (TPS), which synthesizes Tre6P, and trehalose-6-phosphate phosphatase (TPP), which dephosphorylates Tre6P to produce trehalose. These enzymes are encoded by large multigene families that present spatial and temporal specificity. TPS genes are divided into two classes, with only Class I genes encoding active enzymes. In maize (*Zea mays* L.), there are two Class I genes, *ZmTPS1 and ZmTPS12*. *ZmTPS1* encodes a catalytically active enzyme proposed to be the main enzyme for this pathway. *ZmTPS12* is predicted to encode a truncated protein. Here, we used a single-nuclei RNA sequencing approach to determine cell-specific expression patterns of Class I TPS and TPP genes in maize leaf tissue. We found that *ZmTPS1* is expressed in the vascular bundle, specifically in phloem parenchyma, xylem parenchyma, and companion cells/sieve elements complexes. *ZmTPS12* shares this expression pattern but was also found to be expressed in mesophyll cells. *ZmTPS1* is expressed at a highly strategic site for phloem loading and sucrose export to sinks. These results improve our understanding of the possible role of Tre6P as a signaling molecule in maize. We also found that three genes, *ZmTPP2*, *ZmTPP6*, and *ZmTPP14*, are specifically expressed in mesophyll cells. These observations provide insights into the compartmentalization of the Tre6P enzymes in maize source tissue. Here, we show the first evidence of cellular localization of the Tre6P pathway in a crop with strong sink tissues that remobilize sugar. Further studies will be undertaken to translate this novel discovery into crop improvement for abiotic stress tolerance.

Gene / Gene Models described: *TPS1, TPS12, TPP2, TPP6, TPP14*; Zm00001eb353280, Zm00001eb293110, Zm00001eb098650, Zm00001eb249690, Zm00001eb018720
Funding acknowledgement: United States Department of Agriculture (USDA)

P123

Standardized genome-wide function prediction enables comparative functional genomics: a new application area for Gene Ontologies in plants

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The availability of genome-wide gene function annotations enables researchers to generate hypotheses and prioritize candidate genes that may be responsible for phenotypes of interest. In this study, we have functionally annotated 18 crop plant genomes across 14 species using the GOMAP pipeline (Gene Ontology Meta Annotator for Plants; doi.org/10.1186/s13007-021-00754-1). Compared to other GO (Gene Ontology) annotation datasets, GOMAP offers datasets with higher gene coverage and more GO term annotations. We were interested in determining whether the GOMAP-generated datasets could be used to perform comparative functional genomic analyses across the different species of plants. Therefore, we generated dendrograms of functional relatedness based on GO term datasets of the 18 genomes as a proof of concept. The resulting dendrograms were compared to well-established species-level evolutionary phylogenies to assess whether the generated trees were in agreement with known evolutionary relationships, which they largely are. Where discrepancies were observed, we determined branch support based on jack-knifing then removed individual annotation sets by genome to identify the annotation sets causing unexpected relationships. As a conclusion, using GOMAP-generated functional annotations across different plant species generally retain sufficient biological signal to recover known phylogenetics relationships based on genome-wide functional similarities. This shows that comparative functional genomics across species based on GO data hold promise for generating novel hypotheses about comparative gene function and traits.
Funding acknowledgement: ISU PSI, P3 NSF NRT, IOW04714 Hatch funding to ISU

P124 @MASanclemente

Sugar and oxygen responses are modulated by maize NDPK1

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Dual roles in metabolism and gene expression are hypothesized for NDPKs (Nucleoside Diphospho Kinases), enzymes poised to mediate sucrose metabolism and diverse reactions via nucleotide balance (eg. ADP+UTP ATP+UDP), as well as regulate stress-responsive genes by unwinding G4 cis-elements (4-stranded DNA quadruplexes). Here we test whether altered expression of an *Ndpk1* gene can disrupt sugar metabolism, low-oxygen responses, and transcript profiles for G4-containing genes. To do so, we identified two mutant alleles of *ndpk1* from the UniformMu maize population, and utilized a maize root-tip system to precisely control sugar and oxygen availability. The *ndpk1*-1 and *ndpk1*-2 mutations reduced abundance of *Ndpk1* mRNA by ~35% and 70%, respectively, and enzyme activity (for all NDPKs) by ~45%. Independence of the two alleles confirmed genotype-phenotype relationships. Although a knock-down phenotype was not detected in kernels, seed germination was delayed, growth of seedling roots was slowed (*invivo* and *invitro*), and root-tip sugar levels were reduced, especially sucrose. Profiles of these sugars in mutant root tips were largely rescued by exogenous glucose, but growth was not. Responses to low oxygen (0.2% vs 2.0%) were also impaired, with fewer differentially-expressed genes in mutant root tips, and limited involvement of mRNAs for anaerobic proteins central to carbohydrate metabolism. Genes with G4 motifs were enriched in low-oxygen transcriptomes regardless of genotype, but their extent and identities differed. In *ndpk1*-mutant root tips, key responses of G4-containing genes were lacking or markedly attenuated (eg. *shrunken1* sucrose synthase, enolase, pyruvate kinase, and other anaerobic proteins). Collective data demonstrate a role for NDPK1 in sugar metabolism, low-oxygen responses, and transcriptome restructuring consistent with emerging evidence for broader regulatory functions in plants and animals.

Gene / Gene Models described: *Ndpk1*; Zm00001d029969
Funding acknowledgement: National Science Foundation (NSF)

P125
Targeted suppression of gibberellin biosynthetic genes *ZmGA20ox3* and *ZmGA20ox5* produces a short stature maize ideotype

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Maize is one of the world's most widely cultivated crops. As future demands for maize will continue to rise, fields will face ever more frequent and extreme weather patterns that directly affect crop productivity. Development of environmentally resilient crops with improved standability in the field, like wheat and rice, was enabled by shifting the architecture of plants to a short stature ideotype. However, such architectural change has not been implemented in maize due to the unique interactions between gibberellin and floral morphology which limited the use of the same type of mutations as in rice and wheat. Here, we report the development of a short stature maize ideotype in commercial hybrid germplasm, which was generated by targeted suppression of the biosynthetic pathway for the plant hormone gibberellin (GA). To accomplish this, we utilized a dominant, miRNA-based construct expressed in a hemizygous state to selectively reduce expression of the *ZmGA20ox3* and *ZmGA20ox5* genes that control GA biosynthesis primarily in vegetative tissues. Suppression of both genes resulted in the reduction of GA levels leading to inhibition of cell elongation in internodal tissues, which reduced plant height. Expression of the miRNA did not alter GA levels in reproductive tissues, and thus, the reproductive potential of the plants remained unchanged. As a result, we developed a dominant, short stature maize ideotype that is conducive for commercial production of hybrid maize.

Gene / Gene Models described: *ZmGA20ox3, ZmGA20ox5*; GRMZM2G368411, GRMZM2G049418
Funding acknowledgement: Bayer Crop Science, BASF

P126
Targeting meiotic recombination in maize

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Crossovers (CO) produced as a result of meiotic recombination are instrumental in plant breeding. Alas, they are not evenly distributed along chromosomes, leaving extensive chromosome regions CO-depleted. CO formation starts by the production of double strand breaks (DSBs) in chromosomal DNA. These breaks are catalyzed by the SPO11 protein complex. Our goal is to develop a tool based on genome editing techniques to target SPO11 to specific sites on maize chromosomes to trigger meiotic DSBs. SPO11 itself does not confer DNA-binding specificity. Thus, the transgenic maize lines we are generating will express SPO11 fused to an inactivated Cas9 protein that is capable of binding DNA in a sequence-specific manner but lacks endonuclease activity. Inducing DSBs in CO-favorable chromosome environment should lead to CO formation. Being able to target COs to desired chromosome loci will improve selection efficiency and contribute to more effective methods for plant breeding.

Funding acknowledgement: National Science Foundation (NSF)

P127

The auxin response factor *ZmARF27* is required for maize root morphogenesis

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The maize root system is necessary for optimal plant productivity as it functions to uptake water, assimilate essential nutrients from the soil, and provide structural integrity. Therefore, understanding the molecular drivers of root growth and development is key for future crop improvement strategies. The endogenous growth hormone auxin has been shown in other angiosperms to influence root morphogenesis, however there is little known about auxin regulated root development in maize. We aim to fill this knowledge gap by studying the interplay between auxin signaling and the plasticity of maize root architecture. We have identified the auxin regulated maize transcription factor *AUXIN RESPONSE FACTOR 27 (ARF27)* to be enriched in the elongation and meristematic zones of the primary root. Loss-of-function mutations in *ARF27* results in plants with short primary roots, reduced lateral root density and short root hairs. A suite of root growth and auxin-related gene products are altered in *arf27* mutants compared to wild-type W22 based on transcriptomic and proteomic analyses. In addition, *arf27* does not exhibit a robust transcriptional response to auxin when compared to W22. Future work will focus on revealing root-specific *in vivo* target genes of *ARF27* using chromatin immunoprecipitation followed by sequencing (ChIP-seq) as well as identification of *ARF27* interacting proteins by yeast two-hybrid and TurboID approaches.

Gene / Gene Models described: *arftf27*; Zm00001d045026
Funding acknowledgement: United States Department of Agriculture (USDA)

P128

The effect of the ACT-like domain on the C-terminal region of B in *Zea mays*

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The basic helix loop helix (bHLH) transcription factor R is a part of the regulatory network of the anthocyanin biosynthesis pathway in maize. Its homologue B, specifically the allele B-*peru*, controls anthocyanin biosynthesis in the plant and the seed. Previous studies show that both R and B have an ACT-like domain in their C-terminal region adjacent to their bHLH domain. In R, it has been shown that both the bHLH domain and ACT-like domain can dimerize, and that the presence of the ACT like domain affects DNA binding of the bHLH domain to canonical G-box (CACGTG) promoter motifs of the target DNA. When the ACT-like domain dimerizes, the bHLH is monomeric and R is bound to DNA indirectly, through its interaction with C1. When the ACT-like domain is not dimerized, then the bHLH domain dimerizes and R recognizes G-box motifs directly through the dimerized bHLH. However, it is not known whether B exhibits similar dimerization and DNA binding behavior in terms of the relationship between the bHLH and ACT-like domains. We hypothesized that the ACT-like domain of B, like R, affects the ability of B to dimerize, as well as its ability to bind to the G-box motif of target DNA. We performed yeast 2 hybrid assays (Y2H) and amplified luminescent proximity homogeneous assays (ALPHA) in order to examine the dimerization and DNA binding behaviors of B in comparison to R. We performed these assays on various purified fragments of B containing different combinations of the bHLH and ACT-like domain. Our results suggest that the C terminal region of B only shows strong dimerization with the ACT-like domain compared to just the bHLH domain. In addition, our results suggest that the ACT-like domain of B hinders the binding of B to the G-box of the target DNA.

Funding acknowledgement: National Science Foundation (NSF)

P129

The maize *rough endosperm6* (*rgh6*) mutant encodes a predicted Dead-box RNA helicase

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Maize *rough endosperm* (*rgh*) mutants have defective seeds with a rough, etched, or pitted endosperm surface. Molecular genetic analysis of this mutant class has identified multiple RNA processing proteins critical to endosperm development. We isolated the *rgh6* locus from the UniformMu transposon tagging population. Mutant kernels have reduced grain-fill with defective embryos that fail to germinate. Self-pollination of *rgh6* heterozygotes produces mutant seeds at frequency consistent with a single recessive mutation. Molecular mapping localized *rgh6* to a 60 kbp interval on chromosome 5. PCR analysis of gene models within the fine-map interval identified a *Mutator* (*Mu*) transposon insertion within a predicted Dead-box RNA helicase gene. A second allele of *rgh6* was identified from UniformMu population. Reciprocal crosses between *rgh6-umu1* and *rgh6-umu2* heterozygotes produces mutant seeds at a frequency consistent with a 3:1 ratio indicating these mutants are allelic. Both *rgh6-umu1* and *rgh6-umu2* have *Mu*element insertions at the same insertion site within the Dead-box RNA helicase gene. However, the terminal inverted repeat sequences are polymorphic between the alleles showing the alleles are independent mutations. We conclude that mutation of the Dead-box RNA helicase causes the *rgh6* phenotype. Further characterization of the *rgh6* mutant phenotype and biochemical investigation of the RGH6 protein is expected to give further insights on the roles of RNA processing in endosperm development.

Funding acknowledgement: National Science Foundation (NSF), National Institute of Food and Agriculture

P130

The making of a transfer cell. Insights from the transcriptome of CRISPR-generated ZmMRP mutants.

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The endosperm transfer cell layer (BETL) differentiates at the base of the developing maize endosperm. Transfer cells (TC) develop an intricated network of cell wall ingrowths, increasing the available surface to exchange nutrients and other solutes with the mother plant. TC are thus crucial for grain filling, and a critical influence in yield might be anticipated. In addition, the TC transcriptome includes dozens of genes encoding highly abundant small peptides, some of which demonstrated antifungal activity, suggesting an additional role for the BETL as a generator of an antipathogen barrier between the mother plant and the filial tissues.
Years ago, we described a gene encoding a TC-specific transcription factor, ZmMRP-1, which putatively masters the TC differentiation process and controls the expression of many of the TC-specific genes. Now, we have engineered mrp1 KO alleles to find that the function of the gene is entirely dispensable due to complete redundancy with another related maize gene, ZmMRP-4. However, in an RNAseq experiment, mrp1/mrp4 double-KO mutants showed a profoundly modified transcriptome: hundreds of genes appeared downregulated in the KO kernels. We will discuss the extent of the phenotype presented by the double-KO TC and the possible molecular pathways responsible for that phenotype. We will also discuss cases of genes that have been associated over the years with the TC transport role or differentiation process through the ZmMRP-1 regulatory network but now appear not to be regulated by the MRPs.

Gene / Gene Models described: *ZmMRP-1*; GRMZM2G111306
Funding acknowledgement: Spanish MCI: PGC2018-101803-B-I00

P131

Tissue-specific promoters in maize: Identification, cloning, and characterization

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Functional genomic research and genetic enhancement of maize, a locally and globally important crop, relies on having a variety of promoter tools available. Process efficiency and valid interpretation of genetic/epigenetic research is critically dependent on promoters behaving in an expected and specific manner. Currently, the inventory of characterized promoters imparting tissue/organ/timing-specific expression in maize is low, limiting maize genetic research and enhancement efforts. To address the need for a wider array of characterized maize promoter choices, we developed protocols for identifying, cloning, and characterizing promoters in maize and used them to identify and characterize promoters which confer leaf, embryo, and root-specific gene expression. Candidate promoters were identified through analyzing RNAseq data from the Maize Gene Expression Atlas with distance metrics to an idealized promoter profile. While TPM was used for normalization, comparison across tissues may conflict with normalization assumptions and care must be taken when interpreting results. The initial list of candidate genes/promoters was narrowed by eliminating genes with long-range activating regions as well as any documented involvement in stress responses or any pathway which would reduce stability of promoter expression. Once identified, promoter sequences were domesticated for use in Golden Gate cloning and vectors constructed with candidate promoters driving expression of the GUS screenable marker gene. The constructs were then transformed into maize inbred line LH244 for its ease of transformation, sequenced/assembled genome accessibility, and similarity to model maize inbred line B73. In order to standardize characterization, a set of "minimal tissues" were defined through PCA analysis of expression data. These tissues were then assessed with MUG assays. Initial results indicated that 4 out of the 15 promoter sequences tested showed desired tissue/organ-specific expression and will be made available to the maize research community for use in functional genomics research and crop improvement applications.

P132

Transcriptomic signatures of asymptomatic stress in maize

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Recognition of stress before it manifests in adverse plant phenotypes can offer new opportunities for predicting and preventing plant productivity losses. Better understanding of early stages of stress, when there are no apparent changes in plant phenotype, is critical for enabling these future approaches. To this end, we designed and conducted a transcriptomic study that assessed molecular responses of maize plants to mild stress. Results revealed that molecular signatures of mild asymptomatic stress share many similarities with a more severe, symptomatic stress state of the same type. Detected similarities of responses offer an opportunity for further exploration of additional phenotyping and molecular methods that reliably identify early stages of stress in crop plants.

P133

Unlimited maize height

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Maize height was limited, so a stock was made that is not. A plant was culled at 80 ft, which took 1.5 years. It had 200 leaves and roots at 70 ft.

P134 @walk2iron

Using iron transporter genes to enhance the nutritional quality of maize grain

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Corn (*Zea mays*) is a staple food throughout the world, including parts of Africa, Asia, and South and Central America. Because of corn's prominence as a staple grain, the nutritional quality of corn has received substantial attention. The metal micronutrient content of corn, especially iron and zinc, is of considerable interest since breeding for improved micronutrient content and availability would have substantial impacts on human health. HarvestPlus has set a target level for iron (60 ug/g) in maize, but this level may not be achievable through conventional breeding because of naturally low levels of iron in maize grain. A small number of Arabidopsis genes, *i.e.*, particular genes in the the *Yellow striped1-like* (*YSL1* and *YSL3*) and *Oligopeptide Transporter* (*OPT3*) families, affect seed iron deposition. These genes are all internal iron transporters, and are expressed most abundantly in the phloem. This suggests that the amount of iron that the plant deposits into phloem may determine the amount of iron that is eventually accumulated by the grain. However, it remains possible that specific transport either from the maternal tissues close to the developing seed, or in filial tissues of the embryo or endosperm also have an important role. We are investigating the maize orthologs of these genes to determine whether they have roles in iron accumulation in kernels, and ultimately will examine whether targeted over-expression of these genes can cause increased iron accumulation in maize grains. To begin, we have identified transposon insertion alleles of the three maize genes, and are currently assessing whether these represent knock down or loss of function alleles. We will present our current progress. To accelerate identifying homozygous insertion alleles, we have developed a TaqMan-based genotyping strategy. We will also present our strategy for targeted overexpression of these genes.

Gene / Gene Models described: ; GRMZM2G421491, GRMZM2G051179, GRMZM2G026391, Zm00001eb056510
Funding acknowledgement: United States Department of Agriculture (USDA)

P135

What parts of receptor proteins affect the speed of hormone response in corn cells?

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Auxin is a plant hormone known to affect the development of maize ears and tassels by inducing repressor degradation but the full picture of how the repressor and receptor interact remains unclear. Transport Inhibitor Response 1 (TIR1) is the receptor protein that initiates repressor degradation in response to auxin. Previous studies identified a number of amino acid residues in the Arabidopsis TIR1 (AtTIR1) protein that significantly affect repressor degradation rate. We made analogous mutations in maize TIR1 (ZmTIR1) and confirmed similar effects on the degradation rate. Mutations that disrupted sidechain chemical properties–D181A and L484A–showed a drastic drop in degradation rate; mutations that made minor changes–C151S and D181E–resulted in little to no effect on degradation rate. Since these sites are located distal to the auxin and repressor binding site, it is likely that they are affecting interactions with the disordered parts of repressors that flank the auxin binding region, disrupting dimerization of the ZmTIR1, or causing a larger structural change that might affect repressor-receptor binding.

Funding acknowledgement: National Science Foundation (NSF)

P136

Wheat proteomic analysis of salt tolerance in response to Bacillus safensis (ST17) and Bacillus tequilensis (ST25)

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Salinity in one of the major abiotic stresses, affecting the global food crop production. Salt tolerant plant growth-promoting rhizobacteria (PGPR) are prospective bio inoculants for improving crop yield in saline agriculture; however, the molecular mechanism behind the interaction between PGPR and plants is not fully known. In the present research, we studied the role of IAA (indole acetic acid) producing salt tolerant bacterial strains strains ST17 (*Bacillus safensis*) and ST25 (*Bacillus tequilensis*) on the proteome profile of Wheat (*Triticum aestivum* L.) under high salinity (200mM NaCl) stress by using an integrated approach of Tandem Mass Tag labeling and LC-MS/MS. Statistically significant differentially expressed proteins (DEPs) were studied in five different treatments (T1-T5). Based on proteomic data, we explored the adaptation mechanism of wheat seedlings exposed to a high salt concentration of NaCl (200mM) for three weeks in response to bacterial inoculation. Proteomic profiling revealed that changes in the metabolic pathway, defense mechanism, and structural activity might be involved in stress response to PGPR inoculation. Our findings imply that bacterial inoculation improved the wheat plant's ability to withstand salt stress via upregulation of metabolic, structural, photosynthetic proteins and the proteins involved in cold acclimation, DNA strand elongation, protein processing, and post replication repair.

P137 @JinliangYang

A historically balanced locus under recent directional selection in responding to changed nitrogen conditions during modern maize breeding

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Understanding the patterns of selection during plant evolution and recent crop improvement processes is the central topic in population genomics and is important for plant breeding and genetics. As an essential macronutrient for plant growth and development, nitrogen (N) is a key factor in affecting plant adaptation and crop improvement. The widespread adoption of less expensive industrial N fixation has dramatically reshaped plant morphology by favoring compact maize plants to tolerant crowding stress. The associated genetic changes, however, have not been systematically studied. Here, we investigated maize inbred lines developed before and after the 1960s --- the time point when inorganic N fertilizer started to be widely used for maize production. We identified a strong selective sweep exhibiting pronounced genomic differentiation between Old-Era (pre-1960s) and New-Era (post-1960s) inbred lines. Further study revealed population genetics statistics in the sweep exhibited patterns consistent with historical balancing selection. This balanced genomic interval is associated with a number of morphological, physiological, and metabolite traits related to vegetative N responses. A cluster of three glutamate receptor-like (GLR) genes is located within the region targeted by selection. Functional characterizations suggested differences in transcriptional activity of the GLR genes between the haplotypes carried by Old-Era and New-Era inbred lines likely play an essential role in mediating distinct N responses. The identification of both targets of selection and changes in the regulation of N responsive genes between maize lines developed in different eras sheds light on the N sensing and regulation pathways and paves the way to developing N resilient crops.

Gene / Gene Models described: ; Zm00001d014451, Zm00001d014456, Zm00001d014458
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA)

P138
Detection of Abnormal Chromosome 10 in genotype by sequencing data

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Abnormal chromosome 10 (Ab10) is a larger variant of normal chromosome 10 (N10) that drives the transmission of itself, and masses of heterochromatin (knobs) found throughout the genome. It accomplishes this by acting as a meiotic driver during female meiosis. Ab10 likely has environment specific fitness effects as it is more prevalent at lower elevations. There is limited information regarding the effect of Ab10 on maize fitness, and no research about these effects in varying environments. The wealth of publicly available genotype-by-sequencing data for maize landraces combined with the recent assembly of the Ab10 haplotype make it possible to easily detect the presence of Ab10 across large geographic ranges. I am in the process of detecting Ab10 in genotype by sequencing data for ~3000 maize landrace samples. I will then pair this data with traditional geographic variables (altitude, latitude, longitude) as well as climate models to better elucidate the relationship between Ab10 and the environment.

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

P139
Exploring Cis-Regulatory Evolution across Andropogoneae

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Gene expression plasticity is a key mechanism of environmental response in plants. Cis-regulatory changes that alter patterns of gene expression are an important mode of adaptation to environmental conditions, but limited work has explored regulatory evolution on a large scale. This project exploits one billion cumulative years of regulatory evolution across the Andropogoneae grass tribe to characterize cis-regulatory motifs associated with temperature-responsive differential gene expression. We leverage RNA-seq data from diverse grass species to identify genes differentially expressed under cold conditions, and then scan for regulatory motifs enriched near differentially-expressed genes. Presence-absence variation of key motifs will be characterized across hundreds of Andropogoneae species, and motif-environment associations explored. We hypothesize that many regulatory motifs are conserved across Andropogoneae and that motif presence-absence variation is associated with adaptation to divergent temperature regimes across the evolutionary history of the Tribe. By understanding key components of temperature-responsive gene regulation, we could improve the performance of genomic prediction models across environments by better prioritizing functional variants.

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

P140 @snodgrasshopper

Identifying fractionation events across the *Tripsacinae* subtribe

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Polyploidy and fractionation-- the processes by which genomes grow and shrink in size-- characterize plant genome evolution. Researchers have done extensive work on these twin processes, yet questions remain as to the tempo of fractionation and what is lost during genome downsizing. The *Tripsacinae* subtribe, which contains the genera *Tripsacum* and *Zea*, underwent a whole genome duplication event (WGD) ~12MYA after divergence from *Sorghum*. Species of these genera, including *Zea mays* spp. *mays*, have since fractionated back to a diploid-like state and in some cases (ex. *Zea perennis*) have undergone another round of polyploidy. With high-quality, de novo assemblies of species from across both genera, we can begin to tease apart remaining questions regarding the tempo of fractionation and fractionation differences between species descending from the same WGD event. But first, we must accurately call fractionation events across multiple genomes. Previous work identifying fractionation events has relied heavily on manual curation due to issues with automated pipelines. This becomes impractical when scaling to larger numbers of genomes. Thus, we have evaluated the new whole genome alignment program AnchorWave in calling fractionation events, using the maize Nested Association Mapping (NAM) population genome assemblies. Since fractionation events have already been called using manual curation pipelines in the NAM, we can validate AnchorWave deletions calls against this gold standard. Here we describe our evaluations, which demonstrate AnchorWave as a promising tool for automating fractionation event calling, along with preliminary results of fractionation events across diverse *Tripsacinae* species. A scalable pipeline will ease comparisons of divergent genomes and begin to address remaining questions surrounding fractionation within the *Tripsacinae* and more broadly as a complex and dynamic process of genome evolution.

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

P141 @SierraScientist

Lost Phenotypes: Assessing the effect of maize breeding on Rhizosphere Nitrification Suppression

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Maize (*Zea mays subsp. mays*) domestication occurred ~9000 years ago. Substantial modification to the maize genome has since occurred, including introgressions with sympatric *Zea* species and population bottlenecks. Simultaneously, maize reproductive and vegetative architecture was heavily selected to maximize yields. In the 20th century, selective breeding was conducted in agroecosystems artificially fertilized with nitrogenous fertilizers, removing the selective pressures of nitrogen deficiencies from maize germplasm development. In doing so, the directed evolution of maize may have inadvertently selected against microbial nutritional symbioses important for growth and fitness. Nitrification, the chemolithoautotrophic oxidation of ammonium to nitrate, is an important microbial biogeochemical process with the potential to increase rhizosphere nitrogen loss by promoting nitrate leaching and/or nitrous oxide emissions. Numerous grass species, including landrace rice (*Oryza sativa*), perennial wild rye (*Leymus racemosus*), and sorghum (*Sorghum bicolor*), possess the biological nitrification inhibition (BNI) phenotype, allowing plants to enzymatically inhibit nitrification, and increasing rhizosphere nitrogen availability. We hypothesize that maize breeding in replete N conditions has selected against maintenance of the BNI phenotype in modern maize germplasm. The effect of maize genotype and demography on rhizosphere nitrification potential and microbial communities was assessed using rhizosphere nitrification potential enzyme assays and 16S rRNA amplicon sequencing. . Linear mixed effects models revealed that demographic group significantly influenced rhizosphere nitrification potential ($F_{(7,157)} = 12.424$, P

P142

PIF1 helicase phylogeny and function on the maize B chromosome

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The maize supernumerary B chromosome has a unique ability to maintain itself in populations through a drive mechanism. Understanding this mechanism and its many factors has become a point of interest considering the many applications of the B chromosome in maize genetics and genomics. The PIF1 family of helicases is known to unwind nucleic acid secondary structures, most notably G-Quadruplexes and D-loop structures and to play a role in recombination. The maize A chromosomes contain only 3 PIF1 helicase genes, while the B chromosome has a striking 29! A phylogenic analysis of the PIF1 genes contained within *Zea mays*, *Oryza sativa japonica*, and *Sorghum bicolor*, shows two main clusters of PIF1 genes on the B. Each of the two clusters has high relative homology to separate A chromosome PIF1 genes; this phylogenic pattern suggests the occurrence of two independent transposition events of a PIF1 gene to the B. Each PIF1 B gene within a specific cluster also has higher homology to other PIF1 B genes in that cluster relative to the predicted PIF1 A progenitor, which implies a selection for PIF1 genes on the B after transposition. Because purifying selection on the B chromosome serves the B perpetuation and not the plant itself, PIF1 is likely vital to the B's drive mechanism. RNAseq data shows that all but one of the PIF1 B genes produce gene products in leaf tissue, pollen tissue, or both. What subset of these products are actively serving the PIF1 roles, and what this role is, in the drive mechanism are yet to be elucidated. Further analysis will attempt to elucidate a link between the proliferation of PIF1 genes on the B and their potential contribution to the B drive mechanism.

Funding acknowledgement: National Science Foundation (NSF)

P143

Population genomics identifies candidate genes within selective sweeps in sweet corn

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Plant breeding can exert dramatic changes in several plant traits, either intentionally in productivity and quality components or unintentionally in non-targeted traits. Corn (*Zea mays*) is a quintessential case of how domestication/breeding can alter plant architecture, ear traits and ultimately yield. Despite this efficiency, the specific genes or regulatory elements that have been targeted by selection are, in most cases, unknown. Sweet corn has an interesting breeding history as several target traits were different from the ones selected in field corn. Here we sequenced a diversity panel of 693 sweet corn (*Zea mays*) genotypes to identify selective sweeps across the entire genome. This panel includes landraces, old inbred lines (developed >40 years ago) and elite cultivars with both the *sugary1* and *shrunken2* mutations involved in sugar accumulation. A whole genome resequencing analyses and variant calling with GATK identified 28M high-quality SNPs covering all chromosomes with an average density of >12k SNPs/Mb. Using the cross-population composite likelihood ratio method (XP-CLR) with 50kb windows, we explored 85 genome regions with normalized XP-CLR score >20. These regions span all ten chromosomes of sweet corn and most of them were identified contrasting old versus new, elite inbred lines. On the other hand, the contrast of landraces with old inbreds identified the fewest of these sweeps. Among regions with selective sweeps there were known breeding targets involved in kernel sugar accumulation, such us the *sugary1* and *shrunken2* genes. Other candidate genes within sweeps include genes involved signal transduction (e.g. serine/threonine kinases) and transcription factors (e.g. *wrky1*, *ap2-erebp* and *fha13*). By revealing genes that have potentially been targeted by breeding, population genomics studies like this might be paving the way for a future where gene editing-based precision breeding will be commonplace.

Funding acknowledgement: United States Department of Agriculture (USDA), NIFA-SCRI 2018-51181-28419

P144

Using near-isogenic lines to dissect the genetic architecture of evolutionary change following long-term selection for grain protein concentration

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The Illinois Long Term Selection Experiment is the longest-running continuous genetics experiment in higher plants. More than 350 cycles of artificial selection performed over 120 years have generated distinct populations representing the phenotypic extremes of kernel protein concentration in maize. The current working hypothesis for the sustained changes in kernel protein concentration follows the infinitesimal model, in which the phenotype can be attributed to the contributions of countless loci of small effects that are additive. As a corollary, a polygenic architecture also limits the ability to detect signatures of selection. To help unravel regions of the maize genome that contribute to the rapid and dramatic responses to phenotypic selection, we are developing a panel of near-isogenic lines derived from crosses among the four populations subjected to long-term selection. To aid with the phenotypic evaluation of grain protein concentration, these populations also carried the FLOURY2-RFP reporter gene, a visual marker for alpha-zein accumulation. Following six generations of backcrossing, rates of change for both grain protein concentration and RFP intensity varied significantly among the NILs. One conclusion can already be drawn - not all segments of the genome contribute equally to kernel protein concentration, and many have no detectable phenotypic effect. Further genotyping and analysis of these near-isogenic lines will help reveal important genomic segments and selection signatures that contribute to the variation in kernel protein concentration.

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

P145 @tjcteng

24-nt phasiRNA biogenesis and regulation in maize anther

LIGHTNING TALK

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Anthers are the male reproductive floral organs. The loss of the 24-nt class of phased small-interfering RNAs (phasiRNAs) from anthers confers temperature-sensitive male sterility in maize. Their mRNA precursors, *24-PHAS*, are transcribed by RNA polymerase II from 176 unique or low-copied regions on all 10 chromosomes of the maize genome. The precursors are first cleaved, by direction of miR2275, and then the 3'-fragments are converted into double stranded RNAs, and progressively chopped into 24-nt phasiRNAs by a monocot-specific endonuclease *Dicer-like 5* (*Dcl5*). Both *24-PHAS* and *Dcl5* mRNAs are abundant specifically and coordinately in tapetal cells after the last periclinal divisions are complete, all four wall layers are formed, and meiosis has initiated in the pollen mother cell. Notably, the male sterile maize *ms23*, *ms32*, and *bhlh122* mutants with various degrees of tapetal defects lack *24-PHAS* mRNA precursors and 24-nt phasiRNAs, which were not affected in another male sterile mutant, *bhlh51*. Multiple lines of evidence suggest that 24-nt phasiRNA biogenesis is primarily controlled by MS23 and MS32, including expression of most *24-PHAS* precursors and *Dcl5*, with distinctive action in *24-PHAS* transcription by bHLH122.

Gene / Gene Models described: *ms23, ms32, bhlh122, bhlh51*; Zm00001d008174, Zm00001d006564, Zm00001d017724, Zm00001d053895
Funding acknowledgement: National Science Foundation (NSF)

P146

A disordered protein mediates sex determination and auricle development in maize

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The *feminized upright narrow1* mutant (*fun1*) is a unique recessive mutant that affects both sex determination as well as leaf development. *fun1* tassels are feminized, producing perfect flowers with viable silks that can be fertilized and set seed. Moreover, the leaves lack auricles and have a narrow upright habit. Interestingly, the ligule is normal, distinguishing *fun1* from other maize leaf mutants that affect auricle development. *fun1* was cloned by a combination of chromosome walking and expression profiling and encodes a highly disordered hydrophilic protein that may have signaling function during embryogenesis. Two EMS induced *fun1* alleles were sequenced and have mutations resulting in premature stop codons. Moreover, CRISPR mediated frame shift mutations generated in *Setaria* cause seedling lethality, demonstrating that *fun1* may have an essential function. A specific antibody was raised to FUN1 and used for immunolocalization in maize. L1 layer specific expression was observed in mature leaves and floral organs in tassels. Interestingly, no carpel specific expression was observed, supporting the hypothesis that long distance L1 layer signaling may mediate carpel abortion. To discover the nature of this signal, we performed RNA seq analysis and found genes belonging to two hormone classes, ABA and ethylene, as being differentially expressed. Direct measurement of ABA confirmed a three-fold difference in overall levels in wildtype compared to mutants. In addition, low ABA levels were observed in mutant tassel carpels using an ABA specific antibody for immunolocalization. Taken together, these results indicate that sex determination and leaf development share a common pathway that may be controlled through a mechanism mediating long distance signaling through several stress hormones.

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

P147

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

(submitted by Allison Phillips <allison.phillips@wlc.edu>)
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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 mutants phenocopy *stunter1* (*stt1*), a previously characterized maize mutant with viable but reduced seed size 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 have reduced pollen tube germination. Post-fertilization, embryo and endosperm development are delayed in both *stt2* and *stt3* with 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 and protein content in the endosperm compared to wild-type sibling endosperm, while *stt2* has reduced amylose content but elevated levels of endosperm proteins. Whereas *stt2* is likely allelic to *stt1*, *stt3* is unlinked and represents a unique lesion. The *Mu* insertion in *stt3* falls in the predicted first intron of GRMZM2G155387. The effect of the insertion on gene expression is under investigation. These mutants will help elucidate mechanisms for maternal control of seed development and seed size in maize.

Gene / Gene Models described: *stt1, stt2, stt3*; GRMZM2G155387
Funding acknowledgement: National Science Foundation (NSF), United States Department of Agriculture (USDA), Wisconsin Lutheran College Faculty Mini-Grant Program

P148 @edbertolini

Architectural pleiotropy between tassel branching and leaf angle decoded through gene regulatory network rewiring

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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 identified regulatory modules that contribute to architectural pleiotropy between tassel branch number (TBN) and leaf angle (LA) in maize. 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 prioritize novel genes and regulatory elements underlying diversity in LA and tassel branching phenotypes. We 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 thousand diverse maize lines. Our multi-trait network-assisted GWAS approach highlighted polymorphisms in candidate genes associated 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.

Funding acknowledgement: National Science Foundation (NSF)

P149

Boom or Bust: Regulation of meristem size by the REL2 corepressor family

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In cereal crops, yield increases can result from modulating the size of the inflorescence meristem (IM). Prior characterization of *ramosa1 enhancer locus2* (*rel2)* mutants revealed pleiotropic vegetative and reproductive phenotypes such as defective axillary meristem initiation and IM maintenance in single recessive mutants (PMID:30348817). In maize, the REL2 family of transcriptional corepressor proteins is comprised of four members, REL2, REL2-LIKE1 (RELK1), REL2-LIKE2 (RELK2), and REL2-LIKE3 (RELK3). *rel2;relk1* double mutants were identified in an enhancer screen of the *rel2-ref* mutant allele, whereas loss-of-function mutations in *RELK2* and *RELK3*, two paralogous genes co-orthologous to Arabidopsis *TOPLESS* (*TPL*), were generated by CRISPR-Cas9. Interestingly, whereas double *rel2;relk1* mutants had enhanced ear fasciation (among other phenotypes), triple *rel2;relk2;relk3* mutants revealed a shoot apical meristem termination phenotype. Triple mutant seeds either failed to germinate or developed few leaves before apical growth termination. This suggests that while *RELK2* and *RELK3* are functionally homologous to *TPL*, *RELK1* has a partially divergent function, being mainly implicated in the regulation of inflorescence meristem size, together with *REL2*. To this end, we also report a heterozygous effect of the *rel2-ref* allele in the inbred line B73 which revealed an increase in Kernel Row Number (KRN) without morphological deformity in ears. To evaluate the potential of *rel2-ref* to increase yield, we used a panel of NAM founder lines to generate a series of F1 hybrids. Data collected over two growing seasons revealed several hybrid combinations in which the presence of a heterozygous *rel2-ref* allele resulted in an increase in KRN, often accompanied by pleiotropic effects on ear length and seed weight. 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)

P150

Boundary domain genes were recruited to suppress bract growth and promote branching in maize

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Grass inflorescence development is diverse and complex and involves sophisticated but poorly understood interactions of genes regulating branch determinacy and leaf growth. Here, we use a combination of transcript profiling, genetic and phylogenetic analyses to investigate *tasselsheath1* (*tsh1*) and *tsh4*, two maize genes that simultaneously suppress inflorescence leaf growth and promote branching. We identify a regulatory network of inflorescence leaf suppression that involves the phase change gene *tsh4* upstream of *tsh1* and the ligule identity gene *liguleless2* (*lg2*). We also find that a series of duplications in the *tsh1* gene lineage facilitated its shift from boundary domain in non-grasses to suppressed inflorescence leaves of grasses. Collectively, these results suggest that the boundary domain genes *tsh1* and *lg2* were recruited to inflorescence leaves where they suppress growth and regulate a non-autonomous signaling center that promotes inflorescence branching, an important component of yield in cereal grasses.

Gene / Gene Models described: *tsh1, tsh4, lg2*; GRMZM2G325850, GRMZM2G307588, GRMZM2G060216
Funding acknowledgement: National Science Foundation (NSF)

P151 @aminachaudhri

Characterization and positional cloning of the prolific mutant *eary-517**

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Maize architectural genes have been a subject of great interest for understanding regulatory mechanisms underlying shoot development and domestication. Changes in overall plant and inflorescence architecture characterize mutants of domestication loci, such as *grassy tillers 1 (gt1), teosinte branched 1 (tb1)*, and *enhancer of tb1 (etb1.2), all mapped to chromosome 1.* In a forward genetics approach to identify additional architecture-related genes, a novel mutant, provisionally called *eary-517**, was identified in an enhancer EMS-mutagenesis screen of the *ba1-mum1* mutant. *eary-517** presented a striking phenotype in the A619 background producing 4 to 6 small, yet fully fertile ears, each borne on an extremely elongated shank. Mutant plants also exhibited significantly reduced tassel length and plant height with shorter internodes 1 to 4 in comparison to normal plants. When introgressed in B73, the prolific phenotype of eary-517* was completely suppressed. We generated a mapping population and by Whole Genome Sequencing Bulked Segregant Analysis, we mapped the mutation to a small region on chromosome 1L. The mapping interval was further narrowed down using dCAPS markers whereas no mutations were identified in the coding sequences of the *GT1, TB1,* or *ETB1.2* genes. A candidate gene was identified carrying an EMS-induced C>T transition, resulting in the conversion of a highly conserved glutamic acid residue into a lysine. A CRISPR-Cas9 construct targeted to the candidate gene is being transformed to first confirm the identity of the *eary-517** mutant. I am also investigating the subcellular localization of the EARY517* protein using transient expression in *Nicotiana benthamiana* cells. Additionally, I am constructing double mutant combinations with *gt1* and *tb1* to shed light on the role of *EARY-517** in regulating shoot architecture. Understanding the mechanisms of reproductive proliferation could help reshape plant architecture for increasing yield, making maize an even more robust cash crop.

Funding acknowledgement: National Science Foundation (NSF)

P152

Characterization of maize genes that regulate leaf initiation and phyllotaxy

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The regular pattern of leaf initiation at the shoot apical meristem (SAM) determines a plant's arrangement of leaves around a stem, or phyllotaxy. Maize follows a phyllotactic pattern in which leaves are initiated on alternating sides of the plant. Among all plants, few genes have been identified that influence phyllotaxy. In maize, mutants for the *aberrant phyllotaxy1 (abphyl1)* gene have altered phyllotaxy such that leaves are initiated on opposite sides of the plant in pairs. One aspect of a proposed mechanism for this change is an enlarged SAM disrupts the distribution of hormones that determine phyllotactic patterns. The penetrance of the *abph1* mutant is dramatically reduced when converged into standard inbred lines. Our lab has identified mutants that result in irregular leaf initiation and altered phyllotaxy, especially in the upper half of the plant, with relatively high penetrance in standard inbred lines. Named *aphyll* (abp*)*, mutants have altered meristem size, but it does not correlate with a phyllotactic phenotype. Expression analysis indicates there is a potential reduction in the biosynthesis of auxin, a hormone necessary for leaf initiation. In addition to meristem size and altered leaf initiation, *abp** exhibits pleiotropic effects, such as delayed phase change, an increase in total leaf number, and more primary tassel branches. Moreover, closer examination of leaf morphology reveals mutants have decreased leaf width, increased leaf length, and altered leaf angles. Our results indicate that *abp** and related genes function throughout much of plant shoot vegetative development and in the tassel and ear, potentially influencing agronomic traits. The alteration of these traits is dependent upon the allele and allelic combinations of genes, as well as the genetic background of the plant. We have crossed existing mutants into multiple genetic backgrounds and generated new alleles through *Ds* transposon remobilization and CRISPR-Cas9 mediated genome editing.

Gene / Gene Models described: *abph1*; GRMZM2G035688
Funding acknowledgement: National Science Foundation (NSF), Iowa State Crop Bioengineering Center

P153

Characterization of the *tassel-less4* mutant and its interactions with auxin

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The plant growth hormone auxin is a key driver of growth and development in plants, triggering responses such as cell division, elongation, and apical dominance. One of auxin's roles is the establishment of organs on the periphery of the shoot apical meristem (SAM), where transport of auxin from cell to cell and local biosynthesis leads to auxin maxima and differential gene expression as organs initiate. These auxin maxima are mediated by the recruitment of auxin efflux proteins known as PINs to one end of the plasma membrane, allowing for polar auxin transport. In maize, the *tassel-less4 (tls4)* mutant produces semi-dwarf plants and is notable for reproductive defects and dramatically reduced or absent tassels. This phenotype is characteristic of mutants with defects in the auxin pathway. Double mutant analyses with known auxin mutants including the biosynthesis mutant *vanishing-tassel (vt2)* and the transport mutant *barren-inflorescence2 (bif2)* indicated an involvement of *tls4* with auxin transport. Moreover, FM4-64 assays suggested that *tls4* may be involved with vesicle trafficking, which would impair the *tls4* mutant's ability to recycle PIN proteins through endocytosis. Here, we used confocal microscopy to characterize immature tassels of *tls4* plants that contained markers for the ZmPIN1a protein as well as the DR5 marker, which indicates the transcription of auxin responsive genes. Our results show more diffuse localization of ZmPIN1a in *tls4* mutants, providing evidence for the gene's role in vesicle trafficking. Further characterization of *tls4* function could provide insight into the role vesicle trafficking plays in auxin-driven reproductive development.

Funding acknowledgement: National Science Foundation (NSF), University of Missouri Honors College, American Society of Plant Biologists

P154

Characterizing the role of the FDL1 transcription factor in cuticular wax deposition on maize silks

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The hydrophobic cuticle, which covers aerial portions of plants, is the first line of defense against environmental stresses, including drought, UV radiation, temperature, and insects and pathogens. This cuticle is comprised of a cutin polyester matrix that is infused with and laid atop by cuticular waxes, comprised of differing combinations of very long chain fatty acids (VLCFAs), hydrocarbons, aldehydes, alcohols, esters, and ketones. The cuticle on maize silks is rich in hydrocarbons, with minor amounts of VLCFAs and trace aldehydes that provide important protection for this tissue during the pollination period. Cuticle biosynthesis within the epidermal cells is tightly regulated at both the transcriptional and post-transcriptional levels. Many of the transcription factors known to regulate cuticle biosynthesis and deposition in Arabidopsis and in maize are not expressed in silks, suggesting different transcriptional regulation. Specifically, the FDL1 transcription factor has recently been shown to control cuticle deposition, and thereby leaf permeability, in maize seedlings. In fdl1 seedlings, the coleoptile and first leaf show an ~80% decrease in cuticular wax load and ~ 50% decrease in the second leaf. (Castorina et al, 2020 and Liu et al, 2020). To assess the potential function of FDL1 in regulation of cuticle biosynthesis and deposition on silks, in which it is more highly expressed, we have profiled cuticular waxes from fdl1 and wildtype silks via gas chromatography-flame ionization detection. In the fdl1 mutant, the overall abundance of cuticular waxes decreases by ~28%, but is not differentially impacted relative to specific classes of cuticular metabolites (i.e. hydrocarbons, aldehydes or VLCFAs). This reduction in silk cuticular wax is not nearly as drastic as what has previously been observed in fdl1 seedlings. Therefore, it is likely that FDL1 contributes to the regulation of cuticular wax biosynthesis in silks in combination with other transcription factors.

Gene / Gene Models described: *fdl1*; Zm00001d022227
Funding acknowledgement: National Science Foundation (NSF)

P155
Control of maize ear development by a putative receptor-coreceptor pair in a CLAVATA-related signaling pathway

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The maize ear is derived from a stem cell niche within the inflorescence meristem. Meristem size is controlled by the CLAVATA (CLV)-WUSCHEL (WUS) signaling pathway, which involves an interplay between CLV receptors, their ligands, and the mobile transcription factor WUS. Maize mutants lacking CLV receptors have fasciated ears, with flattened tips and disordered kernel rows. One such mutant, *fasciated ear3* (*fea3*), was first described in maize, and lacks a functional copy of a leucine rich receptor-like protein. Weak *fea3* alleles increase ear size without a compensatory loss in seed size, making this an attractive target for yield enhancement. *FEA3* is expressed below the organizing center of the meristem, and *WUS* activity extends downwards in *fea3* mutants, in contrast to other CLV receptor mutants, where *WUS* expression extends upwards. *fea3* interacts synergistically with *td1* and *fea2*, mutants of the maize orthologs of *CLV1* and *CLV2*. Together, these data suggest FEA3 operates in a separate pathway than TD1 and FEA2 to control meristem size. Intriguingly, an ortholog of *TD1*, *ZmBARELY ANY MERISTEM 1D (ZmBAM1D*), is upregulated in *fea3* mutants. *FEA3* and *ZmBAM1D* expression overlaps in the center of spikelet meristems, and the two proteins interact when co-expressed in *N. benthamiana*. These observations suggest FEA3 and ZmBAM1D form a receptor-co-receptor pair. We are further exploring this interaction and discovering additional interactors of FEA3 in maize inflorescence meristems using a proximity labeling approach called Turbo-ID, which can better resolve transient protein-protein interactions compared to immunoprecipitation-based approaches. We are also examining genetic interactions between *FEA3* and *ZmBAM1D*, as well as a higher order *bam* mutant CRISPR population, to understand how these receptor proteins control meristem size. With a deeper understanding of how these receptors regulate meristem activity, we can more precisely engineer this process to enhance yield-related traits.

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

P156 @kswentowsky
Developmental genetics and genomics of perennial regrowth in *Zea diploperennis*

(submitted by Kyle Swentowsky <swentow@cshl.edu>)
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Perennial plants regrow for multiple years and perennial crops are sustainable agriculture systems. However, breeding perennial maize has been largely unsuccessful due to our poor understanding of genes and developmental mechanisms that control perenniality. The teosinte species *Zea diploperennis* is perennial and forms fertile hybrids when crossed with maize. Using *Z. diploperennis*/maize mapping populations, perennial regrowth has been mapped to two loci called *regrowth1* (*reg1*) and *reg2* on chromosomes 2 and 7, respectively. We screened an additional F2 mapping population by QTL-seq and PCR genotyping and also mapped two loci, one of which corresponds to *reg1* on chromosome 2 and the other is a novel locus on chromosome 8 that we named *reg3*. By analyzing individuals that displayed regrowth at early and late time points in the perennial life cycle, we determined that *reg1* affects initial regrowth while *reg3* is necessary for multiple sustained cycles of perennial regrowth. Residual Heterozygous Lines (RHLs) derived from these F2 individuals are being generated to fine-map *reg1* and *reg3* and identify the causal variants. We also plan to study the developmental mechanisms that dictate perennial regrowth in *Z. diploperennis*, and how they differ between perennial and annual grass species. We hypothesize that: (1) the maintenance of *Z. diploperennis* tiller buds in a juvenile and non-flowering state is necessary for perennial regrowth; and (2) dormant tiller buds become reactivated through phytohormone or carbohydrate signaling after flowering to begin the next cycle of perennial growth. To test these hypotheses, we will cross *Z. diploperennis* to maize lines expressing transgenic reporters to visualize phytohormone signals. We will also ectopically express developmental regulators to alter the developmental state of tiller buds and assess whether this affects perenniality. Understanding the developmental genetics of perenniality could aid in breeding of more sustainable maize crops.

Funding acknowledgement: National Science Foundation (NSF)

P157

Disruption of an mRNA processing gene results in bract growth in maize *tassel sheath 5* (*tsh5*) mutants

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Disruption of an mRNA processing gene results in bract growth in maize *tassel sheath 5* (*tsh5*) mutants Brian Cox[1], Yuguo Xiao[1,2], Clinton Whipple[1]1. Department of Biology, Brigham Young University, 4102 LSB, Provo, TU2. Danforth Center, 975 N. Warson Road, St. Louis, MO63132 Maize and other grasses do not produce bracts, or leaves that subtend inflorescence meristems and spikelet meristems, although other plant taxa do produce bracts. Several maize genes are known to affect bract suppression, including *tassel sheath 1*, a GATA-zinc finger transcription factor, *tsh3*, and *tsh4*, a micro-RNA targeted SBP-transcription factor. The maize *tassel sheath 5* (*tsh5*) mutant has leafy bracts in the tassel and ear and exhibits pleiotropic effects including spikelet development defects, reduced tassel branching, and short stature. We have identified *tsh5-1* and *tsh5-2*, two mutants with very similar phenotypes that fail to complement. We conducted BSAseq on a *tsh5-1* mapping population and identified two genomic regions with homozygous SNP peaks, a large peak on chromosome 1 (chr1) and a smaller peak on chromosome 5 (chr5). The chr1 peak includes a canonical EMS G > A mutation which causes a cysteine > tyrosine substitution in cleavage polyadenylation specificity factor 73 – I (CPSF73-I), which SnpEFF predicts to have a moderate effect on the protein. *tsh5-2* has an EMS induced premature stop codon in the same gene. Interestingly, there is a duplicate of this gene within the chr5 peak, and *tsh5-1* segregating populations show variable expressivity for the mutant phenotype, suggesting that another locus modifies the *tsh5* phenotype. We are phenotyping and genotyping an F3 *tsh5-1* population at the chr1 locus and chr5 locus to confirm that chr5 does contribute to the bract phenotype. We were surprised that a "housekeeping" mRNA processing protein would have such a developmentally specific effect on inflorescence development. If CPSF73-I is implicated, poly-A tagging and sequencing will allow us to determine the effects of mutant CPSF73-I on the polyA state of its target genes and provide insight into the role of RNA processing in inflorescence development.

P158

Distinctive features of maize vascular development

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While vascular growth often follows a series of genetically regulated stereotypical steps, maize appears to have spatio-temporal regulatory mechanisms that produce veins with unique functions. We currently do not have a genetic framework for connecting such specialized vein development with maize physiology. To address this, we initiated a mutant screen for novel vascular mutants and uncovered three classes that underline several unique aspects of maize vascular function. We observed that adult protoxylem exhibits thicker secondary cell walls with more helical turns compared to those formed during the juvenile phase. This difference is associated with increased water movement capacity and resistance to heat stress. Differential expression of the *MIR156* microRNA and its target *SBP* box transcription factors appear to be responsible for this phenotype. Post juvenile expression of *necrotic upper tips* and *wilted1* control development of adult phase specific xylem cell wall characteristics. Although vascular patterning appears to be identical across the stem, subtle differences in cell differentiation can be seen in the bundles on the stem periphery compared to the center. These differences are under genetic control since we identified several new mutants that affect only a subset of these bundles based on histology and water movement assays. Fusion of intermediate veins creates auxin pools near the nodal plexus that affect aerial root emergence and axillary branching. Auxin movement visualized using the PIN1 and DR5 fluorescent reporters demonstrate that auxin emanating from vascular fusion travels outwards to the periphery of the stem. Points where two opposing flows of auxin converge correlate with the formation of brace root primordia. Mutants such as *blh12* and *blh14* that lack this peripheral auxin flow also lack ear and tiller development. Alternatively, mutants such as *Corngrass* that produce ectopic brace roots display increased auxin flow at the nodal plexus.

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

P159
Evidence for the developmental homology of the grass ligule

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The grass leaf consists of a proximal sheath and a distal blade. The sheath and blade are partitioned by a hinge-like auricle and an epidermally-derived ligule fringe. Plant morphologists have long-debated the homology of the grass ligule, proposing its derivation from an apical extension of the grass sheath margin, as homologous to the stipules of eudicot leaves, or as an amalgamation of both structures. Others speculated that the ligule is an evolutionary innovation of grass leaves. However, developmental genetic analyses of ligule morphogenesis have played a limited role toward resolving the question of ligule homology. Here we provide evidence that the maize ligule comprises the distal extension of the epidermally-derived sheath margin. Using single-cell transcriptomic analysis, we find that cells from the developing leaf margin share transcriptional signatures with cells from the initiating ligule, suggesting a shared developmental genetic program responsible for patterning both structures. Quadruple mutant analyses reveal a role for the functionally-redundant *WUSCHEL-LIKE HOMEOBOX3* (*WOX3*) transcription factor-encoding genes in regulating outgrowth from both the leaf margins and the ligule. *WOX3* function in the ligule is associated with expression of adaxial-abaxial (top-bottom) patterning genes, as is the case in developing leaf margins. Computational modeling of ligule outgrowth indicates that juxtaposed domains of adaxial and abaxial cell identity, used to model mediolateral outgrowth from leaf margins, are also sufficient to grow a ligule. These findings illustrate the evolutionary repurposing of an extant genetic patterning module to produce a grass-specific morphological innovation. Moreover, we propose a resolution to a nearly two-hundred-year-old controversy on the homology of the grass ligule.

Funding acknowledgement: National Science Foundation (NSF), BBSRC

P160
Exploring the roles of two putative maize BAG (Bcl-2 associated athanogene) family proteins in modulating plant growth, autophagy, and senescence

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BAG (Bcl-2 associated athanogene) family proteins play diverse functions in fungi, animal, and plants. Several BAG proteins function as co-chaperones by interacting with HSP/HSC70. Mammalian BAG proteins have six members and are the most well studied with function ranging from regulating autophagy, apoptosis, and tumorigenesis. Arabidopsis contains seven BAG proteins (AtBAG1-7) with important regulatory functions in plant development and stress responses. AtBAG5-7 contain an N-terminal calmodulin-binding domain, which is not found in any mammalian BAG proteins. AtBAG7 is the most divergent protein within the family and the least similar to mammalian BAG. It localizes to the endoplasmic reticulum (ER) and is required for plant survival under acute ER stress through its critical function in the unfolded protein response. In addition, it has been implicated as a potential autophagy component. Here we study the diversification and function of BAG proteins in maize. We have identified 21 putative ZmBAG proteins. A phylogenetic analysis of Arabidopsis and maize BAG proteins suggest that maize contains several putative isoforms of each of the seven AtBAG proteins. The two putative ZmBAG7 proteins, ZmBAG7 and ZmBAG7-like, show different expression patterns under ER stress and fixed carbon starvation, suggesting that may have non-redundant functions. To analyze the functions of these two genes in plant growth, autophagy, and stress responses, we are characterizing transposon insertion mutants of the two putative genes, examining their subcellular localizations, and testing their interactions with chaperons and autophagy proteins.

Funding acknowledgement: National Science Foundation (NSF)

P161 @NelmsLab

Gametophyte genome activation occurs at pollen mitosis I in maize

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Flowering plants alternate between multicellular haploid (gametophyte) and diploid (sporophyte) generations. Pollen actively transcribes its haploid genome, providing phenotypic diversity even among pollen grains from a single plant. In this study, we used allele-specific RNA sequencing of single pollen precursors to follow the shift to haploid expression in maize pollen. We observed widespread biallelic expression for 11 days after meiosis, indicating that transcripts synthesized by the diploid sporophyte persist long into the haploid phase. Subsequently, there was a rapid and global conversion to monoallelic expression at pollen mitosis I, driven by active new transcription from the haploid genome. Genes showed evidence of increased purifying selection if they were expressed after (but not before) pollen mitosis I. This work establishes the timing during which haploid selection may act in pollen.

Funding acknowledgement: National Science Foundation (NSF)

P162

Genetic transformation of maize progenitor teosinte (*Zea mays* ssp. *parviglumis*)

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Teosinte (*Zea mays* ssp. *parviglumis*) is considered as a progenitor of domesticated maize (*Zea mays* ssp. *mays*) and has ample genetic diversity that can be useful for maize breeding. Here we report the first protocol for genetic transformation of teosinte (*Zea parviglumis)* using biolistic bombardment of seedling-derived callus tissues. We achieved a 4% transformation frequency (percentage of independent transgenic events in total bombarded explants that produced callus) using a reporter plasmid pKL2155, which carries a mutant acetolactate synthase gene (HRA, for resistance of herbicide imazapyr) and a red fluorescent protein marker gene (*tdTomato*). This protocol describes the technical details to produce transgenic teosinte plants, a species recalcitrant to tissue culture and genetic transformation. These steps include sterilization of mature seeds for explant preparation, development of a tissue culture protocol, optimization of biolistic delivery of DNA construct, and verification of transgene insertion in T0 and T1 plants. This protocol provides a major enabling technology for studying domestication, gene function and creation of an ideotype maize plant from its wild progenitor. Zobrist et al. (2021) Transformation of teosinte (*Zea mays* ssp. *parviglumis*) via biolistic bombardment of seedling-derived callus tissues. Frontiers in Plant Science 12: 773419. doi: 10.3389/fpls.2021.773419

Gene / Gene Models described: *n/a*; n/a
Funding acknowledgement: National Science Foundation (NSF)

P163

Impact of heat stress during maize pollen development

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

1. Begcy K, Dresselhaus T. Epigenetic responses to abiotic stresses during reproductive development in cereals. Plant Reproduction. 2018;31:343-55. 2. Begcy K, Dresselhaus T. Tracking maize pollen development by the Leaf Collar Method. Plant Reproduction. 2017;30:171-8. 3. Begcy K, Nosenko T, Zhou L-Z, Fragner L, Weckwerth W, Dresselhaus T. Male sterility in maize after transient heat stress during the tetrad stage of pollen development. Plant Physiology. 2019;181:683-700.

Funding acknowledgement: CSC, BayKLIMAFIT

P164

In vitro kernel culturing of hybrids: Kernel development and fl2-RFP accumulation in response to variable nitrogen

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Nitrogen (N) status strongly influences maize kernel development. However, the genetic factors controlling N use efficiency (NUE) in maize remain elusive because of the complex nature of the genotype-environment interaction. The Illinois Long-Term Selection Experiment (ILTSE) has generated inbred lines representing the extremes of kernel protein concentration, Illinois High Protein 1 (IHP1) and Illinois Low Protein 1 (ILP1)[5]. Preliminary analysis of these lines indicates that free amino acid concentrations of reproductive and photosynthetic tissues at flowering strongly correlate to final yield in hybrids across variable N. Connecting these metabolic associations with genetic factors remains difficult, partially because numerous processes affect NUE across the whole plant. An *in vitro* kernel culturing assay allows direct investigation of kernel developmental N responses and can assess complex N patterns that vary in the timing of N stress[6]. B73 X Mo17, B73 X IHP1, B73 X ILP1, B73 X Ms71, and B73 X Oh7B hybrid kernels were grown under variable field N fertilizers and then cultured in vitro with variable N to create four treatments. Free amino acid profiles and fresh weights assayed three times show highly significant effects of all experimental factors. Additionally, RNA sequencing performed on 5 and 8 days after pollination B73 X Mo17 kernels allows for weighted gene co-expression network analysis (WGCNA)[3] to integrate metabolite and transcript information. Candidate genes and modules include known regulators of kernel development, hormone signaling components, and orthologs to nitrate responsive root development pathways.

Funding acknowledgement: National Science Foundation (NSF), Department of Energy (DOE)

P165
Maize anther development with fixed single-cell RNA-seq

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The *de novo* differentiation and development of somatic cells into reproductive germ cells is of the upmost importance for plant reproduction as plants do not have established germlines, unlike sexual animals. Understanding the genetic cues and evolution of the cell layers that regulate sporogenesis in anthers is therefore fundamental to plant biology, but also to agriculture because controlled pollen production underlies hybrid seed production of most crops. Maize is an ideal system for investigating angiosperm sporogenesis as individual plants have separate male tassels and female ear inflorescences, hundreds of large anthers per tassel, and a large collection of male-sterile and female-fertile mutants. In addition, the ontogeny of these large anthers can be charted based on anther length so that each tassel has hundreds of easily collected, developmentally identical replicates. Here we present the first developmental analysis of the maize anther at the single-cell scale using our novel FX-Cell scRNA-seq protocol. We identify the different cell types of the anther, uncover their developmental trajectories leading to sporogenesis, and identify crucial marker genes for future studies of the maize anther. Notably, we discuss the future of developmental single-cell RNA-seq for studying plant development and evolution.

Funding acknowledgement: National Science Foundation (NSF)

P166
Multiplex genome editing of maize inbred lines via biolistics delivery of CRISPR/Cas9 to Type I embryogenic calli

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Genome editing with CRISPR/Cas9 is a powerful tool for functional genomics, but applications in maize are hindered by historical challenges of maize transformation. Preference for single copy transgenes has favored DNA delivery via *Agrobacterium* instead of biolistics, which further limits genotypes that can be transformed. However, efficient transformation systems using biolistics delivery to Type I embryogenic calli can potentially be established from a broader range of maize genotypes, and offer advantages in delivery of genome editing components that will subsequently be removed via crossing and segregation. We developed an approach for multiplex genome editing with CRISPR/Cas9 that delivers a single compact DNA fragment via biolistics to Type I embryogenic calli, followed by selection of transgenic events using *nptII* as a selectable marker. We demonstrate the creation of heritable mutations at multiple target sites within the same gene, as well as three members of a multigene family. Among the many mutations generated, we observed one transgenic event where CRISPR/Cas9 mutagenesis "proceeded to completion", with bi-allelic mutations created at each of the three paralogs targeted in the experiment. Deep Illumina sequencing of this event did detect rare off-target mutations. Methods were also optimized to use *in vitro* Cas9 cleavage as an initial rapid screen for successful edits and for genotyping edits among progeny. Multiplex genome editing was achieved for both the highly transformable inbred line H99 and Illinois Low Protein1, a unique genotype derived from nearly a century of recurrent selection for low grain protein concentration, where transformation has not previously been reported. Experiments targeting the same two genes in each genotype produced transformation frequencies of approximately 3% in H99 and 7-11% in ILP1, and a majority of the events with intact transgenes produced heritable mutations. These results expand the potential for CRISPR/Cas9 for maize functional genomics.

Funding acknowledgement: United States Department of Agriculture (USDA)

P167

Mutation of a predicted arabinogalactan protein gene (*famp1*) in maize is associated with a significant male-specific transmission defect

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The pollen grain represents the male gametophyte of angiosperm species and mediates the transportation of sperm cells to the female flower (the ear and silks in maize). Successful delivery of sperm cells to their female gametophyte counterpart (the egg & central cell) leads to fertilization and the production of a new generation (the seed). This project explores a specific gene in maize (Zm00001eb236740), designated *famp1*, using a Green Fluorescent Protein (GFP)-marked mutation (Li et al 2013) that likely disrupts the gene's function. This mutation in *famp1* is associated with significantly reduced transmission success (failure to effectively produce the next generation) via the pollen grain (Warman et al 2020). The DNA sequence of the gene predicts that it encodes a transmembrane protein with a large extracellular domain, homologous to fasciclin and arabinogalactan family proteins, which have been linked to cell wall function. A variety of experiments are in progress to test the hypothesis that the famp1 mutant's reduced transmission is caused by defects in the pollen grain or pollen tube cell wall. Controlled pollinations showed that *famp1::Ds-GFP* was associated with ear-to-ear variation in male transmission defects across four field seasons; for example, in 2020, transmission ranged from 30% to 57%. However no significant deviation from 1:1 transmission through the female was observed. Manipulating pollen load indicates that *famp1::Ds-GFP* transmission occurs at a higher rate when sparse pollen is applied to silks, strongly suggesting that that defect is influenced by the level of competition among gametophytes. However, a variety of pollen staining assays have identified no differences between mutant and wild-type pollen. A set of pollen tube growth experiments are currently being analyzed to determine if a *famp1* mutant phenotype is discernible *in vitro*.

Gene / Gene Models described: ; Zm00001eb236740
Funding acknowledgement: National Science Foundation (NSF)

P168

Non-Mendelian segregation for maize embryo-specific mutations

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Our lab has been studying embryo-specific mutations (*emb*s) generated through the application of Ethyl methanesulfonate-treated W22 pollen to B73 ear silks. In a previous abstract, we examined the non-Mendelian transmission patterns of the *emb* UND-9. We found that approximately half of self-pollinated ears from plants carrying this simple, single-gene recessive mutation would segregate mutant embryos in a non-Mendelian frequency (in less than 10 percent of the kernels). We have also obtained ears with much higher, non-Mendelian segregation ratios than would be expected. We have closely examined the relationships between generations of parents and offspring to elucidate the cause of these non-Mendelian transmission patterns. We discovered that transmission via either the male versus the female gametophyte has no significant effect on whether the mutant allele is transmitted to the next generation, nor on the proportion of mutant embryos within an individual ear in subsequent generations. We discovered a slight positive correlation between the proportion of mutant embryos in a parent ear with the proportion of mutant embryos in the offspring ear. Further, the non-Mendelian transmission pattern of UND-9 has been observed in the four other *emb* mutants that we have analyzed for this effect. We suspect that this indicates that the abnormal transmission is common, if not universal, for these embryo-specific mutations involving crosses between B73 and W22. Because it appears that this is a common effect, we speculate that the abnormal transmission phenomena may be a result of genomic interaction between the two parent inbreds.

P169

Rebuilding Zea mays Ab10 meiotic drive system in budding yeast using kindr and trkin kinesins

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Preferential segregation of chromosomes during meiosis occurs through a process known as meiotic drive. Maize abnormal chromosome 10 (Ab10) engages in meiotic drive when its heterochromatic DNA elements called knobs behave as motile neocentromeres. These heterochromatic knobs consist of two repeating tandem DNA sequences, the knob180 sequence and the TR-1 sequence. Mobilization of knobs is made possible by kinesins that localize on knobs and interact with the spindle, preferentially transmitting the chromosome to the oocyte. Two kinesins have been identified that are involved in the Ab10 meiotic drive system: Trkin, which localizes on the TR-1 DNA sequences; and Kindr, which localizes on knob180 DNA sequences. Kindr has previously been identified as an independent meiotic driver capable of producing preferential segregation, but it is unclear whether Trkin can do the same in the absence of Kindr. In order to dissect the molecular mechanism, we are expressing the maize kinesin components in the yeast model system S. cerevisiae and probing their ability to recreate the chromosome drive phenotype through a plasmid loss assay. The ability for Trkin and Kindr to segregate the acentric plasmid is measured by its inheritance in the offspring.

Funding acknowledgement: National Science Foundation (NSF)

P170

Roles of putative auxin transporters in root growth and development

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The phytohormone auxin is essential for regulating plant growth and development. Auxin accumulation in meristems is required to maintain stem cell populations and auxin transport can facilitate cellular differentiation during organogenesis. Auxin transporters are required for maize shoot development but those that underpin maize root development are not known. Within the primary root, free indole-3-acetic acid (auxin) levels are asymmetrically distributed, suggesting that this pattern is established by regulated transport and/or biosynthesis. Using a reverse genetics approach we have identified two putative candidate auxin efflux carriers, annotated as PINFORMED 14 (PIN14) and PIN-LIKES 2 (PILS2), that are required for primary root morphogenesis, lateral root formation and proper auxin transport *in vivo*. Loss of function UniformMu transposon alleles of *pin14* and *pils2* display altered auxin response in rolled towel assays performed in a controlled environment. Transient and stable expression of fluorescently tagged PIN14 proteins is underway to determine if this protein is localized to the plasma membrane or endoplasmic reticulum, which is well established for PIN and PILS orthologs in Arabidopsis. Using an integrated transcriptome, proteome and phosphoproteome profiling approach we have characterized gene expression differences in both mutants compared to W22, both with and without exogenous auxin treatment, which will be used for network analyses. Based on these findings we present a working model for PIN14 and PILS2 in regulating maize root morphogenesis, which may inform strategies to generate desirable root system architecture traits.

Gene / Gene Models described: *umc1142, pin14*; Zm00001d051044, Zm00001d043083
Funding acknowledgement: United States Department of Agriculture (USDA)

P171
Single cell analysis of maize embryo development

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Maize embryo development is a complex process that initiates the histological and organogenic patterns that are reiterated throughout plant morphogenesis. Diverse cell types, tissues and organs are formed in dynamic, spatial-temporal manners. Cell-specific transcriptomic analyses are key to understanding the differentiation of different tissues in the maize embryo. In previous studies, laser-microdissection (LM) RNAseq was used to investigate the transcriptomics during development of the maize embryo and related lateral organs. Nonetheless, the gene expression profiles specific to the diverse cell types comprising the maize coleoptile, scutellum, vasculature, and epidermis are unclear. Moreover, LM-RNAseq analyses are unable to identify previously uncharacterized cell types. In this project, cell type-specific transcriptomic analyses (single cell RNA-seq) of maize L1-staged embryos that have formed a shoot meristem and the first foliar leaf were performed at single-cell resolution. Twelve distinct cell clusters were identified in the L1-staged embryo: cell type identities were assigned for 11 clusters based on differential expression of previously described marker genes. Uniform Manifold Approximation and Projection (UMAP) graph showed two, distinctly separate groups of cell clusters: 1) shoot meristem, leaf primordia, and basal region; and 2) scutellum and coleoptile. In addition, UMAP analysis identified one previously-undescribed cell cluster of ambiguous identity. Intriguingly, this unknown cluster contains many more genes overlapping with the basal region cluster and expresses an abundance of cell-cycle genes and auxin-response genes, suggesting the potential for cell proliferation and differentiation. Pseudotime analysis suggests that foliar leaves may differentiate from the shoot apical meristem (SAM), whereas the coleoptile may differentiate from the adaxial region of the scutellum. Lastly, to explore the use of spatial, cell-type specific, gene-expression profiles, we are adapting the 10X Genomics Visium© protocol to conduct RNAseq analyses of plant tissues. This new technology enables mapping of cell type-specific transcriptomes within a spatio-morphological context, and promises to provide deeper insight into cell-specific gene expression.

Funding acknowledgement: National Science Foundation (NSF)

P172 @_DianaRuggiero
Single cell genomics and high-throughput phenotyping for determining the quantitative genetics of maize leaf vascular development.

LIGHTNING TALK

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Vascular density varies across the sheath, auricle, and blade compartments of the maize leaf such that tissues engaging in efficient C4 photosynthesis have the highest vein density. Although mature leaf veins are anatomically similar, maize and other C4 grasses possess several different vein types that can be identified by their stereotypical developmental sequence and spatial configuration throughout the sheath, auricle, and blade. To characterize the changes in gene expression that distinguish these diverse vein types, we used combinatorial barcoding to perform single nucleus RNA sequencing (snRNA-seq). We collected transcriptomic profiles from over 7000 individual nuclei from leaf 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 developmental trajectories of intermediate cell types transitioning into vascular tissue. As a complementary approach, we are conducting a GWAS correlating vein density phenotypes with genetic markers from the Wisconsin Diversity Panel (WiDiv). Last field season, we collected over 4000 leaf samples from 700 WiDiv inbred lines. To facilitate such a large-scale study of microscopic traits, we have devised a deep-learning based automated phenotyping system for estimating type-specific vein density in cleared leaf images. This system employs an implementation of U-Net, a convolutional neural network (CNN) architecture for semantic segmentation. This system classifies and masks the various vein types in each sample, allowing for quantification of unambiguous connected components. By identifying key marker genes for specific vein types and alleles which correlate with natural variation in vein abundance and organization, we hope to reveal methods of fine-tuning vascular density and plant photosynthetic performance.

P173

The *enhancer of spi1* (*eos1*) gene affects inflorescence development in maize.

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Auxin regulates reproductive development in maize. *sparse inflorescence 1 (spi1)* and *barren inflorescence 2 (bif2)* are involved in auxin synthesis and transport respectively. *spi1* mutants have few tassel branches and spikelets. *bif2* mutants have an unbranched tassel with few spikelets. *eos1* mutants also have fewer tassel branches compared to wildtype, but more branches compared to *spi1* mutants. However, *eos1*;*spi1* double mutants have an enhanced phenotype with a short tassel, few or no branches and wispy empty spikelets. The severity in phenotype suggests a synergistic interaction between *eos1*and *spi1*. *eos1*;*bif2* double mutants have a thick *bif2* like tassel. The *eos1* mutation is caused by a *Mu* insertion as confirmed by PCR using a *Mu*-specific and *eos1* specific primer pair and co-segregation with the mutant phenotype. In the future, we will be performing Scanning Electron Microscopy in double mutant tassels to dissect the defects.

Funding acknowledgement: National Science Foundation (NSF)

P174

The *tls5* gene functions in vegetative and reproductive development in maize

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Auxin affects vegetative and reproductive development in maize. We isolated a mutant, *tasselless* 5 (*tls5*), that has defects in internode length and tassel development. The *tls5* mutant was generated by EMS mutagenesis in the Mo17 background and was backcrossed to B73 five times. Compared to wild type, the mutant plants are short and internode length is reduced either at the bottom, middle or top of the plants. The tassel is thin, with few branches and sometimes fails to emerge from the whorl. In some cases, the leaves are split or deformed. These phenotypes indicate that the mutant plants have an abnormal pattern of development in early vegetative and reproductive stages. In addition, the variability in phenotypes is typical of auxin mutants. The candidate gene was mapped to the short arm of chromosome 9 between SSR marker bnlg1372 and indel marker idp8586. We are using a whole genome resequencing approach to identify candidate genes by SNP identification.

Funding acknowledgement: National Science Foundation (NSF)

P175

The transcription factor gene *GRASSY TILLERS1 (GT1)* and the trehalose-6-phosphate phosphatase gene *RAMOSA3 (RA3)* interact to regulate carpel suppression in maize flowers

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Floral morphology is immensely diverse. One developmental process acting to shape this diversity is growth suppression. For example, grass flowers exhibit extreme diversity in floral sexuality, arising through differential suppression of stamens or carpels. In maize, carpels undergo programmed cell death in half of the flowers initiated in ears and in all flowers in tassels. The HD-ZIP I transcription factor gene *GRASSY TILLERS1 (GT1)* is one of only a few genes known to regulate this process. To identify additional regulators of carpel suppression, we performed a *gt1* enhancer screen and identified a class of mutants we call '*rapunzel (rzl)'* because of the long silks emerging from otherwise normal tassel spikelets. We have cloned one *rzl* gene, and discovered that it encoded the trehalose-6-phosphate (T6P) phosphatase (TPP), RAMOSA3 (RA3). Our results revealed surprising roles for *GT1* in regulating meristem determinacy, and for *RA3* in regulating carpel suppression, and suggest ancient roles for *GT1*-like genes and trehalose metabolism that have been more recently recruited to regulating floral development. Here, we discuss our ongoing work cloning and characterizing the *rzl* genes, and dissecting the evolution of genes regulating flower development in the grasses.

Gene / Gene Models described: *gt1, ra3*; GRMZM2G005624, GRMZM2G014729
Funding acknowledgement: National Science Foundation (NSF)

P176

Uncovering the genetic basis for diversity of moisture regulated root branching in maize

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Plants that exhibit moisture regulated root branching, called 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. Tuning of root branching and development is pivotal for efficient nutrient and water uptake in a changing environment and hydropatterning may explain some of the environmental plasticity that has been observed. While hydropatterning has been observed in maize, rice, and Arabidopsis, our mechanistic understanding of this process is limited to research in Arabidopsis.
We recently discovered significant variation of hydropatterning in a diverse population of 250 maize inbred lines using a novel high-throughput phenotyping platform. SNP-based GWAS and TWAS (transcriptome-wide association studies) collectively identified 19 hydropatterning-associated genes. Some of these genes had previously been identified as being differentially regulated across the root growth zone during hydropatterning in the maize inbred B73 or had been reported in other studies in relation to water deficit responses making them good candidates for validation. Using transposon insertion lines from the UniformMu and BonnMu collections, we are in the process of experimentally validating some of these candidates. Preliminary results suggest that effects can be observed but depend significantly on the genetic background. Additionally, soil experiments are being carried out with a subset of inbred lines that diverge in their propensity for hydropatterning to study previously observed correlations between hydropatterning, in-field root architecture, and above-ground traits.
Future investigation of the discovered genetic loci will illuminate the molecular mechanisms controlling moisture-regulated root branching and their role as potential targets for breeding more water-use efficient crops.

Funding acknowledgement: Department of Energy (DOE)

P177 @Xiaosa_Xu

Uncovering the non-enzymatic function of RAMOSA3 in maize inflorescence branching

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A fundamental understanding of plant development requires insight into the molecular mechanisms regulated by different signaling pathways. Plant development emerges from stem cell populations called meristems, that control organ initiation and branching. *RAMOSA3* (*RA3*), a classical maize developmental gene, controls maize inflorescence branching, and encodes a trehalose phosphate phosphatase enzyme. Our recent genetic and cell biology studies found that RA3 has a potentially non-enzymatic moonlighting function, since its phenotype can be uncoupled from catalytic activity. Furthermore, RA3 protein forms nuclear speckles, suggesting that it associates with transcriptional regulatory machinery. To tackle the mystery of the non-enzymatic function of RA3, we performed ethyl methyl sulfonate (EMS) screening of *ra3* mutant, and identified an enhancer, *indeterminate spikelet1* (*ids1*). IDS1 is an AP2 type transcription factor, and is the orthologue of the major wheat domestication gene *Q* that controls spikelet density and yield. By carefully examining the early developmental stages of *ra3;ids1* double mutants, we found that floral meristems were transformed into branches, rather than forming florets. We confirmed these findings by crossing *ra3* with additional *ids1* alleles and conducting allelism tests. Using *in-situ* hybridization, we found RA3 and IDS1 were co-expressed around the boundary regions between floral meristems, which was also supported by our single-cell transcriptomic profiling data. To further examine if IDS1 might be involved in the hypothetical transcriptional regulatory function of RA3, we checked for physical interactions between RA3 and IDS1 *in planta* by Bimolecular Fluorescence Complementation assays. Indeed, we found that RA3 and IDS1 proteins interact in nuclear speckles, reminiscent of the nuclear speckle localization of RA3. We further performed RNA-seq for *ra3;ids1* double mutants and identified a list of candidate genes regulated by the RA3-IDS1 complex. Together, our data suggest that RA3 had a potential regulatory role in controlling inflorescence branching by interacting with the transcription factor, IDS1.

Gene / Gene Models described: *RAMOSA3, INDETERMINATE SPIKELET1*; GRMZM2G014729, GRMZM5G862109
Funding acknowledgement: National Science Foundation (NSF)

P178 @sabrinals_chin

Unraveling the maze of gravity-sensing cells in maize with RNA-Seq to discover gravity- specific transcriptional networks

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Gravitropism is a fundamental plant growth response that orients the root to grow downward into the soil for nutrient and water uptake. Gravity in roots is sensed by columella cells of the root cap, and the associated cellular perception events occur immediately after the gravity vector changes. This is followed by a signal transduction to the elongation zone, and finally, differential cell elongation of the root elongation zone, which leads to downward root growth. Although there are numerous studies that investigated transcriptomic changes in roots during the gravity response, they were limited because they did not specify gene expression changes in the root cap, nor addressed the timing of transcriptional changes during gravitropism. To fill these knowledge gaps, roots of 4-day-old maize seedlings were gravity stimulated by rotating them 90°. Root cap cells were harvested after 5, 10, and 30 minutes, and 1 and 2 hours of gravity stimulation for RNA-Seq analysis. Differentially expressed genes (DEGs) for each time point were obtained by comparing transcripts of gravity-stimulated root caps to those of vertical controls. Root cap cells at the 5- and 10-minute time points had the lowest number of DEGs. The number of DEGs in the root cap cells peaked at 30 minutes after gravity stimulation, which corresponded to the time when the elongation zone visibly began to bend. Genes upregulated at 30 minutes were associated with developmental processes, including RNA biosynthesis, mitotic cell cycle, and metabolism. Interestingly, the number of DEGs declined after an hour of gravity stimulation and increased again at two hours. Our findings reveal the dynamic transcriptional responses in root cap cells that corresponds to the different stages of gravitropism. This work is supported by NASA 80NSSC19K0129.

Funding acknowledgement: NASA

P179

Using molecular genetics and precision phenotyping to map gene function contributing to drought resilience in sorghum

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Development of the next generation of bioenergy feedstocks will require strategies that utilize resource-limited agricultural lands, including the introduction of novel traits into crops to increase abiotic stress tolerance. This project investigates the innate drought resilience of sorghum (*Sorghum bicolor*), a bioenergy feedstock and cereal crop. Drought is a complex trait and identifying the genes underlying sorghum's innate drought tolerance and how they are regulated in the broader context of the whole plant and its environment requires advanced approaches in genetics, genomics, and phenotyping. This project leverages a sequence-indexed population of EMS mutagenized sorghum and a field-based phenotyping infrastructure at Maricopa, AZ, which provides an exceptional capability for managed stress trials in a hot and arid environment through controlled irrigation. An automated field scanner system collects high-resolution phenotyping data using a variety of sensors throughout the growing season. 430 EMS families in the tx623 background were phenotyped under the field scanner to compare drought-stressed and well-watered plants. Each mutant's genome has been sequenced so that sequence variants can be linked with phenotypes. Being able to assess the genotype-to-phenotype link in response to drought over the life cycle of the plant will facilitate discovery of genes and their functions. To accelerate mapping of causal loci that underlie mutants of interest, we use bulked segregant analysis-seq. So far, we've identified candidate genes underlying defects in leaf senescence, plant architecture and male fertility. Regulatory maps generated from diverse sorghum lines in response to stress are being used to nominate gene candidates and place them in the larger context of a drought response network. This work will identify control points for enhancing the productivity of bioenergy crops in marginal environments through precision breeding or engineering, and thus accelerate the development of improved varieties that are high-yielding with limited water resources.

Funding acknowledgement: National Science Foundation (NSF), Department of Energy (DOE)

P180 @StephEMartinez

Using an allelic series of katanin mutants in maize to determine the role of KATANIN in plant growth

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Microtubule dynamics and organization influence cell shape and are essential for proper cell division. Several proteins are involved in this process, such as KATANIN, a microtubule severing AAA ATPase protein complex composed of a catalytic p60 and a regulatory WD-40-containing p80 subunit. The p60 catalytic subunit forms hexamers and is sufficient to sever microtubules through ATP hydrolysis. However, binding of the KATANIN protein complex to microtubule severing sites is mediated by the p80 subunit, allowing for higher severing efficiency of microtubules. In maize, several katanin (p60) mutants have been identified, including a loss-of-function double mutant, discordia3a discordia3b (dcd3a dcd3b), and a unique semi-dominant mutant, Clumped tassel 1 (Clt1). The Clt1 mutant contains a missense mutation in the ATPase domain, which may disrupt functional ATP-hydrolysis and microtubule severing. However, it is not yet known how the mutation affects protein function. To determine how each of these katanin mutant alleles impacts microtubule severing, time-lapse imaging of microtubules in dcd3a dcd3b mutants was done and severing events counted. This work provides insight into how these mutations affect microtubule severing. In vitro ATPase activity assays of these mutant proteins will be used to characterize ATPase activity between wild-type and mutant KATANIN p60 subunits. Characterizing KATANIN function will lead to greater understanding of the impacts of microtubule dynamics and organization on plant growth and development.

Gene / Gene Models described: *clt1, ktn2*; GRMZM2G017305, GRMZM2G054715
Funding acknowledgement: National Science Foundation (NSF)

P181 @williangviana

Using the *crown root defective* mutant to understand crown root development under well-watered and drought-stressed conditions

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Crown roots make up the bulk of the mature root system in grasses and are essential for anchorage and water and nutrient absorption. While they play a critical role in crop productivity, little is known about the genetic pathways that control their development in response to environmental cues. Recent work in the panicoid grass model system *Setaria viridis* has shown that sufficient soil moisture (WW) around the crown promotes crown root development, while drought stress (DS) leads to arrest of crown root growth, which allows plants to conserve water stored in the soil. However, if rewatered, DS plants will rapidly resume crown root growth within hours of treatment. We have isolated a *S. viridis* mutant, designated *crown root defective (crd-1),* that specifically affects crown root development under WW conditions. While *crd-1* develops very few crown roots under WW conditions, DS followed by rewatering leads to an induction of new crown roots in *crd-1* plants, which can produce almost as many crown roots as wild-type plants grown under WW conditions. Interestingly, this response in *crd-1* is temporary, and the mutant eventually stops making crown roots if it continues to grow under WW conditions. Bulk Segregant Mapping of the *crd-1* mutation identified a single SNP disrupting the splice site of a gene encoding a WD-repeat protein. Secondary alleles, generated by CRISPR/Cas9 gene editing, showed a similar crown root phenotype. Additionally, T-DNA insertions in the Arabidopsis ortholog resulted in a reduction of lateral root density, suggesting that the function of this gene in modulating root system architecture is conserved in different species. Histological, molecular, and chemical-genetic characterization of the *crd-1* mutant will facilitate understanding of the mechanism of crown root pre-emergence regulation under WW conditions and how DS can modulate crown root growth, possibly via an alternate pathway.

Funding acknowledgement: Department of Energy (DOE)

P182

Vgt1: a *cis*-regulatory element regulating flowering time and growth speed

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The identification and characterization of *cis*-regulatory DNA sequences and how they coordinate responses to developmental and environmental cues is of paramount importance to plant biology. Although thousands of candidate *cis*-regulatory sequences have been identified in maize, few *cis*-regulatory elements have been well characterized. We are studying the function of *Vgt1*, a predicted regulatory element located about 70 kb upstream of the floral repressor gene *ZmRap2.7*, in more detail. Consistent with a function as enhancer of *ZmRap2.7*, *Vgt1* contains an accessible chromatin region. It however lacks significant enrichment of H3K9ac, a histone modification often observed at active regulatory sequences. Silencing of *Vgt1* by RNA-directed DNA methylation results in earlier flowering, and in addition accelerates the growth speed. Expression analysis by RT-qPCR on different leaf areas during plant development indicated *ZmRap2.7* downregulation in leaf 4 of V3 plants upon silencing of *Vgt1*. Consistent with the role of *ZmRap2.7* in flowering, DNA methylation of *Vgt1* resulted in earlier and higher *ZCN8*, *MADS67* and *ZMM4* expression in leaves during plant development. RNA-seq analyses confirmed the downregulation of *ZmRap2.7* expression in *Vgt1* silenced plants. Altogether our data are consistent with *Vgt1* acting as enhancer of *ZmRap2.7*. · Weber et al. (2016) Trends in Plant Science 21, 974. doi: 10.1016/j.tplants.2016.07.013. · Oka et al. (2017) Genome Biology 18:137. doi: 10.1186/s13059-017-1273-4. · Schmitz, Grotewold, Stam (2022) The Plant Cell 34:718. doi.org/10.1093/plcell/koab281.

Gene / Gene Models described: *ZmRap2.7*; Zm00001eb355240

P183

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 optimized for photosynthesis, a sheath that tightly wraps around the stem, and a ligule/auricle that acts as a hinge between 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. 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 170Mbp interval on chromosome 4, suggesting recombination is suppressed in this region, supported by a predicted inversion overlapping the region in low coverage nanopore long-read sequencing. Genetic analysis of 108 double mutants shows that the *wab1-rev* loss-of-function allele (reversion of the dominant *Wavy Auricle in Blade1* [*Wab1-R*]) is epistatic to *Wab2* and that one copy of the *wab1-rev* allele completely suppresses 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. The anatomical features of Wab2 mutants and preliminary mapping data suggest that *WAB2*, like *WAB1*, encodes a TCP transcription factor. We hypothesize that WAB2 and WAB1 TCP transcription factors interact in the nucleus to cooperatively regulate cell proliferation during maize development. Future work will examine potential interactions between these proteins as well as other genetic loci.

P184

bds1 and bds2 function redundantly to regulate inflorescence and shoot architecture in maize via brassinosteroid biosynthesis

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Shoot architecture is a key determinant of grain yield in maize. Among the major plant growth regulators, brassinosteroids (BRs) affect multiple developmental processes and plant architecture traits, including organ size, sex determination, and leaf angle. However, the genetic mechanisms by which BRs regulate plant architecture traits in maize remain poorly understood. We recently generated, identified, and characterized a recessive, EMS-induced maize mutant, which we tentatively named *brassinosteroid deficient semi-dwarf mutant1* (*bds1*). Mutants have a semi dwarf stature due to compressed internodes and are partially rescued by brassinolide. *bds1-ref* mutants also have short leaf sheaths and twisted leaf blades and display a partial tassel-seed phenotype in the Mo17 background and reduced tassel branch number in B73. We localized *bds1* to a small genomic region containing a point-nonsense mutation in a gene involved in brassinosteroid biosynthesis using map-based cloning and whole-genome sequencing. Non-complementation of *bds1-ref* and a *bds1-Mu* allele confirmed that *bds1* encodes an enzyme likely involved in brassinosteroid biosynthesis. Using phylogenetic and blast analysis, we identified that *bds1* has a close homolog we have named *bds2*, and in which we generated several mutant alleles by remobilizing a nearby *Ds* transposable element. Contrary to *bds1* mutants, the *bds2* single mutants are indistinguishable from wild-type plants, while the *bds1-ref*;*bds2-Ds* double mutants are severely dwarfed with other defects similar to those observed with BR-deficient mutants *nana1* and *nana2*. Metabolite accumulation profiling analysis and feeding experiments are ongoing to confirm and characterize how the *bds1* and *bds2* mutants disrupt brassinosteroid biosynthesis. Based on these results, we propose that *bds1* and *bds2* cooperatively regulate shoot architecture and brassinosteroid biosynthesis.

Funding acknowledgement: National Science Foundation (NSF)

P185

101 Evolutions: Evaluating maize gene annotations with genome sequences across the Andropogoneae

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Understanding how species adapt to the environment and the identification of adaptation-related genes relies heavily upon accurate gene models. These models have traditionally been predicted by algorithms that leverage the conservation of proteins from a few well-studied, but often not closely related, species. As a result, gene models can be inaccurate due to reference biases, pseudogene misclassification, transposon activity, and multiple predicted transcripts. To evaluate maize gene model predictions, we leveraged extensive evolutionary depth. By moving out of the genus *Zea* and into the encompassing Andropogoneae tribe, we capture over one billion years of evolution to assess conservation of the B73 v5 gene models. We sequenced 101 Andropogoneae species using short reads, and assembled their genomes to a gene-space level. We then developed a pipeline to systematically evaluate each maize gene model against all Andropogoneae species. Alignments of each gene model to the Andropogoneae species were evaluated to determine conservation scores of each gene model, exon, and codon position. Each gene model in all Andropogoneae species was then evaluated using machine learning models to determine the conservation of UTRs and splice sites. These results were used in our own random forest model to evaluate the quality of each gene model and underlying exons. Our model predicts pseudogenes, private and non-private genes, and genes showing proteome evidence. These results highlight that gene model accuracy can be evaluated by leveraging short read assemblies of a large cohort of related species. Our pipeline will allow a more in depth knowledge of evolution, gene families, and help improve gene model annotation for the maize community.

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

P186

A novel paradigm for optimal mass-feature peak picking in large scale LC-MS datasets using the 'isopair': isoLock, autocredential and anovAlign; and application to plant metabolite genome wide association studies (GWAS)

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Untargeted metabolomics aims to quantify **all** metabolites in a biological sample without any a priori knowledge. This ambition makes it an excellent candidate technology to link biochemistry to genes via quantitative genetics (i.e. genome wide association studies (GWAS)). However, untargeted metabolomics remains **hard** because its dominant application, via liquid chromatography mass spectrometry (**LC-MS**), produces large and noisy data files. LC-MS signal-to-noise issues become worse as datasets grow in size, severely limiting untargeted metabolomics on population scale datasets (> hundreds of samples) in which billions of machine signals must be reduced to several thousand metabolite signatures. This is particularly difficult because LC-MS identifies metabolites as features in a two-dimensional space (journey time through a chromatography tube (retention time) **AND** mass (m/z)) and noise is present in both measurement domains. I will present a novel software program (the I.A.A suite) that addresses all sources of noise in LC-MS data, through a unique approach of signal amplification and alignment. The I.A.A suite quantifies more metabolites with better reproducibility than existing approaches, on datasets of unprecedented size. The I.A.A suite contains three algorithms: **i**solock, **a**utocredential and **a**novAlign. isoLock corrects for drift in the m/z domain, while autoCredential identifies metabolite m/z signals, and anovAlign determines accurate retention time windows for each metabolite's m/z signal. The I.A.A suite is optimized for quantifying metabolites at the scale needed for metabolite GWAS, making it a foundational plant genetics resource. To demonstrate this, I will present preliminary applications of the tool to metabolomics GWAS in *S. viridis* and *S bicolor* during water deficit, as part of a DOE funded effort to understand conserved drought biology of the C4 grasees.

Funding acknowledgement: Department of Energy (DOE)

P187

Bioinformatic identification and analysis of microRNAs associated with maize male gametophyte development

(submitted by Daniel Hickey <hickeyda@oregonstate.edu>)
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MicroRNAs (miRNAs) play a key role in the regulation of development and stress response in plants, targeting sets of mRNA transcripts for cleavage or translational inhibition. The miRNAs involved in male gametophyte development in maize and the specific function(s) they provide are unknown. To initiate an investigation in this area, small RNA sequencing (sRNA-seq) libraries were generated for five stages of male gametophyte development from staged, purified samples (tassel primordia, microspores, bicellular pollen, mature pollen, and sperm cells), in parallel with a set of standard mRNA-seq libraries (Warman et al. 2020). Analysis of the sRNA-seq libraries with the ShortStack, miRador, and miRDeep-P2 tools, based on annotation criteria previously established by Axtell and Meyers (2018), led to the discovery of 156 predicted miRNA loci across the five stages. Based on sequence comparison of the predicted mature miRNA sequences, these were assigned into 44 miRNA families, each potentially sharing a set of target mRNA transcripts. Approximately half of these miRNAs were not annotated in the miRNA database miRBase22, highlighting the potential for miRNA discovery in these datasets. A variety of expression patterns were observed among the identified miRNAs, e.g., peak expression at one particular stage of gametogenesis, or more constitutive across the entire developmental series. To better address possible functional roles, potential target genes (i.e., protein-coding transcripts) of miRNAs were identified using TargetFinder. Ongoing approaches to identify the best miRNA and target gene pairs for experimental validation using expression data and TargetFinder scores will be presented.

Funding acknowledgement: National Science Foundation (NSF)

P188

Cell size and RNA transcriptome size measurements in hybrids and inbreds in diploid and tetraploid maize

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Heterosis refers to the phenomenon that progeny of diverse varieties of a species or crosses between species exhibit greater biomass, speed of development, and fertility compared to both parents. Various models have been posited to explain heterosis, including dominance, overdominance, and pseudo-overdominance, but these models appear inadequate particularly with regard to the behavior of heterosis in polyploids. In plants, ploidy series studies show a positive correlation between cell size and transcriptome size. By comparing the leaf cell size of ploidies of maize and its relatives, we found a significant increase of the cell size in a triploid Maize/Tripsacum/*Zea diploperennis* plant compared to a triploid Maize/Tripsacum (one maize; two Tripsacum genomes) suggesting that diverse genetic backgrounds might increase the cell size. We further compared the cell size of inbreds and hybrids in both diploid and tetraploid levels in maize with varying magnitudes of heterosis and found that the hybrids showed mid-parent heterosis in cell size. Further, comparing the gene expression per cell (normalized by genomic DNA) to the gene expression per transcriptome showed that the transcriptome size in diploid hybrids is higher than the mid-parent value. Our results suggest that heterosis is associated with an increase of the whole transcriptome size, which is not easily reconciled by classical models and suggests a need for new models to explain heterosis.

Funding acknowledgement: National Science Foundation (NSF)

P189

Characterization of the gene networks underlying cuticle production in maize via systems' biology approaches

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The plant cuticle is a hydrophobic barrier deposited on the extracellular epidermis, and constitutes a first line of defense against numerous abiotic and biotic stresses. It is comprised by a cutin polyester matrix infused with and laid atop by cuticular waxes including very long chain fatty acids and fatty acid derivatives. Herein, two model systems are investigated to gain a better understanding of cuticle biosynthesis in maize: 1) the reproductive silks; and 2) developing seedlings that initiate vegetative growth. A systems' biology approach is employed to identify specific gene-to-metabolite associations through joint statistical analysis of cuticle metabolomes and companion transcriptomes. Approximately 300 genes are significantly associated with silk cuticular wax variation between B73 and Mo17, and along the silk length. These candidates include genes known to participate in cuticular wax biosynthesis and genes from the pathways that directly or indirectly interact with cuticular wax biosynthesis, including cell wall biogenesis, proteasome-mediated protein degradation, and flavonoid biosynthesis. Using a similar multi-omics integration pipeline, cuticle constituents including cutin and cuticular waxes are examined during early seedling establishment. A gene network comprised of ~1900 candidate genes is identified as associated with the compositional changes of cutin monomers and/or cuticular waxes among six seedling organs from B73 and Mo17, and their reciprocal hybrids, capturing a transition from heterotrophic growth to autotrophic growth. This work suggests that cuticle production during early seedling establishment may be stimulated by repression of fatty acid beta-oxidation, and by establishment of photosynthetic machinery. In conclusion, this work provides putative networks of numerous candidate genes derived from two independent studies, demonstrating the complexity in metabolic pathways underlying cuticle composition.

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

P190 @nbornowski

Comparative genomics of expired Plant Variety Protected maize lines in the Stiff Stalk heterotic germplasm pool

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The Stiff Stalk heterotic group is an important source of inbreds used in U.S. commercial hybrid production. Founder inbreds B14, B37, B73, and to a lesser extent B84, are found in the pedigrees of a majority of commercial seed parent inbred lines. We created high-quality genome assemblies of B84 and four ex-Plant Variety Protection lines LH145 representing B14, NKH8431 of mixed descent, PHB47 representing B37, and PHJ40 which is a Pioneer Hi-Bred early Stiff Stalk type. Sequence was generated using long-read sequencing achieving highly contiguous assemblies of 2.13 to 2.18 Gbp with N50 scaffold lengths greater than 200 Mbp. Inbred-specific gene annotations were generated using a core five-tissue gene expression atlas while transposable element annotation was conducted using de novo and homology-directed methodologies. In comparison to the reference inbred B73, synteny analyses revealed extensive collinearity across the five Stiff Stalk genomes, although unique components of the maize pan-genome were detected. Comparison of this set of Stiff Stalk inbreds with the original Iowa Stiff Stalk Synthetic breeding population revealed that these inbreds represent only a proportion of variation in the original Stiff Stalk pool and there are highly conserved haplotypes in released public and ex-Plant Variety Protection inbreds. Despite the reduction in variation from the original Stiff Stalk population, substantial genetic and genomic variation was identified supporting the potential for continued breeding success in this pool. The assemblies described here represent Stiff Stalk inbreds that have historical and commercial relevance and provide further insight into the emerging maize pan-genome.

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

P191 @shanwai1234

Deciphering the contribution of 3D genome organization to gene regulation in maize inbreds and hybrids

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Three-dimensional (3D) chromatin organization plays a key role in gene regulation of plants. The substantial structural variation among maize inbreds offers the opportunity to assess how structural variants influence long distance interactions. In addition, comparisons of long distance interactions in hybrids and inbred parents allows the chance to assess potential influences of heterosis and potential for interallelic interactions. The 3D genome architecture in multiple maize genotypes with assembled genomes and derived hybrids remains unclear. In this study, we primarily applied HiChIP (H3K4me3 and H3K27ac) technology to assess the variation of long-range interactions and their associated gene regulation in B73, Mo17 and their reciprocal F1 hybrids (B73xMo17 and Mo17xB73). The substantial structural variation between two inbreds can influence the ability to detect leads to the biased detections of interactions. However, a detailed analysis of interactions between interacted syntenic genes between B73 and Mo17 finds that many interactions persist even when the physical distance is quite different in the two inbreds were not affected by their physical interaction distance. The genes involved targets of inbred-specific interactions are enriched for genes that show differences in expression between the two genotypes tended to enrich more inbred-specific expressed genes. Comparisons of the ng interactions found in between inbreds and hybrids revealed , we identified a set of high-confident hybrid-specific interactions in both F1 hybrids that often result in non-additive expression pattern were likely to produce genes in non-additive expression patterns. We identified a set of interactions that are gained or lost in hybrids relative to the inbred parents and these often were found near genes with non-cis regulatory variation in expression levels. The analysis of hybrids also offered the potential to identify interactions between the alleles present on homologous chromosomes. Using SNPs to classify genetic sources of two anchors of interactions in the hybrid data, we identified a few potential inter-allelic interactions in maize hybrids. These interallelic interactions suggest limited pairing of homologus chromosomes in somatic tissues and could result in transvection-like gene regulation. , suggesting a possibility of genetic complementation in plants.

P192

Desiccation tolerance in grasses: two methods evolved to limit damage and survive

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Desiccation tolerance has evolved recurrently in C4 grasses as a common adaptation to survival in seasonally dry regions. Desiccation tolerant grasses such as Oropetium thomaeum and Eragrostis nindensis can quickly re-establish photosynthesis, in leaves that have completely desiccated, when water becomes available. Interestingly these grasses use different processes to accomplish this amazing feat. O. thomaeum retains chlorophyll during desiccation while E. nindensis degrades then reconstitutes its chlorophyll. We used a combination of gene expression (RNAseq) and chromatin accessibility (ATACseq) in order to identify genes involved in these processes and look for potential regulatory motifs. We observed a strong association with differential gene expression and accessibility of nearby chromatin. While both species had a down regulation of photosystem I, O. thomaeum showed an up regulation of photoprotection and protein localization to the chloroplast, whereas E. nindensis had an upregulation of endocytic recycling and a down regulation of photosystem II. Analysis of motifs near open chromatin regions indicate that an ABI5-like transcription factor may be driving some of the common gene expression changes in desiccation. Some non-syntenic gene expression differences in these species are also correlated with different nearby motifs and chromatin openness reflecting neofunctionalization during desiccation tolerance evolution. Our results provide a glimpse at how some grasses evolved desiccation tolerance, the different strategies to avoid photooxidative damage, and the genome-wide regulatory elements impacting the genes involved.

Funding acknowledgement: National Science Foundation (NSF)

P193 @plantbiojordan

Detection and quantification of tar spot foliar infection in maize using machine learning, object detection, and application development framework

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The expanding geographic range of Phyllachora maydis, the fungus that causes tar spot of maize, is increasingly threatening a Michigan industry that provides $1 billion to the state's economy annually. Foliar infection of maize by P. maydis is often difficult to detect early. Visible lesions initially appear tiny, ambiguous, and are sparse in distribution, making them difficult to quantify with the naked eye. In 2020, one-third of all farms around the world cultivated maize, and this number is expected to increase globally by 5% by 2030. Both farmers and breeders of maize desperately need better tools that allow early, definitive detection of tar spot lesions and provide more time for management decisions. This tool must verify presence of P. maydis and quantify infection severity as quickly as possible to allow growers the most options for treatment. Advances in machine learning now enable quantification of crop disease incidence and severity using powerful object detection packages including Tensorflow and PyTorch. Tensorflow, specifically, has developed Application Programming Interface tools to connect powerful object detection capabilities with streamlined usability. I propose an application developed by weaving the transferrable, containerized infrastructure of Docker with the powerful machine learning platform Tensorflow, thereby allowing users to analyze images of maize foliage using their preferred operating system. By implementing both complementary platforms, farmers and breeders will be afforded a tool specifically designed for assessing tar spot severity, leading to a more tailored and informed approach for disease mitigation.

Funding acknowledgement: Bayer Crop Science, Michigan State University Plant Science Fellowship

P194

Development of an instance segmentation pipeline for comprehensive high-throughput phenotyping of maize ears

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Maize yield is a complex trait determined by both genetic and environmental factors (including management decisions) as well as interactions between the two. Because final yield is determined by many different properties of the plant, each governed by many distinct genetic loci, the individual contribution of a single locus to final yield outcomes is typically quite small. By partitioning yield into different component traits, it may be possible to increase the proportion of variation for individual traits explained by specific genetic loci. However, scoring yield component traits such as ear length, ear row number, ear fill, kernel number, and kernel dimensions across hundreds or thousands of ears can be time consuming and logistically unfeasible. Some high-throughput phenotyping to quantify kernels in maize ears currently exist, but they are incomplete, as they do not collect data from the entire ear. To provide a wholistic view of the ear, these ears were first placed on a rotational scanner, and then projected into two-dimensional images. We then sought to implement an instance segmentation model to classify objects from each two-dimensional image as either kernel or cob space. Following the optimization of this model, ear yield component traits of interest will be extracted, which will enable future comprehensive, high-throughput ear phenotyping for large field experiments.

Funding acknowledgement: National Science Foundation (NSF)

P195

Dynamic maize metabolic gene regulatory network construction by regression analysis

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Gene regulatory networks represent relationships between regulator genes, such as transcription factors, and target genes. These networks are dynamic when the individual impact of a regulator on the expression of its target gene is inferred by statistical modeling. As dynamic gene regulatory networks scale in accordance to genome size, their predictive power lessens. With maize's large and complex genome, smaller modules must be constructed individually and connected in order to build a complete network.
Maize whole transcriptome sequencing data of leaf tissue samples was gathered from several publicly available experiments, representing many environmental perturbations during vegetative development. The samples from these experiments were compiled with alignment to the B73_v5 reference genome and read quantification with batch normalization. Target genes for gene regulatory modules were grouped based on their function within specific metabolic processes. Starting with nitrogen assimilation enzymes as targets, linear regression models were built to predict each gene's expression based on the expression values of all transcription factors. This was then repeated to create an amino acid synthesis module. We identified transcription factors regulating both which act as connectors similarly to metabolite connections in metabolic network modules. To validate the regulatory links within our modules, we predicted gene expression values based on transcription factor expression values from a separate maize leaf development experiment involving varying nitrogen treatment.
Dynamics gene regulatory networks allow us to bridge the gap in plant models between genotype and phenotype. By building a robust network of enzymatic gene targets, we are able to explore and build future hypotheses on why certain variants such as differing alleles or gene knockouts have significant impacts on overall plant phenotype.

Funding acknowledgement: Foundation for Food and Agriculture Research (FFAR)

P196

Global Run-On sequencing identifies nascent transcription in the B73 genome

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Identifying nascent transcripts is critical for validating and enhancing genome annotations. Such profiles reveal the entirety of transcription across gene models including the extensive pretermination regions absent from RNA-seq data. These profiles can also identify RNA species having high turnover rates including non-coding RNAs like those produced from enhancers.
We evaluated the potential role of sarkosyl detergent in inhibiting RNA polymerase II re-initiation events and modified our published Global Run-On (GRO)-seq protocol to use biotin-based purification of nascent RNAs and sRNA-type library preparation. We applied these improved procedures to define the nascent RNA transcriptome of B73 6-day post-imbibition seedlings.
These new datasets provide >90M mapped reads (>45M uniquely) which promise to identify novel intergenic transcription. We identified sense transcription from over 75% of all annotated B73 v5 gene models and low levels of antisense transcription from ~72%. Additionally, 74% of all currently annotated ncRNA transcription units were also represented. GRO-seq profiles of other tissues, developmental timepoints, and diverse genomes, promise to deliver an unparalleled view of transcriptional dynamics available in the maize pan-genome.

Funding acknowledgement: National Science Foundation (NSF)

P197

Identification of potential haploid expressed genes in maize

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The purpose of this project is to develop a pipeline capable of taking in published RNA-Seq data and return a list of prospective reading frames that are either overexpressed or uniquely expressed in haploid tissue compared to diploid tissue. Additionally, that list further curated by comparison to transposon databases such that the final list can have their function characterized by a follow up knock out experiment.

Funding acknowledgement: National Science Foundation (NSF)

P198

MaizeGDB's MaizeMine: New genome and community data sets

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The availability of high-throughput genomic technologies has facilitated the generation of massive quantities of genomic datasets. Increasingly, there is a need for maize researchers to conduct comparative analysis between their own datasets and published or publicly available data. MaizeGDB's data mining warehouse, MaizeMine (http://maizemine.maizegdb.org), utilizes the InterMine data warehousing platform which empowers researchers without bioinformatics skills to integrate their data with publicly available data and perform meta-analyses using a suite of tools, including a keyword search, built-in template queries with intuitive search menus, and a QueryBuilder tool for creating custom queries. The List tool allows users to upload identifiers to perform set operations and to execute template queries with lists. Users can easily compare their results with published results by uploading genomic coordinates or identifiers. MaizeMine integrates the Zea mays B73 genome and genes with Gene Ontology (GO), protein annotations (UniProt), protein families and domains (InterPro), homologs (Ensembl Plants), SNP (Ensembl Plants) and pathways (KEGG, Plant Reactome) as well as project-specific data generated by the maize research community. Here we report the updating of MaizeMine (v1.4) to include the most recent B73 genome assembly (Zm-B73-REFERENCE-NAM-5.0) and associated annotations (Zm00001eb.1). MaizeMine v1.4 includes both updated older data sets and new community data, including gene expression from MaizeGDB's qTeller, GWAS and QTL loci with associated traits, transcription start sites, and enhancers. Gene alias identifiers facilitate easy conversion between the old and new gene sets, and cross references are provided to link Zm00001eb.1 genes with RefSeq.

Funding acknowledgement: United States Department of Agriculture (USDA)

P199

Mapping and functional characterization of cis-regulatory variation in maize

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The domestication and improvement of many plant species has frequently involved modulation of transcriptional outputs and continues to offer much promise for targeted trait engineering. The cis-regulatory elements (CREs) controlling these trait-associated transcriptional variants however reside within non-coding regions that are currently poorly annotated in most plant species. This is particularly true in large crop genomes such as maize where regulatory regions constitute only a small fraction of the total genomic space. Transcription factors (TFs) bind to short DNA sequence motifs in regulatory regions of target genes and control the gene expression changes responsible for plant developmental programs and environmental responses. 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. Our goal is to identify functional CREs and to understand how TF-DNA binding and its variability in different genetic backgrounds affects gene regulation and ultimately phenotypic outcomes in maize. Using a combination of DAP-seq and ATAC-seq for detecting TF binding and open chromatin regions, respectively, we are generating CRE maps in different maize inbred lines. Guided by these high-resolution maps of proximal and distal CREs, we are disrupting specific CREs and measuring phenotypic outcomes, with a focus on genes regulating plant architecture and biotic stresses.

Funding acknowledgement: National Science Foundation (NSF)

P200

Modeling chromatin state from sequence across angiosperms using recurrent convolutional neural networks

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Accessible chromatin regions are critical components of gene regulation but modeling them directly from sequence remains challenging, especially within plants, whose mechanisms of chromatin remodeling are less understood than in animals. We trained an existing deep learning architecture, DanQ, on leaf ATAC-seq data from 12 angiosperm species to predict the chromatin accessibility of sequence windows within and across species. We also trained DanQ on DNA methylation data from 10 angiosperms, because unmethylated regions have been shown to overlap significantly with accessible chromatin regions in some plants. The across-species models have comparable or even superior performance to a model trained within species, suggesting strong conservation of chromatin mechanisms across angiosperms. Testing a maize held out model on a multi-tissue scATAC panel revealed our models are best at predicting constitutively-accessible chromatin regions, with diminishing performance as cell-type specificity increases. Using a combination of interpretation methods, we ranked JASPAR motifs by their importance to each model and saw that the TCP and AP2/ERF transcription factor families consistently ranked highly. We embedded the top three JASPAR motifs for each model at all possible positions on both strands in our sequence window and observed position- and strand-specific patterns in their importance to the model. With our cross-species "a2z" model it is now feasible to predict the chromatin accessibility and methylation landscape of any angiosperm genome.

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

P201

North American maize yield prediction contest

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We invite individuals or teams from all disciplines to apply quantitative genetic, machine learning, deep learning, or any other method to the largest publicly available maize field productivity trial and related datasets and make predictions for the 2022 season! Improving crop productivity prediction across genetics and environmental conditions has far reaching economic, scientific, and societal implications. However, crop performance is influenced by numerous interdependent factors, many of which are difficult to measure at scale. The use of large-scale datasets, improved quantitative methods and novel approaches will be required to improve prediction accuracy. The Maize GXE project within the Genome to Fields (G2F) initiative has collected the largest and most comprehensive publicly available maize productivity data set to date with phenotypic, genotypic, soil, weather, and management information from approximately 180,000 field plots across 162 distinct environments and involving 2,500 diverse hybrid varieties. A cash prize, opportunity to present at an international meeting, and primary authorship on a resulting manuscript will be offered to the individual or team with the highest accuracy predictions! See poster for conditions, schedule, and further details.

P202 @GrameneDatabase

One-stop pan-genome 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 the current status on the maize pan-genome subsite (https://maize-pangenome.gramene.org) that supports recent work to develop reference assemblies for 26 maize accessions parents of the nested association mapping (NAM) population. A key feature of the 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 30K GeneTree families were constructed comprising over 1 million individual genes from 34 genomes. In addition to the 26 NAM lines including B73 (v5) and two older versions (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). New supporting data tracks for repeat masking features and gene model annotation are available. Additionally the pan-site will support the reference genome of teosinte, the wild ancestor of maize, BLAST service and supporting annotation tracks for the NAM genomes. We gratefully acknowledge funding from NSF awards #1744001 and #1127112, and USDA-ARS award #001-8062-505 002.

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

P203 @Yan_Geneticist

Phenotyping and dissecting genetic variations of maize self-organized azimuthal canopy orientation and its impacts on light interception

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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 (SAS), 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, our mutants exhibited altered canopy architectures. Specifically, in both of *phyC2* and *liguleless1* 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. Further, we show such alteration on canopy orientation varies in different planting density. 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 SAS.

Funding acknowledgement: National Science Foundation (NSF)

P204

Regulatory networks governing nitrogen use efficiency in maize and sorghum

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Nitrogen (N) is an essential macronutrient for plant growth and development. Maximizing Nitrogen Use Efficiency (NUE) in plants is vital to increase crop production and reduce negative impacts on the environment. Previous studies in Arabidopsis using an enhanced yeast-one hybrid assay indicated that genetic perturbation of twenty-one transcription factors (TFs) regulates response to N limitations in root and shoot. In this study, we aim to explore the gene regulatory network (GRN) that controls NUE related processes in maize and sorghum. We constructed and projected GRN for maize, profiled the transcriptome of maize in response to N limitation, and ran a gini correlation analysis. Our data suggested more than ⅓ of the edges in the projected GRN is conserved based on the expression data, with the nitrate assimilation subnetwork being more conserved. We are also conducting this analysis in sorghum with the target to functionally validate candidate genes using sorghum EMS mutants. Conservation of these shared NUE modules may provide critical insight into nitrogen regulation for agronomically important crops. This work is funded by the US Department of Agriculture, Agriculture Research Service under Award Number 8062-21000-041-00D.

Funding acknowledgement: United States Department of Agriculture (USDA)

P205

Simulating breeding effect of recombination landscapes in maize.

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Recombination is the most important process in generating new allelic combinations in sexually reproducing organisms. In crop plants, recombination creates novel combinations, which confer improvement of agronomic traits. Recombination can also break up unfavorable allele combinations by decreasing undesirable genetic linkages. Unfortunately, there are normally very few crossovers (CO) events along a chromosome, which is a limiting factor in generating new varieties quickly. Furthermore, in many crops, including maize, CO distribution is skewed towards chromosome ends, leaving large pericentromeric segments practically devoid of COs. Altering recombination either in specific regions or genome-wide would aid breeding efforts. However, there have been no studies so far that have shown effective control over specific recombination events in plants. Mutants of maize genes responsible for DNA methylation such, as *Decrease in DNA Methylation I* (*ddm1*) and *Zea methyltransferase2* (*zmet2*) confer increases in CO events and alter genome-wide CO distributions. This project explores effects of recombination landscapes conferred by *ddm1* and *zmet2* on genetic gain in various breeding scenarios. We are exploring scenarios such as differences in trait architectures, SNP densities, and heritability to discover which recombination landscapes can help maximize genetic gain.

Funding acknowledgement: National Science Foundation (NSF)

P206 @1amandagilbert

Structural and functional properties of core and dispensable genes in maize

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Plant genomes are dynamic, with variation arising through polyploidization, differential genome fractionation following whole-genome duplication events, transposable element activity, and more. The breadth of genetic variability present within a species is rarely represented by a single reference genome, necessitating the move from a single reference to a multi-reference pan-genome approach. The core genome describes the set of genes shared by all sequenced individuals, while the dispensable genome refers to a subset of sequences shared by only some individuals. The pan-genome, consisting of the core and dispensable genes, represents the full complement of genetic material present in a species. The chromosome level assemblies of the 26 maize NAM founder lines were used to identify 32,052 core and 71,486 dispensable genes within maize. To gain further insights into the properties and functions of these two sets of genes, we are exploring and comparing nucleotide diversity, methylation state, gene expression and connectivity networks, and functional domains.

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

P207

Systematic exploration of transcription factor function in maize

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Transcription factors (TFs) control many important processes in plants such as abiotic stress tolerance and pollen development. The large number of TFs in the genome – over 2000 in maize – have left many of these key regulators poorly understood. We are exploring TF function in maize more systematically using gain-of-function assays in leaf mesophyll protoplasts. By ectopically expressing different TFs, it is possible to induce their target pathways. We then measure the full transcriptome-wide response to each TF by RNA-sequencing, using high-throughput methods originally developed for single-cell RNA-seq. We have optimized our protocols with robotics so that all steps can be performed rapidly in 384-well plates, with 1000s of assays in parallel. We are first testing a pilot set of 163 cloned TFs, and plan to scale to the full cloned TFome – providing a valuable resource to the maize community. These data will provide insight into native TF function and help identify TFs that can reprogram plant form and function for synthetic biology.

P208

Temporal and spatial auxin responsive networks in maize primary roots

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Auxin is a key regulator of root morphogenesis across angiosperms. To better understand auxin regulated networks underlying maize root development we have characterized auxin responsive transcription across two time points (30 and 120 minutes) and four regions of the primary root: the meristematic zone, elongation zone, cortex, and stele. Hundreds of auxin-regulated genes involved in diverse biological processes were quantified in these different root regions. In general, most auxin regulated genes are region unique and are predominantly observed in differentiated tissues compared to the root meristem. Auxin gene regulatory networks (GRNs) were reconstructed with these data to identify key transcription factors that may underlie auxin responses in maize roots. Additionally, Auxin Response Factor (ARF) subnetworks were generated to identify target genes which exhibit tissue or temporal specificity in response to auxin. These networks describe novel molecular connections underlying maize root development and provide a foundation for functional genomic studies in a key crop.

Funding acknowledgement: United States Department of Agriculture (USDA)

P209

The classic maize mutant *Rootless1* impairs in shoot-borne root formation and affects the root system architecture

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Monocots can develop shoot-borne "nodal" roots that influence major root architectural traits important for plant anchorage and resource capture. Yet, the genetic and molecular control of nodal root development remains largely unknown, especially in maize, one of the world's most important crops. The basic-helix-loop-helix (bHLH) family of transcription factors (TFs) play crucial roles in lateral organ development, but their functions in nodal root development are elusive. Here we identified the molecular basis of the classic maize *rootless1 (rt1)* mutant as a loss-of-function mutation in a gene encoding a bHLH TF (named here as *ZmRt1*). Nanopore sequencing of *rt1* revealed an Indel in the 5' region of *ZmRt1* leading to its reduced expression. Histology and X-ray tomography analyses of below and above ground mutant nodes respectively revealed defects in the nodal root primordia initiation process. *ZmRt1* mRNA accumulates in distinct domains of nodal root meristems in Wild-type, consistent with tissues affected by the mutation. Protein sequence analyses and localization of *Rt1* to the nucleus suggest that it may function as a transcriptional regulator similar to its related family members. Genetic complementation tests with additional mutant alleles confirm *ZmRt1* as the causative locus for the defective nodal root developmental phenotypes in greenhouse and field experiments. Phylogenetic analyses predicted a *ZmRt1* paralog designated as *ZmRt2,* which is unique to maize and absent in closely related Poaceae. Expression analyses suggest *ZmRt2* may compensate in weak *rt1* mutants. Population genetics coupled with 3D X-ray root phenotyping experiments involving maize inbred and its wild teosinte ancestor lines in the context of *rt1* mutation and natural genetic variations at *Rt1* loci confirmed the presence of potential adaptive alleles for *ZmRt1* that contribute to the robustness of variations in global root system architecture observed. In support, several transposon insertions were identified in the promoter of *Rt1* in teosinte and maize NAM inbred lines that correlated with variation in nodal root numbers and *ZmRt1* expression. We conclude that *ZmRt1* plays a key role in nodal root development in maize with allelic variations that could be targeted for maize root system ideotype development.

Funding acknowledgement: National Science Foundation (NSF)

P210
The effect of maize genotypes, enviroments, and GXE interactions on maize endophytes

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Recent studies in the plant-associated microbiome have pointed out that rhizosphere microbiome and endophytes, organisms that live inside plants without causing disease, can benefit their host plant by supplying vital nutrients, manipulating plant hormone levels, and producing secondary metabolites. However, most discoveries focus on an individual plant-microbes relationship and fail to translate into an agricultural benefit in the real world. This simplified view on plant-microbes interaction ignores the fact that plants are under the consistent influence of the environment and change their metabolism to react to environmental stimuli, which indirectly affects the endophyte community. To understand the environmental context of plant-microbe interaction, we sample 25 maize pedigrees in more than 15 locations in 2019 and 2020 and analyze the effect of locations, genotypes, and GXE interaction on the diversity of endophyte and individual taxa. We found the huge GXE and location effect on both alpha diversity of the endophyte community and individual taxa abundance, but a little effect of maize genotypes. We also found the environment factors such as soil pH are associated with community composition.

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

P211 @JoeGage10
Variation in upstream open reading frames contributes to allelic diversity in protein abundance

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The 5' untranslated region (UTR) sequence of eukaryotic mRNAs may contain upstream open reading frames (uORFs), which can regulate translation of the main open reading frame (mORF). The current model of translational regulation by uORFs posits that when a ribosome scans an mRNA and encounters a uORF, translation of that uORF can prevent ribosomes from reaching the mORF and cause decreased mORF translation. In this study, we first observed that rare variants in the 5' UTR dysregulate maize protein abundance. Upon further investigation, we found that rare variants near the start codon of uORFs can repress or derepress mORF translation, causing allelic changes in protein abundance. This finding holds for common variants as well, and >80% of common variants that disrupt uORF start codons are GWAS hits for metabolic and whole-plant phenotypes, suggesting that translational regulation by uORFs serves an adaptive function. These results provide evidence for the mechanisms by which natural sequence variation modulates gene expression, and ultimately, phenotype.

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

P212

B chromosome induced ploidy variation in high loss lines

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The B chromosome of maize is a nonvital chromosome, but has a drive mechanism consisting of nondisjunction at the second pollen mitosis that produces the two sperm and then the sperm with the B chromosomes preferentially fertilizes the egg rather than the central cell. The so-called High Loss line discovered by Rhoades and Dempsey shows breakage of heterochromatic knobs on the A chromosomes at the second pollen mitosis when B chromosomes are present, which was suggested as a related phenomenon to the B nondisjunction. The High Loss line also shows the production of trisomies and triploids when B chromosomes are present, but this aspect had not been examined in detail previously. Examination of mature pollen of this line with B chromosomes shows chromatin bridges between sperm and a high percentage (up to 20%) of grains with only a single diploid sperm, the latter explaining the production of triploids. When the High Loss line with B chromosomes is crossed to tetraploid females, a higher percentage than normal of fully developed endosperms is produced suggesting the joining of the polar nuclei with a diploid sperm. Examination of the chromosome number in the embryos showed diploids, triploids, and tetraploids confirming that the diploid sperm are functional. Some of the diploids produced contain B chromosomes indicating fertilization occurred but there has been a loss of the A chromosomes. The reference inbred line B73 shows properties of being a High Loss line, when several B chromosomes are introduced, although to a lesser degree. It has long been recognized that in Native American collections there is a negative correlation between B chromosomes and a high number of knobs, which suggests that some level of the high loss phenomenon occurs in many lines.

Funding acknowledgement: National Science Foundation (NSF)

P213

Can sorghum ethanol production be increased with colchicine induced autotetraploid lines?

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Biofuel crops have the potential to provide energy with a smaller carbon footprint. *Sorghum bicolor* is a highly stress tolerant C4 crop that can synthesize and accumulate sugars in the stem that can be used for ethanol production. However, the current ethanol production rate of sorghum is yet to be optimized. Past studies in other species have shown that higher ploidy levels can lead to increased cell size and vigor. In an effort to increase the sugar accumulation in sorghum stems, we induced an autotetraploid line using colchicine. Colchicine is known to disrupt microtubule formation and chromosome segregation during cell division and has been used to double the ploidy level of many plant species. Colchicine concentrations, and treatments exposures times were modified from previous studies to determine which combination would be optimal for inducing autotetraploid sorghum. Three total trials were conducted using a single genotype. Two trials used a 0.1% colchicine solution, diluted in water, with exposure times of 2.5 and 5.5 hours. The third trial used a 0.05% colchicine solution exposed for 5.5 hours. Meristematic tissue of sorghum coleoptiles was directly targeted in these colchicine treatments by cutting the tips and inverting seedlings into a colchicine solution. Flow cytometry of nuclei extracted from leaf tissue showed chimeric plants with diploid and tetraploid cells. The resulting seeds are currently being grown to validate that the polyploid event was inherited. Stem cross sections of verified autotetraploids and diploid controls will be used to investigate cell size and sugar accumulation. Field trials will evaluate autotetraploid agronomic performance compared to diploids and will also measure ethanol production. More genotypes will undergo colchicine treatment to produce additional autotetraploid lines. The development of multiple autotetraploid lines will allow us to test the effect of progressive heterosis on biofuel traits.

Funding acknowledgement: Department of Energy (DOE)

P214

Copy number variation analysis of the cis factor (region) 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, both cis factor and trans factors are required for the unequal allocation of the B chromosome. Previous analyses demonstrated that the cis factor required for nondisjunction resides in the centromeric region (Han et al., 2009). Three EMS TB-9Sb recovered by Wayne Carlson in the 1970's that have lost nondisjunction were analyzed and found to have the same mutated trans factor. An analysis of TB-9Sb mutant hybrids or hybrids between mutants and normal 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. Copy number variation (CNV) analysis of mutants and normal using mature pollen gDNA-seq data suggests an over-replication of the B repeats in the B centromere region in the normal B chromosome. While in the nearby centromeric heterochromatin region, the knob sequences might replicate slower in the normal B chromosome in pollen than the same region in the mutated B chromosomes. Our results indicate that the different replication rates of the knob and the B repeats around the B centromere region might cause the nondisjunction of the B chromosome at the second pollen mitosis.

Funding acknowledgement: National Science Foundation (NSF)

P215

Frequent spindle assembly errors require structural rearrangement to complete meiosis in Zea mays

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The success of an organism is contingent upon its ability to faithfully pass on its genetic material. In meiosis of many species, the process of chromosome segregation requires that bipolar spindles be formed without the aid of dedicated microtubule organizing centers such as centrosomes. Here we describe detailed analyses of acentrosomal spindle assembly and disassembly in time-lapse images from live meiotic cells of *Zea mays*. Microtubules organized on the nuclear envelope with a perinuclear ring structure until nuclear envelope breakdown, at which point microtubules began bundling into a bipolar form. However, the process and timing of spindle assembly was highly variable with frequent assembly errors in both meiosis I and II. Approximately 61% of cells formed incorrect spindle morphologies, with the most prevalent being tripolar spindles. The erroneous spindles were actively rearranged to bipolar through a coalescence of poles before proceeding to anaphase. Spindle disassembly occurred as a two-state process with a slow depolymerization followed by a quick collapse. The results demonstrate that maize meiosis I and II spindle assembly is remarkably fluid in the early assembly stages, but otherwise proceeds through a predictable series of events.

Funding acknowledgement: National Science Foundation (NSF)

P216

Further genomic and cytological investigation of *RAD51* and *BRCA2* genes in maize

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RAD51 and BRCA2 are double-strand break repair proteins. In most eukaryotes, including maize, RAD51 and BRCA2 form a complex to repair double-strand breaks using a homologous template. This form of repair occurs both in somatic and meiotic cells, in which it forms the basis of crossing over of chromosomes. Therefore, *rad51* and *brca2* mutations are associated with reproductive defects in many organisms. Maize *rad51a1/rad51a2* double mutants and *brca2* single mutants were previously shown to have full male sterility and low female fertility. The reproductive functions of *RAD51A1*, *RAD51A2*, and *BRCA2* were further investigated through pollen viability analysis and immunolocalization of ZYP1, a protein involved in chromosome synapsis in meiosis I. Additionally, the phylogenetic relations of *RAD51* and *BRCA2* genes in maize and other plant species, which have been represented differently by different sources, were investigated. In addition to the expected finding of almost completely sterile pollen in *rad51a1/rad51a2* and *brca2* mutants, there was reduced fertility in *rad51a1* single mutants, although *RAD51A1* and *RAD51A2* were previously thought to be redundant. Immunolocalization revealed that in *rad51a1/rad51a2* and *brca2* mutants, ZYP1 co-localized with DNA but with a more punctate and fragmented appearance compared to wild type. This suggests faulty pairing of chromosomes in these mutants, which could explain their failure to form fertile pollen. Two trials showed that *rad51a1/rad51a2* mutants, but not *brca2* mutants, have reduced height. Phylogenic analysis confirmed other recent findings of *RAD51A*, *RAD51B*, *RAD51C*, and *RAD51D* genes in all plants surveyed, with a *RAD51A* duplication in both maize and rice; *BRCA2* was a single-copy gene in all species analyzed except for Arabidopsis.

Gene / Gene Models described: *RAD51A1, RAD51A2, BRCA2*; Zm00001d021898, Zm00001d041757, Zm00001d024953
Funding acknowledgement: National Science Foundation (NSF)

P217 @citogenetica

Satellites DNA evolution might be affected by their position into the genome suggest analysis of the K180 and Cent-C sequences

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Repetitive sequences evolve in concert following two main ways, either by the library or molecular driver. Despite the model chosen, the evolution of repetitive sequences is assumed to operate in the same way through the genome. Maize genome represents a unique opportunity to investigate whether the repetitive sequences variability has the same behavior in different genome contexts. Two repetitive fractions of satellite DNAs in maize are in specific genome environments. Cent-C is into centromeric heterochromatic regions, while K180 is the main component of heterochromatic knobs immersed in rich gene regions of the maize genome. Using the genome data available to B73, each of these fractions was analyzed using different approaches to measure and compare nucleotide diversity. In general, knobs present a higher nucleotide variability when compared to centromeric regions concerning the two satDNAs used. Curiously, the Cent-C distribution along the centromeric regions seems not random, with some highly conserved cores mapped in well-defined regions. The variability tends to increase from these conserved cores to the borders of the arrays. K180 does not show any pattern of nucleotide variability distribution along with the arrays. Due to the location of the Cent-C repeats inside the centromere, which has a well-defined function resulting from DNA and protein interactions, this functional environment seems to interfere with the taxes of nucleotide polymorphism. Once knobs are not associated with specific functions and are not linked to functional proteins, it has a random variation of their nucleotide composition. These results suggest that the satellite DNA orchestrated evolution is affected by genome location, especially those interacting with functional proteins.

P218

Smd13 is a candidate adaptor protein that mediates the interaction between KINDR and knob180 repeats, facilitating meiotic drive of abnormal chromosome 10

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Maize abnormal chromosome 10 (Ab10) encodes a meiotic drive system that converts two types of heterochromatic knobs into neocentromeres that are preferentially transmitted to progeny. In recent years, two neocentromere-activating genes encoding kinesins were discovered on Ab10, *Kinesin driver (Kindr)* and *TR-1 kinesin* (*Trkin*). *Kindr* activates knob180 knobs, and *Trkin* activates TR-1 knobs. However, it is unclear how either kinesin physically associates with knobs. Here, we describe a mutant of Ab10 called *smd13* in which KINDR is expressed normally but does not show meiotic drive. We hypothesize that this mutant lacks a gene required for KINDR association with knob180 knobs. We identified a gene with homology to *Kinesin-10* that has lower expression in the *smd13* mutant relative to wildtype Ab10. It is encoded in six tandem copies immediately adjacent to the ten tandem copies of *Kindr* on the distal tip of Ab10. Three of the copies are deleted in the *smd13* mutant. Our further study of the *smd13* mutant and the candidate *smd13* gene will reveal how *Kindr* physically associates with knobs to cause meiotic drive.

Funding acknowledgement: National Science Foundation (NSF)

P219

Investigating Dynamic Heat Stress Responses with Diverse Maize Inbreds through Transcriptome Analyses

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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. Our project objectives 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 responses. For transcriptome profiling, 3' mRNA-seq method was applied using 432 leaf samples from 27 inbred lines, collected at 4 time points from developing and mature leaves in a greenhouse heat stress experiment. Samples for lipid profiling and physiological characterization came from the same greenhouse experiment setting. Under natural environments, two existing and three newly developed mapping populations were used for heat tolerance QTL mapping. With the primary focus on generic heat response mechanisms, we classified the 27 inbred lines into two groups (heat-tolerant and heat-sensitive) for downstream investigation. Differential expression analyses and co-expression network analyses consistently uncovered shared and unique heat response patterns for the two groups along the time series. Gene co-expression modules were associated with other traits to bridge the understanding of heat responses from molecular, biochemical, and whole-plant levels. Among the unique modules from the heat-tolerant group, we highlighted one that was identified to be associated with unfolded protein response, supported by gene ontology (GO) enrichment analysis and cis-element over-representation analysis. This module consisted of a high proportion (37.8%) of heat shock transcription factors (HSFs) and heat shock protein genes (HSPs). Through strategic pattern mining from complex datasets, this study provided insights into maize heat stress response mechanisms at multiple levels.

Funding acknowledgement: United States Department of Agriculture (USDA)

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History of the Maize Genetics Conference

Year	Annual	Location	Dates	Chair
2022	64	St. Louis, Missouri	March 31 – April 3	Erin Sparks
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

Year	Annual	Location	Dates	Chair
1978	20	Allerton Park, IL	March 11-12	Earl Patterson
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

Notes

Notes

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Notes

This conference received financial support from:

National Science Foundation
Corteva Agriscience, Agriculture Division of DowDuPont
Syngenta
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We thank these contributors for their generosity!

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

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